ASSOCIATION BETWEEN *NAT2* POLYMORPHISMS AND ANTI-TUBERCULOSIS DRUG-INDUCED LIVER INJURY IN MYANMAR PATIENTS IN THAILAND

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy in Pharmacology and Toxicology
Department of Pharmacology and Physiology
Faculty of Pharmaceutical Sciences
Chulalongkorn University
Academic Year 2019

ความสัมพันธ์ระหว่างพหุสัณฐานของยีน *NAT2*
และการบาดเจ็บของตับที่เหนี่ยวนำด้วยยาต้านวัณโรคในผู้ป่วยชาวเมียนมาร์ในประเทศไทย

น.ส.ขิ่น แซนดิ ธอว์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชวิทยาและพิษวิทยา ภาควิชาเภสัชวิทยาและสรีรวิทยา
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ปีการศึกษา 2562

Thesis Title	ASSOCIATION BETWEEN *NAT2* POLYMORPHISMS AND ANTI-TUBERCULOSIS DRUG-INDUCED LIVER INJURY IN MYANMAR PATIENTS IN THAILAND
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ชิ่น แซนดิ ธอว์ : ความสัมพันธ์ระหว่างพหุสัณฐานของยีน *NAT2*
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BETWEEN *NAT2* POLYMORPHISMS AND ANTI-TUBERCULOSIS DRUG-INDUCED LIVER INJURY IN MYANMAR PATIENTS IN
THAILAND) อ.ที่ปรึกษาหลัก : ผศ. ภญ. ดร.รัชนี รอดศิริ, อ.ที่ปรึกษาร่วม : ผศ. ภญ. ดร.พรพิมล กิจสนาโยธิน

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาการกระจายของพหุสัณฐานของยีน N-acetyl transferase 2 (*NAT2*) ในผู้ป่วยวัณโรคชาวเมียนมาร์และประเมินความสัมพันธ์ระหว่างพหุสัณฐานของยีน *NAT2* และการบาดเจ็บของตับที่เหนี่ยวนำด้วยยาต้านวัณโรคในผู้ป่วยชาวเมียนมาร์ การศึกษานี้เป็นแบบ case-control ทำการศึกษาในผู้ป่วยวัณโรคผู้ใหญ่ชาวเมียนมาร์ที่โรงพยาบาลสมุทรสาคร ผู้ป่วยที่ได้รับยาต้านวัณโรคจนครบการรักษาโดยไม่เกิดการบาดเจ็บของตับ (control) จำนวน 54 คนและผู้ป่วยที่เกิดการบาดเจ็บของตับในระหว่างได้รับยาต้านวัณโรค (case) จำนวน 5 คนเข้าร่วมการศึกษานี้ ข้อมูลคุณสมบัติทั่วไปของผู้ป่วย ขนาดยาต้านวัณโรค และระดับเอนไซม์ตับได้มาจากฐานข้อมูลของโรงพยาบาล เก็บเลือดผู้ป่วยและตรวจความแปรผันของยีน *NAT2* จำนวน 4 SNPs ได้แก่ rs1041983, rs1799929, rs1799930 และ rs1799931 ด้วยเทคนิค allele-specific polymerase chain reaction (AS-PCR) ในการศึกษานี้ผู้ป่วยทั้งหมดได้รับยาต้านวัณโรคสูตรมาตรฐานและอยู่ในช่วงขนาดยาที่องค์การอนามัยโลกแนะนำ ข้อมูลพื้นฐานของกลุ่ม control และ case ไม่แตกต่างกัน ในผู้ป่วยทั้งหมดพบความถี่ของ *NAT2* rs1041983 มากที่สุด (56%) แต่พบความถี่ของ *NAT2* rs1799929 ใน case สูงกว่า control (50% vs 10%) แอลลีลที่พบมากที่สุดคือ *NAT2*6A และจีโนไทป์ที่พบมากที่สุดคือ NAT2*4/*7B* ผู้ป่วย 47% (28 คน) มีฟีโนไทป์แบบ slow acetylator และผู้ป่วย 44% (26 คน) มีฟีโนไทป์แบบ intermediate acetylator ซึ่งการกระจายของฟีโนไทป์ในการศึกษานี้ใกล้เคียงกับการศึกษาในชาวไทยและชาวอินโดนีเซีย ไม่มีความสัมพันธ์ระหว่างปัจจัยที่ไม่ใช่พันธุกรรมกับการเกิดการบาดเจ็บของตับที่เหนี่ยวนำด้วยยาต้านวัณโรค ผู้ป่วยที่มี *NAT2* rs1799929 CT และ TT มีความเสี่ยงในการเกิดการบาดเจ็บของตับสูงกว่า *NAT2* rs1799929 CC (OR= 19.2865, 95% C.I =1.751-212.417, p = 0.016 และ OR = 22.50, 95% C.I = 1.001– 505.846, p = 0.005 ตามลำดับ) อย่างไรก็ตามไม่มีความสัมพันธ์ระหว่างจีโนไทป์และฟีโนไทป์ของ *NAT2* กับการบาดเจ็บของตับที่เหนี่ยวนำด้วยยาต้านวัณโรค เมื่อแบ่งผู้ป่วยตามระดับเอนไซม์ตับเป็นกลุ่มระดับเอนไซม์ปกติและกลุ่มระดับเอนไซม์สูงพบว่า *NAT2* rs1799929 CT พบมากในผู้ป่วยที่มีระดับ AST สูง (OR=4.625, 95% C.I=1.078-19.840, p = 0.039) นอกจากนี้พบว่าผู้ที่มีฟีโนไทป์แบบ slow acetylator และมีจีโนไทป์แบบ *NAT2*5B/*5B* มีระดับ AST และ ALT สูงที่สุด เมื่อวิเคราะห์ด้วย multiple linear regression พบว่า *NAT2* SNPs rs1799929 ขนาดยาของ isoniazid และอายุมีความสัมพันธ์กับระดับ AST อย่างมีนัยสำคัญ (B=49.334, p <0.001, B=22.241, p=0.007 และ B=1.991, p=0.025 ตามลำดับ) ซึ่งปัจจัยเหล่านี้สามารถอธิบายความแปรผันของระดับ AST ได้ 35% นอกจากนี้ฟีโนไทป์ของ *NAT2* ขนาดยาของ isoniazid และอายุสามารถอธิบายความแปรผันของระดับ AST ได้ 22% (B=23.781, p=0.038, B=22.003, p=0.016 และ B=2.581, p=0.008 ตามลำดับ) *NAT2* SNPs rs1799929 ยังสามารถอธิบายความแปรผันของระดับ ALT ได้ 22% (B=85.944, p < 0.001) โดยสรุปการศึกษานี้แสดงอิทธิพลของ *NAT2* SNPs rs1799929 ต่อ การบาดเจ็บของตับที่เหนี่ยวนำด้วยยาต้านวัณโรคในผู้ป่วยชาวเมียนมาร์และการเพิ่มขึ้นของเอนไซม์ตับ เนื่องจากการกระจายของฟีโนไทป์ของ *NAT2* ในประชากรชาวเมียนมาร์ใกล้เคียงกับประชากรไทย การศึกษานี้สนับสนุนการใช้แนวทางการวินิจฉัยและแนวทางการรักษาผู้ป่วยวัณโรคในประเทศไทยในการติดตามอาการไม่พึงประสงค์จากยาต้านวัณโรคในผู้ป่วยชาวเมียนมาร์ในประเทศไทย

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6076124433 : MAJOR PHARMACOLOGY AND TOXICOLOGY
KEYWORD: Drug-induced liver injury, Myanmar, N-acetyltransferase 2 polymorphism, Tuberculosis

Khin Sandi Thaw : ASSOCIATION BETWEEN *NAT2* POLYMORPHISMS AND ANTI-TUBERCULOSIS DRUG-INDUCED LIVER INJURY IN MYANMAR PATIENTS IN THAILAND. Advisor: Asst. Prof. RATCHANEE RODSIRI, Ph.D. Co-advisor: Asst. Prof. PORNPIMOL KIJSANAYOTIN, Ph.D.

The aims of this study were to determine the N-acetyl transferase 2 (*NAT2*) genetic distribution of Myanmar tuberculosis (TB) patients and evaluate the association of *NAT2* polymorphisms and antituberculosis drug-induced liver injury (AT-DILI) in Myanmar patients. A case-control study was conducted in adult Myanmar TB patients at Samut Sakorn Hospital. Fifty-four patients who completed anti-TB treatment without liver injury (controls) and five patients with AT-DILI during their anti-TB treatment (cases) were enrolled. Patients' baseline characteristics, anti-TB doses, and liver enzyme levels were collected from the hospital's record. Blood samples were collected and four *NAT2* SNPs, rs1041983, rs1799929, rs1799930, and rs1799931, were genotyped using allele-specific polymerase chain reaction (AS-PCR). In this study, all patients received the standard anti-TB regimen with anti-TB doses in World Health Organization (WHO) recommended dose range. Baseline characteristics of cases and controls were not different. *NAT2* rs1041983 was highly found in all patients (56%), while cases had higher *NAT2* rs1799929 frequency than control (50% vs 10%). The most common allele was *NAT2*6A*, and the most common genotype was *NAT2*4/*7B*. Forty-seven percent (n=28) were slow acetylators, while 44% (n=26) were intermediate acetylators. The phenotype distribution in this study is in concordance with Thai and Indonesian studies. There was no association of non-genetic factors and AT-DILI. *NAT2* rs1799929 (CT and TT genotypes) showed higher risk to get liver injury than CC genotypes (OR= 19.2865, 95% C.I =1.751-212.417, p = 0.016 and OR = 22.50, 95% C.I = 1.001– 505.846, p = 0.005, respectively). However, there was no association between *NAT2* genotype and phenotype and AT-DILI. When stratified patients according to their liver enzyme levels to normal and elevated levels, CT genotype of *NAT2* rs1799929 was highly found in patients with elevated AST levels (OR=4.625, 95% C.I=1.078-19.840, p = 0.039). In addition, the highest AST and ALT levels were found in slow acetylators with *NAT2**5B/*5B. Multiple linear regression analysis showed that *NAT2* SNPs rs1799929, isoniazid dose, and age significantly associated with AST levels (B=49.334, p < 0.001, B=22.241, p=0.007 and B=1.991, p=0.025 respectively). These factors can describe 35% of AST level variation. Furthermore, *NAT2* phenotype, isoniazid dose, and age also explained 22% of AST level variation (B=23.781, p=0.038, B=22.003, p=0.016 and B=2.581, p=0.008 respectively). In addition, *NAT2* SNPs rs1799929 also exhibited 22% of ALT level variation (B=85.944, p < 0.001). To summarize, this study demonstrated a strong influence of *NAT2* rs1799929 on AT-DILI and elevated liver enzymes in Myanmar patients receiving anti-TB treatment. As the *NAT2* phenotype distribution of Myanmar patients is similar to the Thai population, this study supports the use of Thai TB guidelines in anti-TB adverse drug reaction monitoring for Myanmar TB patients in Thailand.

Field of Study:	Pharmacology and Toxicology	Student's Signature .....
Academic Year:	2019	Advisor's Signature .....
		Co-advisor's Signature .....

ACKNOWLEDGEMENTS

First of all, I would like to express my sincere gratitude to my advisor Assistant Professor. Dr. Ratchanee Rodsiri and my co-advisor Assistant Professor Dr. Pornpimol Kijsanayotin for the continuous support of my master's study and research, for their patience, motivation, enthusiasm, supervision, precious advice, and immense knowledge. Their guidance helped me in all the time of research and writing of this thesis. I could not have imagined how this dissertation would be possible without their kindness and understanding.

And, I would like to give my deep appreciation to Mrs. Chanchira Choppradit at Samut Sakhon Hospital for her kindness, helpfulness and perfect fieldwork for sample collection. In addition, I do thank all the pharmacists, staff, and translators of TB clinics, Samut Sakhon Hospital. Without their help, my work would not be possible.

I would like to extend my sincere gratitude to the personals of the Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand and the personals of the Department of medical services, Ministry of Public Health, Thailand. My special thanks to all my friends at Chulalongkorn University, and everyone who helped to contribute to this project. I would like to thank my thesis committee members for their encouragement, valuable advice, and insightful comments.

In addition, my sincere thanks to the Thailand International Development Cooperation Agency (TICA), Ministry of Foreign Affairs for a scholarship award to study at Chulalongkorn University. Last of all, I would like to acknowledge with gratitude, the support, and love of my parents.

Financial support was provided by the 90th Anniversary of Chulalongkorn University Scholarship under Ratchadapisek Somphot Fund, Chulalongkorn University (GCUGR1125622052M).

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Chapter I

INTRODUCTION

1.1 Background and Rationale

In the 19th and early 20th centuries, the incidence of tuberculosis (TB) has reached its peak level and become a notifiable disease. World Health Organization (WHO) reported an estimated 10.4 million TB cases in 2017 (1). In 1993, the WHO made a global health emergency for tuberculosis and implemented at the national and international level strategy (2). Furthermore, Directly Observed Treatment Short-Course (DOTS) has been developed to provide diagnosing, enough supply of drugs, short-course therapy and standard system for recording and reporting detected cases (2).

The standard treatment for tuberculosis consists of two phases for a six-month course of four first-line drugs; isoniazid, rifampicin, pyrazinamide and ethambutol (3, 4). This standard anti-TB regimen is effective for newly infected drug-sensitive tuberculosis. However, adverse drug reactions and noncompliance occurred during the long periods of treatment (5) and could lead to discontinuation of drug treatment resulting in the increased risk of treatment failure and anti-TB drug resistance (6).

Among the adverse effects of anti-TB drugs, drug-induced liver injury (DILI) is the most common (5, 7). The risk factors associated with the anti-TB drug-induced liver injury include race, age, nutrition plans, alcohol intake, the severity of disease conditions, co-morbidity and genetic polymorphism (8). Even though isoniazid, rifampicin, and pyrazinamide can cause hepatotoxicity (9-11), many studies have

suggested the association between N-acetyltransferase 2 (*NAT2*) polymorphisms and isoniazid-induced liver injury (12).

Isoniazid (INH) is metabolized by NAT2, cytochrome P450 2E1 (CYP2E1) and glutathione S-transferase (GST1) (13). INH is hydrolyzed to hydrazine which is a hepatotoxic metabolite. NAT2 converts hydrazine to acetyl hydrazine. NAT2 also metabolizes isoniazid to acetyl isoniazid. Acetyl isoniazid undergoes further hydrolysis to acetyl hydrazine. Acetyl hydrazine is also a hepatotoxic metabolite. NAT2 can convert acetyl hydrazine to a non-toxic metabolite, di-acetyl hydrazine. Hydrazine and acetyl hydrazine are further metabolized into toxic reactive metabolites by CYP2E1. These reactive toxic metabolites can be removed by GST (Figure 1) (2, 6, 14).

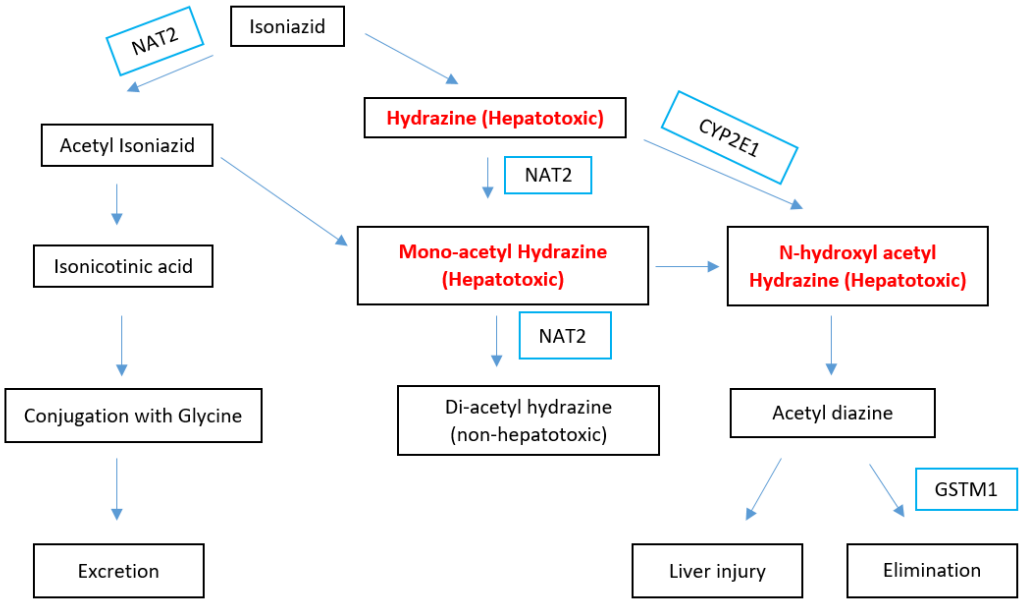


Figure 1 Metabolic Pathway of Isoniazid

Although the role of NAT2 in the production of toxic metabolites or detoxification of toxic metabolites is unclear, several studies demonstrated the association between *NAT2* polymorphisms and isoniazid-induced liver injury (8, 15-18).

Three phenotypes of acetylator based on *NAT2* haplotypes have been reported (13, 19, 20). Slow acetylators are individuals carrying two slow *NAT2* alleles (*NAT2**5, *6, *7, *14) whereas rapid acetylators are those with two rapid alleles *(NAT2**4, *11, *12, *13, *18). Intermediate acetylators are those carrying one rapid and one slow *NAT2* allele (14, 17). In general, slow acetylators decrease isoniazid acetylation resulting in high plasma concentration of isoniazid and a higher risk of DILI due to the accumulation of acetyl hydrazine (8, 13). On the other hand, rapid acetylators have lower plasma drug concentration but decrease the risk of DILI (8, 21). East Asia populations have higher ratio of rapid acetylators (40%) together with higher allele frequency of *NAT2**4 in Japanese (70%), Korea (60%) and Chinese (50%) populations (6). African and Caucasian populations have higher ratio of slow acetylators (46%, 58%) with the highest frequency of *NAT2**5 allele (6). Thai population had a higher ratio of rapid acetylators (63.8%) in one study (22) but in another study with the DILI group had a higher ratio of slow acetylators (71.7%) (23).

Several findings in mix-ethnicity, Chinese, Brazilian and Singaporean patients suggested that slow acetylators have a higher risk of DILI with TB treatment (14, 16, 18, 24). Slow acetylators (*NAT2**6A/*6A and *NAT2**6A/*7B) are also significant risk factors

for DILI in Thai TB patients (23). The results of this study lead to the implementation of new TB guidelines in 2018. In this guideline, it is stated that *NAT2* polymorphisms should be checked in hepatitis patients who are taken anti-TB regimen to support the decision for INH drug re-challenge and dosage adjustment.

Many Burmese moves to Thailand as migrant workers. Consequently, Samut Sakhon hospital and many other hospitals in Thailand have to serve Myanmar TB patients (25). TB patients have been increasing related to the increasing number of migrant workers. As TB is a transmission disease, it is important to limit the prevalence of the disease. Therefore, reducing adverse reactions of anti-TB drugs could encourage patients to adhere to long-term treatment resulting in the increased rate of successful treatment.

The aim of this research is to determine the association between *NAT2* polymorphisms and anti-tuberculosis drug-induced liver injury in Myanmar patients using case-control study. If the association of *NAT2* polymorphisms and anti-tuberculosis drug-induced liver injury in Myanmar is similar to the Thai population, that guideline can be implemented for pharmaceutical care in Myanmar TB patients. If there are differences between Thai and Myanmar populations, the appropriate pharmaceutical care for Myanmar TB patients will be suggested.

1.2 Research question

Do *NAT2* polymorphisms determine the incidence of anti-tuberculosis drug-induced liver injury during the standard anti-TB treatment in Myanmar patients?

1.3 Objective

1.3.1 To determine the *NAT2* genetic distribution of Myanmar TB patients

1.3.2 To identify the influence of *NAT2* polymorphisms and other non-genetic factors on anti-tuberculosis drug-induced liver injury in Myanmar patients in Thailand

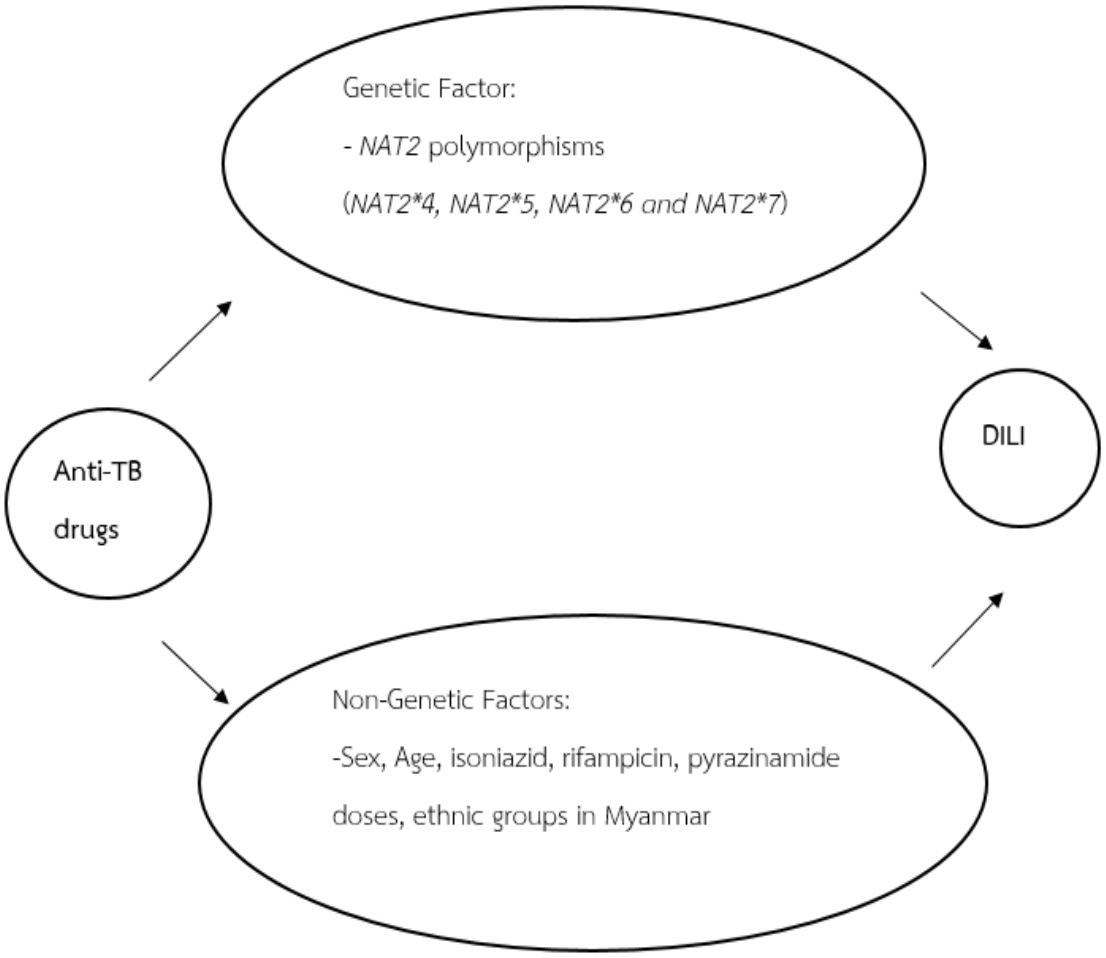
1.4 Hypothesis

NAT2 slow acetylation increases the risk of anti-tuberculosis drug-induced liver injury in Myanmar patients treated with the anti-TB treatment regimen

1.5 Scope of the study

This present study was case-control study in Myanmar TB patients who got DILI from anti-TB treatment compared with patients who completed anti-TB treatment without DILI.

1.6 Conceptual framework



1.7 Expected benefits

The association between *NAT2* polymorphisms and anti-tuberculosis drug-induced liver injury will suggest dosage use and the risk of DILI in Myanmar patients. The finding will provide important information in the pharmacogenetic study of Myanmar population. Moreover, it will give great benefit in establishing TB treatment guidelines of the Myanmar population besides support the appropriate pharmaceutical care to reduce the adverse reaction, increase drug compliance and promote successful treatment.

Chapter II

LITERATURE REVIEWS

2.1 Tuberculosis

Tuberculosis (TB) has existed in human millennia and it remains a global health problem today (2). It is one of the top ten causes of death worldwide, and approximately 10 million people infected each year (1). In 1882, Robert Koch, who received Nobel Prize in physiology and medicine in 1905, identified and described the bacillus that can cause tuberculosis, *Mycobacterium tuberculosis*.

2.1.1 Epidemiology

The incidence of tuberculosis was approximately 10.4 million cases around the world according to the WHO TB report 2017(1). Forty-five percent of global incident cases were from South East Asia. In Southeast Asia, an estimated 4.6 million people were infected with TB and 214,000 people with MDR-TB. Furthermore, 163,000 with TB infection with HIV positive patients were reported. According to WHO, Myanmar and Thailand are on the list of the highest 30 TB burden groups all over the world. In Myanmar, 191,000 people suffered TB infection and in which 13,000 MDR-TB patients and 18,000 HIV positive TB patients were found during 2016. Similarly, 4,700 MDR-TB cases, 10,000 TB plus HIV cases and a total of 119,000 TB infection cases recorded in Thailand during 2016 (1).

The End TB Strategy and Sustainable Development Goals (SDGs) have been developed by WHO aiming: to end the global TB epidemic (1). By 2030 when compared

with 2015, 90% reduction of TB deaths and 80% reduction in TB incidence are set in the End TB Strategy. SDGs consist of 17 goals that are adopted by the United Nations for the period 2016-2030 and include considerable emphasis on disaggregated analysis and reporting of data. The End TB Strategy emphasizes the Urban Health Center (UHC) progressing and actions to address health-related risk factors and provide actions and funding for achieving targets and milestones for the reduction of TB cases (1).

2.1.2 Causative Organism

M. tuberculosis is a rod-shaped, highly aerobic, non-spore forming bacteria (2, 26). It is an acid-fast bacillus with a unique cell wall structure (2). Its cell wall comprises fatty acid, mycolic acid covalently attached to arabinogalactan forming lipid barrier and causing resistance to antibiotics (26, 27).

M. tuberculosis is a slow-growing organism. Four types of this mycobacteria are usually presented in TB patients; the actively growing extracellular organisms in aerated cavities, the slow intermittently growing organisms in unstable parts of the lesions, the organisms in macrophages, phagolysosomes or inflammatory lesions and the dormant organisms in anaerobic conditions (28).

Non-tuberculous mycobacteria (NTM); or atypical mycobacteria; are other mycobacteria that can cause pulmonary diseases like tuberculosis, lymphadenitis, skin and soft tissue infection. NTM are found in soil and water resources at high concentrations and do not spread from person to another (29, 30).

2.1.3 Mode of transmission

Tuberculosis is an airborne disease spreading by coughing, sneezing, talking (27). These airborne droplets can live in the air for minutes to hours after spitting (27, 31). Transmission of the disease is influenced by the number of bacilli in the droplets, the virulence of the bacilli, exposure of the bacilli to UV light, degree of ventilation and occasions for aerosolization (32).

The ciliated cells and secretory cells in the human airway epithelium are the primary defense against inhaled pathogens and particulates (33). After inhaling, the air-borne droplets go down through the respiratory tract. The goblet cells from the upper part of the respiratory tract produce mucus, then, catch the bacillus (27, 28, 34). Consequently, the cilia, hair-like organelles on the surface of the cells, mediate cleaning airways of inhaled particles and pathogens with mucus. In this way, the first-line defense for prevention of infection occurs in most cases of tuberculosis exposure (27, 33, 35).

In addition, the alveolar macrophages from the innate immune system serve as the next host defense (36). The macrophages surround and engulf the droplets containing bacteria. The phagocytosis by macrophages can control the infection and lead to latent infection or develop the active disease (Figure 4) (34, 37). Moreover, the ability of the host defense system and the invading mycobacteria can decide the outcome of infection. The macrophages degrade the bacteria by the release of proteolytic enzymes and cytokines that can attract the T lymphocytes after ingestion.

Accumulation of T lymphocytes and macrophages can form granulomas and solid necrosis. After 2 to 3 weeks, this necrotic environment leads to caseous necrosis. According to the individual immune system, people can control the infection by calcification and fibrosis or can develop primary progressive tuberculosis (4, 26, 27, 36).

Although *M. tuberculosis* primarily infected the respiratory system, it can distribute to the other organs, such as lymph node, pleura, bones, and joints. This type of infection is called extra-pulmonary tuberculosis (32). The most fatal extra-pulmonary tuberculosis can develop in the central nervous system and bloodstream resulting in, meningitis and military tuberculosis (36)

2.1.4 Signs and Symptoms

During latent infection, there is no sign or symptom of the disease but the bacteria can persist in the necrotic stage from year to a lifetime (34). The clinical manifestations of active tuberculosis are productive prolonged cough of three or more weeks, chest pain, and hemoptysis. Moreover, other symptoms of active TB infection are low-grade fever, chills, night sweat, appetite loss and weight loss (27, 34, 37).

2.1.5 Diagnosis

Early diagnosis and appropriate treatment can prevent most deaths from TB. The most common detection of *M. tuberculosis* infection is sputum culturing, chest radiography, Gene Xpert MTB/RIF and tuberculin test (36, 38).

Sputum microscopy for the presence of *M. tuberculosis* is the most common laboratory test for active TB in most high-burden countries (1). According to the WHO guideline, three consecutive sputum samples are needed for the determination of tuberculosis infection in the laboratory. Ziehl-Neelsen staining, Fluorescence microscopy (Auramine-rhodamine staining) and Lowenstein-Jansen medium are also used. As well as, sample centrifuging and fluorescence microscopy improve sensitivity about 10% (27, 38). This test is fast but it is not specific for *M. tuberculosis* as the other mycobacteria can give positive to this test.

Respiratory symptoms abnormality of the people in chest radiography can suggest the active pulmonary tuberculosis. Infiltrates with cavitations in the upper and lower lobes of the lungs are the prominent characteristics of tuberculosis infection. However, in people with co-infection, these may not be shown (1, 36).

Gene Xpert assay in the sputum sample performs well in determining suspected pulmonary TB and specified extra-pulmonary TB. This test is sensitive around 89% for smear-positive and 67% for smear-negative pulmonary tuberculosis (38). This Xpert assay is a cost-effective and quick diagnosis for drug-resistant TB infection (1, 38).

For latent infection, quantifying the amount of memory T cells in the skin by tuberculin skin test (TST) and RD-1 specific skin test or the blood by interferon-gamma release assays (IGRAs) are used. In TST, purified protein derivative (PPD) is injected into

the patient. The area of induration can be examined at the site of injection. However, limitations of TST are low sensitivity and specificity. TST can detect non-mycobacterial infections. In addition, immune compromising conditions can reduce the sensitivity of TST and false-negatives results may develop in those who have been taken BCG vaccine (34, 36). The limitations of IGRAs are similar to TST but IGRAs specificity in active tuberculosis infection is higher than TST (38).

2.1.6 Treatment

Seventeen anti-TB drugs have been currently prescribed in many regimens for different types of TB infections (Table 1) (1). According to WHO guidelines, the standard TB regimen composes of four drugs combination; isoniazid, rifampicin, pyrazinamide, and ethambutol. This regimen must be prescribed for 6 months to treat new TB patients (Table 2). Isoniazid is the most potent bactericidal agent while rifampicin and pyrazinamide can sterilize tuberculous lesions and prevent relapse of disease (1, 34). The goals of TB chemotherapy are

- To treat patients with a short course of treatment therapy with least adverse drug effects
- To stop the relapse of the disease
- To reduce the incidence of drug resistance tuberculosis
- To control the transmission process of tuberculosis

Anti-TB regimens for repeated TB patients, multi-drugs resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) are different (Table 3, 4, 5). MDR-TB is the condition in which the patient is resistant to rifampicin and isoniazid while XDR-TB is the condition in which the patient is resistant not only to rifampicin and isoniazid but also to other second-line anti TB drugs (1). MDR or XDR patients need to take a regimen containing four to five second-line anti TB drugs for at least 18 months. Many challenges are faced in prescribing the individual treatment regimen to the patients such as various drugs in treatment and duration of treatment. In addition to this, the mutation in bacteria replication can lead to drug resistance and poor patient adherence and failure to complete the standard regimen are also the risk factors for drug-resistance (34, 37).

Table 1 Current first-line and second-line anti-TB drugs (1)

First-line drugs	Second-line drugs
▪ Isoniazid (H) ▪ Rifampicin (R) ▪ Pyrazinamide (Z) ▪ Ethambutol (E)	▪ Streptomycin injection (S), Amikacin injection, Kanamycin injection ▪ Capreomycin, Clofazimine ▪ Levofloxacin, moxifloxacin ▪ Cycloserine, P-aminosalicylic acid (PAS) ▪ Ethionamide, Linezolid ▪ Betaquiline, Delanamide

Table 2 Standard regimen and dosing frequency for new TB patients (1)

Intensive phase	Continuation phase	Comments
2 months of HRZE	4 months of HR	
2 months of HRZE	4 months of HRE	Applies only in countries with a high level of isoniazid resistance in new TB patients, and where isoniazid drug susceptibility testing in new patients is not (or results are unavailable) before the continuation phase begins

Table 3 Standard regimen and dosing frequency for repeated TB patients (1)

Intensive phase	Continuation phase	Comments
2 months of HRZES	1 month of HRZE + 5 months of HRE	Country specific data show low or medium levels of MDR-TB in these patients or if such data is not available

Table 4 Standard regimen and dosing frequency for MDR-TB patients (1)

	Shorter MDR-TB regimen	Individualized MDR-TB/RR-TB regimen
Intensive phase		
Duration	4-6 months	Up to 8 months
Composition	4 second-line drugs	4 or more second-line drugs
Continuation phase		
Duration	5 months	12 or more months
Composition	2 second-line drugs	3 or more second-line drugs

Table 5 Composition of regimen for MDR-TB patients (1)

Phase	Drugs (according to Bodyweight)
Intensive phase	Kanamycin, Moxifloxacin, Prothiaonamide, Clofazimine, Pyrazinamide, High dose isoniazid
Continuation phase	Moxifloxacin, Clofazimine, Pyrazinamide, Ethambutol

2.1.7 Drug-induced liver injury

The most frequent adverse reactions of anti-tuberculosis treatment are hepatotoxicity, allergic reactions, gastrointestinal and neurological disorders (12). Asymptomatic transaminase elevations are common during treatment but the liver injury is the most serious adverse effect (3, 39, 40).

Drug-induced liver injury (DILI) is defined by many guidelines and literature based on different hepatotoxicity definitions. Chinese Society of Hepatology Guidelines describes four types of liver injury including hepatocellular injury, cholestatic injury, and hepatocellular-cholestatic mixed injury and vascular liver injury (Table 6) (41). In the American College of Gastroenterology (ACG) clinical guidelines, the definitions of hepatocellular injury, cholestatic injury, and hepatocellular-cholestatic mixed injury are similar to those of CSH guidelines. Moreover, the WHO classifies the severity of hepatotoxicity by respective alanine aminotransferase (ALT) levels (Table 7).

Incidences of anti-tuberculosis drug-induced hepatotoxicity vary in different regions of the world from 2% to 28% according to each researcher's definition of criteria as well as the population studied (Table 8) (12). Isoniazid, rifampicin, and pyrazinamide are hepatotoxic drugs in standard TB regimen. With isoniazid monotherapy, significant elevations of transaminase found in about 0.5% of all patients (12). Approximately 1-2% of patients with prophylactic rifampicin monotherapy experienced hepatotoxicity. Moreover, 58% of patients taking pyrazinamide and ethambutol in latent TB infection developed transaminase elevation (12).

Table 6 Type of liver injury according to CSH Guidelines (41)

Hepatocellular injury	ALT ≥ 3 × ULN or R ≥ 5
Cholestatic injury	ALP ≥ 2 × ULN or R ≤ 2
Hepatocellular-cholestatic mixed injury	ALT ≥ 3 × ULN, ALP ≥ 2 × ULN and 2 < R < 5
Vascular liver injury	Sinusoidal Obstruction Syndrome (SOS)/ Hepatic Veno-occlusive disease (VOD)

R - Ratio of serum ALT results to serum ALP results ULN – The upper limit of normal

Table 7 Definition of hepatotoxicity according to the WHO Adverse Drug Reaction (12)

WHO definition of hepatotoxicity	
Grade 1 (mild)	<2.5 times ULN (ALT 51-125 U/L)
Grade 2 (mild)	2.5-5 times ULN (ALT 126-250 U/L)
Grade 3 (moderate)	5-10 times ULN (ALT 251-500 U/L)
Grade 4 (severe)	>10 times ULN (ALT > 500 U/L)

Table 8 Incidence of anti-tuberculosis drug-induced hepatotoxicity (ATDH) with regimens containing INH, R, and PZA (12)

Proportion ATDH (%)	Definition of Hepatotoxicity	Population
2.0	AST > 6 × ULN and confirmed by re-challenge	E 78%, As 17%, Af 4%, NA+SA 1%
2.3	ALT > 5 × pretreatment level	As (Indian, Pakistan)70%, E 30%,
2.6	ALT/AST > 10 × ULN	E (Spain) 76%, C/SA 14%
3.0	ALT > 3 × ULN	As 42%, E+C/SA 29%, Af 16%, NA 12%
3.4	ALT > 5 × ULN	Dutch 94%, non-Dutch 6%
5.3	ALT/AST > 3 × ULN	As (Singaporean)
8.1	ALT/AST > 5 × ULN	Not mentioned
10.7	ALT > 5 × ULN	E 60%, Af 34%, Other 5%
11.0	ALT/AST > 3 × ULN	E 90%, As 6%, Af 3%, SA 1%
13.0	ALT/AST > 5 × ULN	Af 60%, As 15%, E 24%, other 3%
15.0	ALT > 3 × ULN	As (Taiwan)
16.1	ALT/AST > 5 × ULN, or any increase + symptoms	As (India)
19.0	ALT/AST > 3 × ULN	Not mentioned
27.7	ALT > 3 × ULN with or > 5 x ULN without any symptoms	Iran

Af- Africa, As- Asia, C/SA- Central and South America, E- Europe, NA- North America, SA-South America

2.2 Isoniazid

Isoniazid is one of the first-line anti-TB drugs in the standard TB regimen for drug-susceptible TB patients. Isoniazid is a highly selective anti-TB drug as *M. bovis* and *M. tuberculosis* are highly susceptible to isoniazid with IC_{50} of 0.02-0.2 μg/ml while non-mycobacterial tuberculosis (*M. avium)* is not sensitive to isoniazid (37, 42). INH presents the bactericidal action to the actively dividing organisms but has no effect on the stationary phase of bacteria and those under anaerobic conditions (2, 42).

The mechanism of action of INH is inhibition of mycolic acid synthesis (42). Mycolic acid is long-chain, branched, and saturated fatty acid which is one of the components of *M. tuberculosis* cell wall. Mycolic acid helps to support the bacteria envelope impermeability and plays an important role in virulence of this bacteria (34, 42-44). INH is a prodrug that can enter into the cytoplasm of *M. tuberculosis* by passive diffusion. Inside bacteria, INH is activated by the bacterial catalase-peroxidase enzyme (KatG) and formed NAD and NADP-adducts with NAD^+ and $NADP^+$ respectively (Figure 5) (42). These adducts can bind to enoyl acyl carrier protein (ACP) reductase (InhA) and interrupt mycolic acid elongation.

INH is readily absorbed from the GI tract and undergoes significant first-pass metabolism. The pharmacokinetic profile of INH presents in Table 9. INH is mainly metabolized in the liver by N-acetyltransferase 2 (NAT2), cytochrome P450 2E1 (CYP2E1) and glutathione S- transferase (GST1) (Figure 1). The predominant metabolic

pathway of isoniazid is acetylation by NAT2 (12, 15). INH acetylation produces acetyl isoniazid. Acetyl isoniazid is then undergone hydrolysis to produce acetyl hydrazine. INH is also hydrolyzed to hydrazine which is further acetylated by NAT2 to produce acetyl hydrazine. Consequently, acetyl hydrazine is acetylated and formed di-acetyl hydrazine (12, 28, 45, 46). Hydrazine and acetyl hydrazine are the toxic metabolites of INH. Moreover, CYP2E1 can also convert hydrazine and acetyl hydrazine to other toxic reactive metabolites. On the other hand, GST can remove these toxic metabolites (38, 47, 48).

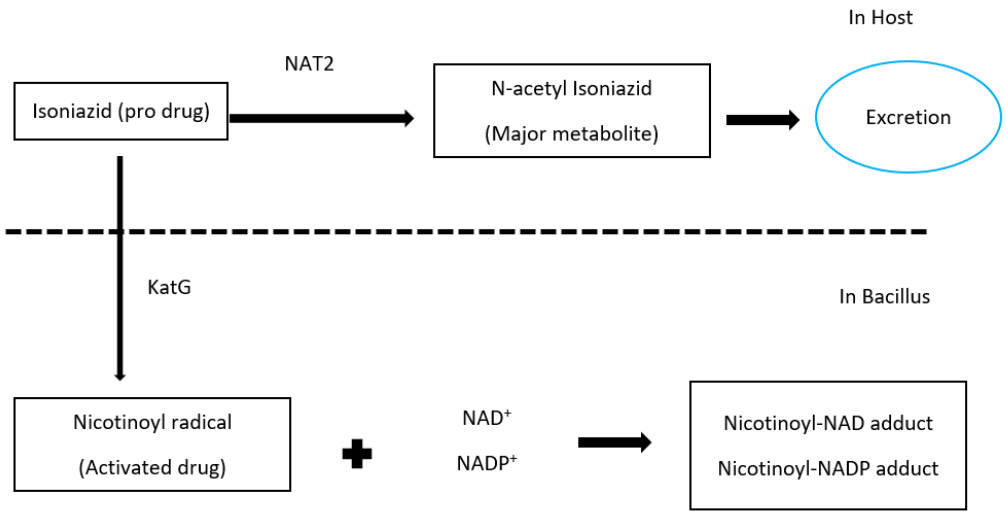


Figure 2 Biotransformation of isoniazid in M. tuberculosis

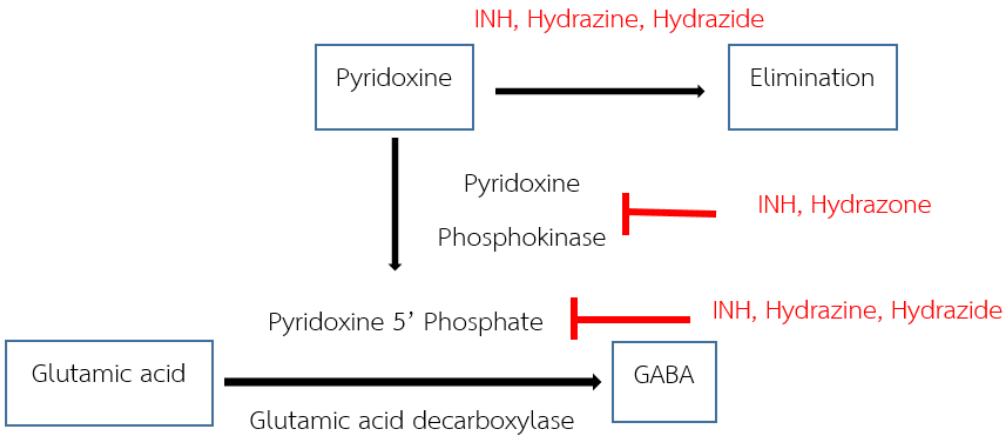


Figure 3 Effect of isoniazid on Pyridoxine deficiency

Table 9 Pharmacokinetic characteristics of isoniazid (6)

Isoniazid	
Usual dose	300 mg daily (4-6 mg/kg/day)
Serum C_{max} (µg/ml)	3-6
Serum T_{max} (hour)	0.75-2
Serum $T_{1/2}$ (hour)	Polymorphic
CSF Penetration (%)	20-100
Protein Binding	10 estimated
VD (L/kg)	0.6-1 estimated
Renal excretion (%)	Polymorphic
Hepatic Clearance (%)	Polymorphic
Active metabolites	None
Metabolic excretion (%)	Renal (50-60) and non-renal

2.3 *NAT2* polymorphism

NAT2 encodes for N-acetyl transferase enzymes which are responsible for many environmental toxicants and pharmacological agents' biotransformation, toxicity, and clearance (14). *NAT2* is on chromosome 8p22 and many single nucleotide polymorphisms (SNPs) have been discovered in the *NAT2* coding exon. Some SNPs can change amino acid and some did not (Table 12). These SNPs combined and formed *NAT2* alleles or *NAT2* haplotypes (49). One hundred and eight *NAT2* variants or alleles are discovered with a signature SNP or in combination with others (50). The most common mutations are *NAT2* G191A (R64Q), *NAT2* C282T, *NAT2* T341C (I114T), *NAT2* C481T, *NAT2* G590A (R197Q), *NAT2* A803G (K268R), and *NAT2* G857A (K268R) which can be used to predict polymorphisms (14).

NAT2 polymorphism produces three types of acetylation profiles which are slow, intermediate and rapid acetylators (Table 10). Rapid acetylator has four to five times the quantity of NAT2 enzymes to acetylate INH than the slow acetylator (34). As a result, rapid acetylators have lower plasma concentration of INH which consequently decreases therapeutic outcomes but lower risk of DILI. In contrast, slow acetylators have higher INH plasma concentration and consequently increase treatment outcome and risk of DILI because of the high concentration of toxic metabolites. Two studies in the Polish Population reported that the average concentration of INH in slow acetylators was 2 to 7 fold higher than rapid and intermediate acetylators (15, 51).

Frequencies of *NAT2* phenotypes differ among ethnic groups (Table 11). Almost 50% of European, Africa and Asia populations are slow acetylators while 52% of middle and east America are intermediate acetylators. In contrast, approximately 40% of the East Asia population (Chinese, Korean, and Japanese) are rapid acetylators (6).

Slow acetylators carry two slow *NAT2* alleles (*NAT2*5, *6, *7, *14*) whereas rapid acetylators carry two rapid *NAT2* alleles (*NAT2*4, *11, *12, *13, *18*). Intermediates carry one rapid and one slow allele (Table 10). Each *NAT2* allele results from several nucleotide and amino acid changes (Table 12). The most common mutations are G191A, T341C, G590A, G857A, A803G, C282T and C481T which can be used to predict polymorphisms (14). The wild type allele, *NAT2*4*, has no mutation (14).

NAT2 alleles most frequently found in slow acetylator alleles are *NAT2*5, NAT2*6, NAT2*7*, and *NAT2*14* and those in rapid acetylators are *NAT2*4, NAT2*11, NAT2*12, NAT2*13*, and *NAT2*18* (6, 14). In the East Asia population which rapid acetylators is higher prevalence, the allele frequency of *NAT2*4* is 70% in the Japanese population, 60% in the Korean population and 50% in the Chinese population. In the same way, *NAT2*5* frequency is approximately 40% in African and Caucasian populations (Table 13) (14, 34). However, there is no previous study in the *NAT2* alleles, genotypes and phenotypes distributions in the Myanmar population.

Many studies demonstrated that slow acetylators associates with isoniazid-induced liver injury. In mixed-ethnicity patients, *NAT2* slow acetylator genotypes (*NAT2*5/*6/*7*) have a significantly higher risk for moderate and severe liver injury (OR = 7.6) (52). The study in the Chinese population suggested that TB patients with slow *NAT2* alleles (282TT, 590AA and 857GA) together with *CYP2E1 C1/C1* increased the risk of liver injury (OR = 5.33) (24). In the Brazilian population, slow acetylator genotypes (*NAT2*5B/*5B, NAT2*6A/*6A, NAT2*6A/*5A,* and *NAT2*6A/*5B*) had higher incidence of hepatitis than intermediate and rapid acetylators (48). However, there was no association between the *CYP2E1* genotype and incidence of hepatotoxicity in Brazilian individuals (48). A study in Singaporean patients showed that two SNPs (rs1041983 and rs1495741) related to *NAT2* slow acetylators were linked with INH-induced liver injury (18). Recently, a study in the Thai population suggested that slow *NAT2* alleles (*NAT2*6A/*6A* and *NAT2*6A/*7B*) are the significant risk factor for DILI in Thai TB patients treated with the standard anti-TB regimen. However, one study in the North Indian TB patients reported no association between *NAT2* genotype (*NAT2*5/*7*) and drug-induced hepatotoxicity (17).

Current management for DILI in tuberculosis patients are re-challenge of all drugs one by one, then, omitting of drugs that cause DILI. In addition to this, genotyping of NAT2 genes and adjusting the appropriate dosage of isoniazid according to their NAT2 phenotypes profiles should be done. In Japanese tuberculosis patients, the researcher conducted the pharmacogenetics based anti-tuberculosis therapy in order

to determine the effectiveness of this therapy. In this study, they used half of the standard dose of isoniazid for slow acetylator and 1.5 times the standard dose of isoniazid for rapid acetylators. Finally, the researchers suggested that these dosage regimens of isoniazid therapy increased therapeutic outcomes and reduced adverse effects of isoniazid in both acetylator phenotypes (53).

Table 10 Likely phenotypes and genotypes of *NAT2* polymorphism (6, 14)

Likely phenotype	Genotype	INH plasma concentration	Treatment outcome	DILI risk
Low activity (slow acetylator)	2 slow *NAT2* alleles (*5, *6, *7, *14)	Higher	Higher	Higher
Intermediate activity (Intermediate acetylator)	1 rapid *NAT2* allele and 1 slow *NAT2* allele	Intermediate	Intermediate	Intermediate
Normal/high activity (rapid acetylator)	2 rapid *NAT2* alleles (*4, *11, *12, *13, *18)	Lower	Lower	Lower

Table 11 Frequencies of *NAT2* phenotypes in different Ethnic groups (6, 22, 23)

Region	Subjects(N)	Slow acetylator	Intermediate acetylator	Rapid acetylator	Reference
Europe (Caucasian)	5382	58.0	34.0	8.0	(6)
Africa	1034	46.0	40.0	14.0	(6)
Asia	1790	45.0	37.0	18.0	(6)
Middle and South America	824	27.0	52.0	21.0	(6)
East Asia (Chinese, Korean, Japanese)	2062	14.0	46.0	40.0	(6)
Korean	1000	9.6	46.9	42.8	(6)
Thai (DILI case group)	53	71.1	22.6	5.7	(23)
Thai	235	36.2	-	63.8	(22)

Table 12 Human *NAT2* Alleles (14)

Allele	Nucleotide change	Amino acid change
*NAT2*4*	None	None
*NAT2*5A*	341T>C	I114T
	481C>T	
*NAT2*5B*	341T>C	I114T
	481C>T	K268R
	803A>G	
*NAT2*5C*	341T>C	I114T
	803A>G	K268R
*NAT2*5D*	341T>C	I114T
*NAT2*5E*	341T>C	I114T
	590G>A	R197Q
*NAT2*5F*	341T>C	I114T
	481C>T	K268R
	759C>T	
	803A>G	
*NAT2*6A*	282C>T	R197Q
	590G>A	
*NAT2*6B*	590G>A	R197Q
*NAT2*6C*	282C>T	R197Q
	590G>A	K268R
	803A>G	
*NAT2*6D*	111T>C	R197Q
	282C>T	
	590G>A	
*NAT2*6E*	481C>T	R197Q
	590G>A	
*NAT2*7A*	857G>A	G286E
*NAT2*7B*	282C>T	G286E
	857G>A	
*NAT2*10*	499G>A	E167K
*NAT2*11*	481C>T	None
*NAT2*12A*	803A>G	K268R

Table 12 (Continued)

Allele	Nucleotide change	Amino acid change
*NAT2*12B*	282C>T	K268R
	803A>G	
*NAT2*12C*	481C>T	K268R
	803A>G	
*NAT2*13*	282C>T	None
*NAT2*14A*	191G>A	R64Q
*NAT2*14B*	191G>A	R64Q
	282C>T	
*NAT2*14C*	191G>A	R64Q
	341T>C	I114T
	481C>T	K268R
	803A>G	
*NAT2*14D*	191G>A	R64Q
	282C>T	R197Q
	590G>A	
*NAT2*14E*	191G>A	R64Q
	803A>G	K268R
*NAT2*14F*	191G>A	R64Q
	341T>C	I114T
	803A>G	K268R
*NAT2*14G*	191G>A	R64Q
	282C>T	K268R
	803A>G	
*NAT2*17*	434A>C	Q145P
*NAT2*18*	845A>C	K282T
*NAT2*19*	190C>T	R64W

Table 13 *NAT2* allele frequencies in Ethnic Groups (14)

Ethnic group	No: of subject	*4	*5	*6	*7	*12	*14
Caucasian	3979	0.23	0.46	0.29	0.03	NR	NR
Caucasian	372	0.25	0.44	0.28	0.02	NR	NR
Caucasian	174	0.23	0.44	0.31	0.02	NR	NR
Caucasian	222	0.23	0.44	0.27	0.02	NR	NR
Japanese	297	0.67	0.02	0.22	0.09	NR	NR
Japanese	145	0.69	0.02	0.19	0.1	NR	NR
Taiwanese	133	0.53	0.05	0.24	0.17	NR	0.08
Chinese (Hong Kong)	70	0.48	0.06	0.31	0.16	NR	NR
Chinese	103	0.51	0.06	0.23	0.19	NR	NR
Korean American	98	0.66	0.03	0.19	0.11	NR	NR
Gabonese	104	0.06	0.41	0.22	0.02	0.13	0.09
African American	128	0.36	0.3	0.22	0.02	NR	0.09
African American	96	0.41	0.31	0.23	0.06	NR	NR
Hispanic	148	0.41	0.27	0.18	0.14	NR	NR

Table 14 Previous Study between genetic polymorphism in different ethnic groups

Ethnicity	Number of patients	Polymorphism	Results	Ref
Singaporean	103	rs1041983	OR=6.34 (C.I 2.54-15.82) $p=7.667 \times 10^{-5}$	(18)
		rs1495741	OR=0.21 (C.I 0.09-0.52) $p=6.267 \times 10^{-4}$	
Thai	158	*NAT2*6A/*6A*	OR=6.32 (C.I 2.14- 18.66) $p=4.16 \times 10^{-4}$	(23)
		*NAT2*6A/*7B*	OR=16.38 (C.I 3.57-75.24) $p=9.83 \times 10^{-6}$	
India	290	*NAT2*5/*7*	OR=2.02 p=o.oo6	(17)
European	65	*NAT2*5/*6/*7*	OR=3.33 (C.I 0.82-13.52) p=0.12	(52)
Indian Subcontinent	62	*NAT2*5/*6/*7*	OR=7.6 (C.I 0.91-63.37) p=0.045	
India	185	*NAT2*5*	OR=3.1 (C.I 1.16-8.32) p=0.02	(54)
		*NAT2*6*	OR=2.3 (C.I 1.16-4.62) p=0.01	
India	300	*NAT2*5/*7*	42.86%>>16.67%	(55)
		*NAT2*6/*7*	33.33%>>8.33%	
Polish	130	*NAT2* slow acetylator	2 to 7 folds increased risk	(15)
Taiwan	348	*NAT2 rs1495741*	OR=14.068 p<0.05	(56)
Chinese	208	*NAT2* 6A/*7B*	OR=9.57, p<0.001	(24)
		*NAT2*6A/*6A*	OR=5.24 p=0.02	
		*CYP 2E1 C1/C1+ NAT2**	OR=5.33 p=0.003	
Brazilian	167	*NAT2*5B/*5B*	OR=1.74 (C.I 0.58-5.27)	(48)
		*NAT2*6A/*6A*	OR=1.40 (C.I 0.37-5.36)	
		*NAT2*6A/*5A*	OR=0.98 (C.I 0.21-4.73)	
		*NAT2*6A/*5B*	OR=3.55 (C.I 0.79-15.87)	
Korea	132	*NAT2*5B/*6A/*7B*	OR=5.41 (C.I 1.76-16.59) p=0.005	(57)
Japanese	77	*NAT2*5/*6/*7*	RR=28 (C.I 26-30)	(58)

Chapter III

MATERIALS AND METHODS

Low incidence of anti-TB drug-induced liver injury have been reported in United States (11) and Thailand (23). In addition, severe liver enzymes elevation found in 2% of patients receiving isoniazid therapy (52). Therefore, case-control study was used to determine the association of *NAT2* polymorphisms and AT-DILI of Myanmar TB patients.

3.1 Sample size calculation

On the basis of data from the previous study in mix-ethnicity group, we assumed that genetic characteristics of patients such as the *NAT2* genotypes (especially a slow acetylator phenotype) were found to be associated with an increased risk of isoniazid-related liver injury (OR (Odds ratio) = 7.6, 95% C.I (Confidence interval) = 0.91 - 63.31, p =0.045). In this previous study of the Indian subcontinent patient group, 0.92 of the cases were slow acetylators and 0.61 of the controls were also slow acetylators (45). Moreover, this previous study also suggested 13 out of 62 got the signs and symptoms of hepatotoxicity and the proportion of the risk of liver injury is about 21%. To achieve a power of 0.8 with an alpha of 0.05, a sample size of at least 27 patients was required in the case group and 54 patients in control group by using Power and Sample Size Program Version 3.1.2 (Figure 6) (52, 59, 60) in using Fisher's exact test.

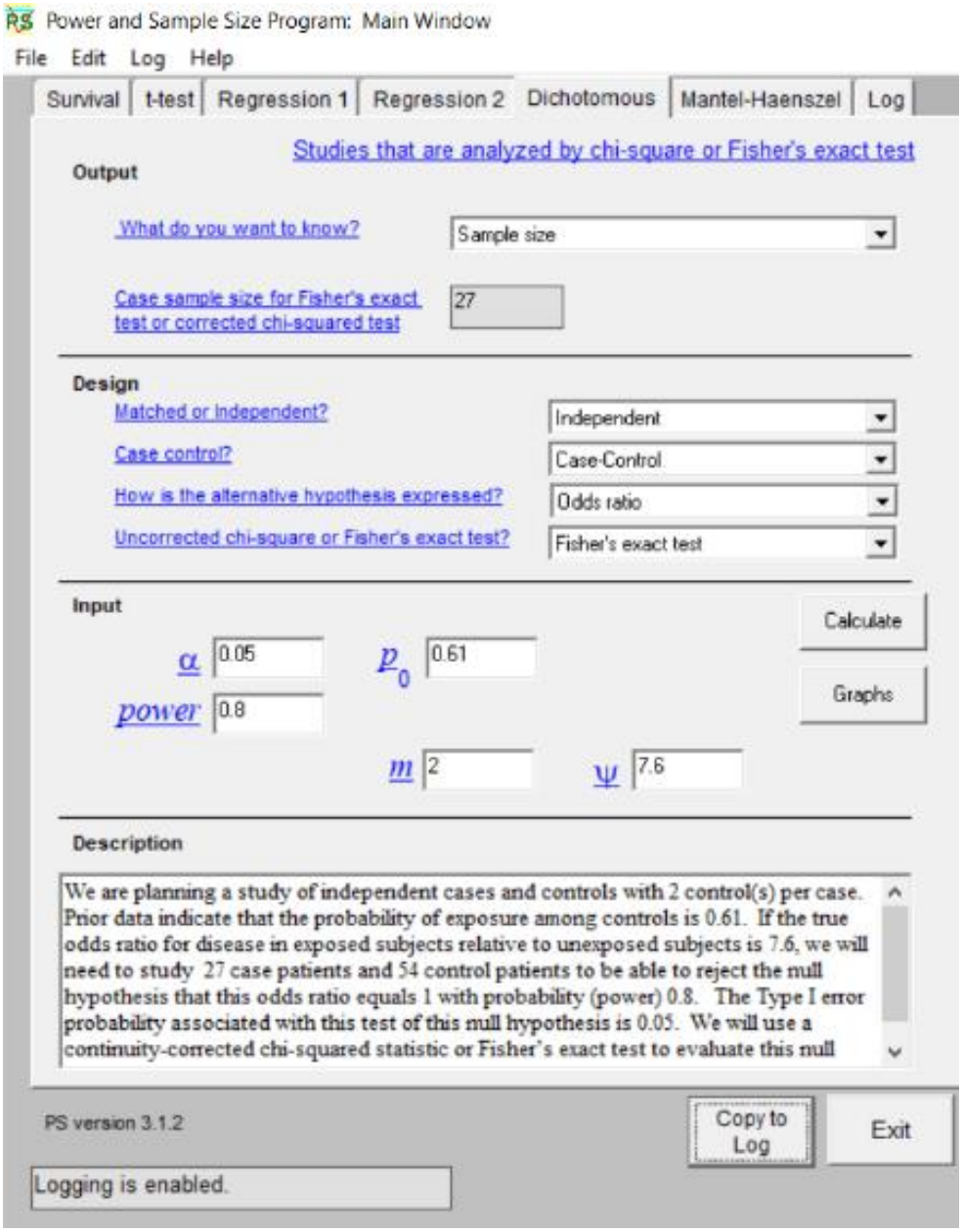


Figure 4 Sample size calculation using "Power and Sample Size Program Version 3.1.2"

3.2 Patients selection

3.2.1 Inclusion and exclusion criteria

This was a case-control study. The inclusion and exclusion criteria were as follow;

Inclusion criteria:

1. Male and female Myanmar TB patients age more than 15-year old
2. First-time treatment with a standard 6-month TB regimen
3. First-time treatment with shorter MDR-TB regimen
4. First-time treatment with TB meningitis regimen

Exclusion criteria:

1. Non-tuberculous mycobacterial infection
2. Alcoholism and alcoholic liver disease
3. Pregnancy and lactation
4. Treatment with other potentially hepatotoxic drugs (Table 15)

Table 15 Drugs that have the potential for liver injury (10, 23)

Drugs	Indication
Verapamil, quinidine	Anti-arrhythmia
Allopurinol, probenecid	Gout
Methotrexate,	Immunosuppressive agent
Carbamazepine, phenytoin, valproate, phenobarbitone	Anti-epileptic
Atenolol, labetalol	Anti-hypertensive
Fluconazole	Anti-fungal
Salicylates	Anti-inflammatory
Quinine	Anti-malaria
Cimetidine	H_2 receptor antagonist
Ethionamide	Second-line anti-TB
Halothane	General anesthesia

3.2.2 Anti-tuberculosis drug-induced liver injury definition

Criteria of DILI in this study based on the American College of Gastroenterology (ACG) and American Thoracic Society (ATS) clinical guidelines. DILI usually develops from direct toxicity of the primary compounds, metabolites or from immunologically mediated response which consequently affects hepatocytes, biliary epithelial cells and liver vasculature (61). The onset of live injury of patients was from 13 days to 58 days (median 26 days) after starting anti-TB treatment (62). In this study, the incidence of DILI based on the liver function tests was determined 2-week after anti-TB treatment.

Three types of injury are defined by these guidelines based on the liver enzymes at laboratory diagnostic results. They are hepatocellular injury; ALT ≥ 3 × ULN

or R ratio ≥ 5 (the ratio of serum ALT results to ALP results), R ratio ≤ 2 or ALP ≥ 2 x ULN as cholestasis injury and 2 < R ratio < 5 as mixed injury (63-65) (66).

3.3 Ethical consideration

The study was approved by the ethics review committee for research involving human research subjects, Health Science Group, Chulalongkorn University, and Samut Sakhon Hospital. All volunteers were informed about the experiment and the purpose of the study and signed informed consent forms before participating in the study.

3.4 Blood samplings

Patients with TB infection from January 2017 to July 2019 were identified from the hospital's patient record system. The patients who treated at the TB clinics of the hospital were screened. Patients who fulfilled the described inclusion criteria enrolled in the study when they had read and signed the informed consent form (Appendix II). The study included 54 Myanmar TB patients without liver injury and 5 patients with a liver injury during their anti-TB treatment

Intravenous blood sample (5 ml) of each patient was collected and kept in a vacationer with EDTA anticoagulant at 4°C. Genomic DNA was extracted from patients' blood by QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). The quality of DNA was checked by NanoDrop spectrophotometer measurement.

3.5 Genotyping by allele-specific polymerase chain reaction (AS-PCR)

Allele-specific polymerase chain reaction (AS-PCR) is one of the popular techniques for genetic analysis and used to detect the mutation of human DNA in

ethidium bromide-stained agarose or polyacrylamide gel (67). This method is very robust and can differentiate the nucleotide differences of each allele on PCR amplification (68). In this PCR, allele-specific primers which are 3' end base complementary to SNP sites produce specific PCR products.

These *NAT2* SNPs which are commonly found in Asian and South-east Asian populations; rs1041983 (*NAT2* C282T), rs1799929 (*NAT2* C481T), rs1799930 (*NAT2* G590A), and rs1799931 (*NAT2* G857A), were sequenced based on previous analysis using AS-PCR (67, 69). Haplotype was determined from the four SNPs including rs1041983, rs 1799929, rs 1799930, and rs1799931. Patients with two slow *NAT2* alleles (*NAT2** 5B, *NAT2** 6A, *NAT2** 7B) were phenotyped into slow acetylator whereas patients with two rapid *NAT2* alleles (*NAT2** 4) as rapid acetylators. In addition, intermediate acetylators were the patients with one rapid *NAT2* allele and one slow *NAT2* allele. After all the processes had done, the blood samples were destroyed.

Table 16 Determination of *NAT2* haplotypes based on *NAT2* SNPs

***NAT2* SNPs**	rs1041983	rs1799929	rs1799930	rs1799931
Nucleotide change	*NAT2* C282T	*NAT2* C481T	*NAT2* G590A	*NAT2* G857A
***NAT2* Haplotypes**				
***NAT2**4 (reference)**	C	C	G	G
***NAT2**5B**	C	T	G	G
***NAT2**6A**	T	C	A	G
***NAT2**7B**	T	C	G	A

3.6 Statistical analysis

In this study, the clinical characteristics of all patients were represented as mean, median and frequencies with standard deviation and percentage. Mann-Whitney U test and Fisher's exact test were used to compare the baseline characteristics of patients between case and control patients.

The expected allele and genotype frequencies were calculated from each single allele frequency and Chi-square was used to test deviation from Hardy-Weinberg equilibrium.

Logistic regression was used to study the association of non-genetic and genetic characteristics of patients and DILI where $p\text{-}value < 0.05$ was considered as statistically significant.

The required patients with DILI for the analysis had not been collected but some patients showed increased liver enzymes without meeting DILI criteria. Then, all patients were stratified into two groups by their liver enzymes levels; normal liver enzymes and elevated liver enzymes. Then, the association of elevated liver enzymes with the genetic and non-genetic characteristics of all patients was performed.

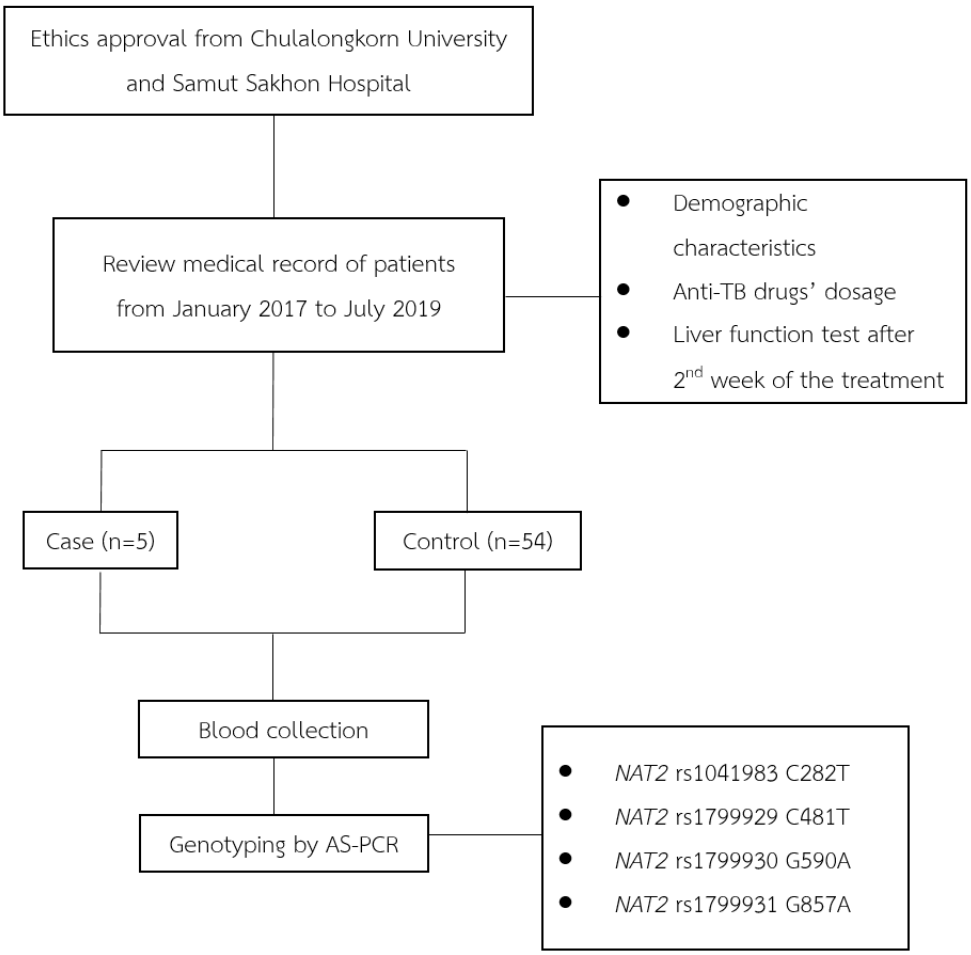
Then, a comparison of characteristics of patients between each liver enzyme level was determined by the Students' T-test and Fisher's exact test.

After this, one-way ANOVA and binary logistic regression were also applied to determine the association of *NAT2* SNPs genotype distribution and elevated AST, ALT, and ALP liver enzymes of patients.

One-way ANOVA test was also applied to compare the liver enzymes of patients between each *NAT2* genotype. Pearson's Chi-square test or Fisher's exact test, ANOVA, and Kruskal-Wallis H test were used to compare the demographic and clinical characteristics of patients between each *NAT2* phenotype where *p-value* < 0.05 was considered as statistically significant.

Then, linear regression was applied to determine the relationship of genetic and non-genetic factors and elevated liver enzymes of patients. The variables that had potential to effect on liver enzymes of patients and the variables which showed a *p-value* less than 0.15 were entered the multiple linear regression analysis. Data were coded and analyzed with IB SPSS version 22.

3.7 Research methodology flow chat



Chapter IV

RESULTS

4.1 Characteristics of patients

4.1.1 Comparison of baseline characteristics between cases and controls

In this study, 54 newly diagnosed adult TB patients without the incidence of liver injury and 5 patients with liver injury from TB clinic of Samut Sakhon Hospital, Thailand were enrolled. All patients met the inclusion and exclusion criteria of the study. Mann-Whitney U test and Fisher's exact test were used to compare the baseline characteristics of patients between cases and controls. There was no difference characteristic between these two groups (Table 17).

Table 17 Baseline characteristics of Myanmar patients with tuberculosis infection

Patients' characteristics	Control (n=54)	Case (n=5)	*p*-value
Age, median (SD)	30 (7.71)	34 (9.81)	0.124[a]
Body weight, median (SD)	50 (7.95)	45 (6.72)	0.165[a]
Height, median (SD)	160 (8)	160 (3.65)	0.469[a]
Body mass index (kg/m^2), median (SD)	19.74 (3.13)	16.93 (3.08)	0.092[a]
Gender, n (%)			1.000[b]
Male	22 (40.7%)	2 (40%)	
Female	32 (59.3%)	3 (60%)	
Ethnicity, n (%)			0.432[b]
Burma	30 (55.6%)	2 (40%)	
Mon	14 (25.9%)	2 (40%)	
Da-wal	5 (9.3%)	0	
Ka-yin	2 (3.7%)	0	
Ya-khaing	1 (1.9%)	1 (20%)	
Shan	1 (1.9%)	0	
Pa-O	1 (1.9%)	0	

Table 17 (Continued)

Patients' characteristics	Control (n=54)	Case (n=5)	*p*-value
Co-existing disease, n (%)			NA
Yes	0	0	
No	54 (100%)	5 (100%)	
Vitamin supplement, n (%)			NA
Yes	54 (100%)	5 (100%)	
No	0	0	
Drinking, n (%)			0.237[b]
Yes	2 (3.7%)	1 (20%)	
No	52 (96.3%)	4 (80%)	
Smoking, n (%)			NA
Yes	0	0	
No	54 (100%)	5 (100%)	
TB history, n (%)			NA
Yes	0	0	
No	54 (100%)	5 (100%)	
Types of TB, n (%)			0.531[b]
Pulmonary TB	47 (87%)	4 (80%)	
Extra-pulmonary TB	7 (13%)	1 (20%)	
Sputum AFB, n (%)			1.000[b]
Positive	20 (37%)	2 (40%)	
Negative	34 (63%)	3 (60%)	
Chest x-ray, n (%)			0.481[b]
Positive	48 (88.9%)	4 (80%)	
Negative	6 (11.1%)	1 (20%)	

AFB: Acid-fast bacilli, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: alkaline phosphatase, TB: Tuberculosis, SD: Standard deviation

[a]Mann-Whitney U test, [b]Fisher's exact test for comparison between groups

4.1.2 Comparison of doses of anti-TB drugs between cases and controls

Doses of anti-TB drugs between cases and controls were compared and analyzed by Mann-Whitney U test. There was no significant difference between two groups (Table 18). In addition, the doses were in the standard dosage range according to the WHO treatment guidelines (70).

Table 18 Doses of anti-TB drugs of Myanmar TB patients

Anti-TB drugs	Control (n=54)	Case (n=5)	*p*-value
Isoniazid dose (mg/kg), median (SD)	5.94 (0.85)	6.67 (0.84)	0.131[a]
Rifampicin dose (mg/kg), median (SD)	10.29 (1.1)	10.09 (0.57)	0.957[a]
Pyrazinamide dose (mg/kg), median (SD)	24.75 (3.76)	22.42 (1.85)	0.369[a]
Ethambutol dose (mg/kg), median (SD)	18.52 (1.69)	17.78 (1.1)	0.539[a]

[a]Mann-Whitney U test for comparison between groups

4.2 Comparison of liver enzymes between cases and controls

Liver enzymes of patients in cases and controls after the second week of the treatment were determined by Mann-Whitney U test. It was shown that cases had significantly higher AST and ALT levels than controls ($p < 0.001$) (Table 19).

Table 19 Liver enzymes of Myanmar TB patients

Liver enzymes	Control (n=54)	Case (n=5)	*p*-value
AST*, median (range)	24.00 (15-80)	170.00 (117-408)	<0.001[a#]
ALT*, median (range)	18.00 (7-160)	114.00 (72-763)	<0.001[a#]
ALP*, median (range)	84.00 (46-388)	112.00 (77-191)	0.076[a]

[a]Mann-Whitney U test for comparison between groups, [#]$p<0.05$

4.3 *NAT2* SNPs, haplotype, genotype, and phenotype distribution of Myanmar TB patients

4.3.1 *NAT2* SNPs distribution in Myanmar TB patients

Chi-square was used to test the deviation from Hardy-Weinberg equilibrium and rs1799929 was found to deviate from equilibrium (Table 20 and 21). Overall, the highest *NAT2* SNP was *NAT2* rs1041983 (56%) (Table 20). However, *NAT2* rs1799929 was highly found in cases (50%) (Table 21), suggesting that patients with this *NAT2* SNP had a higher risk to get liver injury than the other *NAT2* SNPs.

Table 20 SNP-based allele distribution of *NAT2* gene polymorphisms of Myanmar TB patients *(n=59)*

SNP ID	Nucleotide change	Amino acid change	Allele 1/2	11 (n)	12 (n)	22 (n)	VAF	HWE*	
								χ^2	*p-value*
rs1041983	*NAT2* C282T	-	C/T	11	30	18	0.56 (T)	0.0584	0.809
rs1799929	*NAT2* C481T	-	C/T	46	10	3	0.14 (T)	4.526	0.03
rs1799930	*NAT2* G590A	R197Q	G/A	26	24	9	0.36 (A)	0.75	0.386
rs1799931	*NAT2* G857A	G286E	G/A	36	22	1	0.20 (A)	1.34	0.25

*Hardy-Weinberg Equilibrium, VAF= Variance allele frequency

Table 21 SNP-based allele distribution of *NAT2* gene polymorphisms between cases and controls

SNP ID	Nucleotide change	Amino acid change	Allele 1/2	11 (n)		12 (n)		22 (n)		VAF			HWE* χ^2		*p-value*	
				A	B	A	B	A	B		A	B	A	B	A	B
rs1041983	*NAT2* C282T	-	C/T	10	1	26	4	18	0	T	0.57	0.4	0.013	2.22	0.91	0.14
rs1799929	*NAT2* C481T	-	C/T	45	1	7	3	2	1	T	0.10	0.5	4.59	0.2	0.03	0.65
rs1799930	*NAT2* G590A	R197Q	G/A	24	2	21	3	9	0	A	0.36	0.3	1.33	0.918	0.25	0.34
rs1799931	*NAT2* G857A	G286E	G/A	32	4	21	1	1	0	A	0.21	0.1	1.38	0.062	0.24	0.804

A= controls (n=54), B = cases (n=5), *Hardy-Weinberg Equilibrium, VAF= Variance allele frequency

4.3.2 *NAT2* haplotype distribution in Myanmar TB patients

When we examined *NAT2* haplotype distribution in Myanmar TB patients, *NAT2**6A was the most common allele in this population. However, *NAT2**5B was allele which highly found in patients with DILI (Table 22). Therefore, *NAT2**5B may be the risk *NAT2* allele of the incidence of liver injury in this Myanmar population.

Table 22 *NAT2* haplotype distribution in Myanmar TB patients (n=59)

NAT2 allele	Haplotype*	Total		Control		Case	
		2n=118	Frequency	2n=108	Frequency	2n=10	Frequency
*NAT2**4	C-C-G-G	36	0.305	35	0.324	1	0.1
*NAT2**5B	C-T-G-G	16	0.136	11	0.102	5	0.5
*NAT2**6A	T-C-A-G	42	0.356	39	0.361	3	0.3
*NAT2**7B	T-C-G-A	24	0.203	23	0.213	1	0.1

*Haplotype was determined from the four SNPs including rs1041983, rs 1799929, rs 1799930, and rs1799931

4.3.3 *NAT2* genotype, and phenotype distribution of Myanmar TB patients

To examine the deviation from Hardy-Weinberg equilibrium, Chi-square was used and all *NAT2* genotypes were found in equilibrium (Table 23). In all patients, *NAT2**4/*7B was the most common genotype. There were five patients with rapid acetylator phenotypes, twenty-six patients with intermediate acetylator phenotype, and twenty-eight patients with slow acetylator phenotype. Interestingly, there was no rapid acetylator phenotype in patients with liver injury. This distribution suggested that the slow acetylator phenotype had an influence on the anti-tuberculosis drug-induced liver injury.

Table 23 *NAT2* phenotype and genotype distribution of Myanmar TB patients

Phenotype	Genotype		Total (n=59)				Control (n=54)			Case (n=5)		
			n	n (%)	HWE* χ^2	*p-value*	n (%)	HWE* χ^2	*p-value*	n (%)	HWE* χ^2	*p-value*
Rapid acetylator	Rapid/ rapid allele	*4/*4	5	5 (8.47)	0.091	0.763	5 (9.3)	0.184	0.912	0	0.062	0.804
Intermediate acetylator	Rapid/ slow allele	*4/*5B	3	26 (44.07)			25 (46.3)			1 (20)		
		*4/*6A	11									
		*4/*7B	12									
Slow acetylator	Slow/ slow allele	*5B/*5B	3	28 (47.46)			24 (44.4)			4 (80)		
		*5B/*6A	5									
		*5B/*7B	2									
		*6A/*6A	9									
		*6A/*7B	8									
		*7B/*7B	1									

*Hardy-Weinberg equilibrium

4.3.4 Comparison of *NAT2* alleles and phenotypes of Myanmar TB patients with other ethnic groups

NAT2 acetylator phenotype of the present study and other studies was presented in Table 24. It was noticeable that the distributions of *NAT2* allele and *NAT2* phenotypes of the Myanmar population were similar with the Thai and Indonesia populations.

Table 24 Comparison of *NAT2* alleles and phenotypes of Myanmar TB patients with other ethnic groups

Year	Country	Sample size	*NAT2* allele				Acetylator			Ref
			*4	*5	*6	*7	Slow	Inter	Rapid	
2018	Greenland	260	0.64	0.29	0.14	0.06	-	-	-	(71)
2007	Japan	100	0.68	-	0.215	0.105	0.1	0.44	0.46	(72)
2016	Indonesia	241	0.35	0.13	0.35	0.15	0.40	0.46	0.14	(73)
2003	Thailand	235	0.381	0.038	0.326	0.204	0.362	-	0.638	(22)
2016	Thailand	138	0.33	0.08	0.35	0.22	0.41	0.47	0.12	(23)
2019	Myanmar (in this study)	59	0.305	0.136	0.356	0.203	0.4746	0.4407	0.0847	

4.4 Association of non-genetic characteristics of patients and the incidence of AT-DILI between cases and controls

Binary logistic regression reported no association of the non-genetic characteristics of patients and the incidence of DILI between cases and controls (Table 25). Similarly, the doses of anti-TB drugs showed no association with the incidence of AT-DILI (Table 26).

Table 25 Binary logistic regression analysis of non-genetic characteristics of patients between cases and controls

Variables	B	S.E	Wald	*p*-value*	OR	C.I 95% (Lower – Upper)	
Age	0.114	0.065	3.090	0.079	1.120	0.987	1.271
Gender	0.031	0.954	0.001	0.974	1.031	0.159	0.689
Body weight	-0.084	0.071	1.416	0.234	0.919	0.800	1.056
Height	0.026	0.059	0.196	0.658	1.027	0.914	1.154
BMI	-0.288	0.203	2.023	0.155	0.749	0.504	1.115
TB types	0.518	1.189	0.190	0.663	1.679	0.163	17.625

*Binary logistic regression for comparison between groups, $p < 0.05$ considered significant,

OR: odds ratio, C.I (95%): 95% confidence interval

Table 26 Binary logistic regression analysis of doses of anti-TB drugs of patients between cases and controls

Variables	B	S.E	Wald	*p*-value*	OR	C.I 95% (Lower – Upper)	
INH doses	0.816	0.588	1.922	0.166	2.261	0.714	7.162
RIF doses	-0.068	0.446	0.023	0.878	0.934	0.390	2.239
PZA doses	-0.088	0.478	0.478	0.489	0.915	0.713	1.176
ETB doses	-0.058	0.045	0.045	0.832	0.943	0.551	1.614

*Binary logistic regression for comparison between groups, $p < 0.05$ considered significant,

OR: odds ratio, C.I (95%): 95% confidence interval

4.5 Association of genetic characteristics of patients and the incidence of AT-DILI between cases and controls

4.5.1 Association of *NAT2* SNPs allele of patients and incidence of AT-DILI between cases and controls

The association of *NAT2* SNPs allele and the incidence of AT-DILI were also analyzed by binary logistic regression. In *NAT2* rs1799929, the distributions of CT and TT genotype were more than the reference genotype (CC genotype) distribution (OR = 19.286, 95% C.I = 1.751 – 212.417, p = 0.016 and OR = 22.50, 95% C.I = 1.001– 505.846, p = 0.005, respectively) (Table 27). This result indicated that *NAT2* rs1799929 was associated with the incidence of AT-DILI and patients with *NAT2* rs1799929 had higher risk to get liver injury.

Table 27 Binary logistic regression analysis of the distribution of *NAT2* SNPs allele of patients between cases and controls

Variables		B	S.E	Wald	*p*-value*	OR	C.I 95% (lower-Upper)	
rs1041983	CC	reference						
	CT	0.431	1.178	0.134	0.715	1.538	0.153	15.491
	TT	-18.900	9473.574	0.000	0.998	0	0	NA
rs1799929	CC	reference						
	CT	2.959	1.224	5.845	0.016#	19.286	1.751	212.417
	TT	3.114	1.588	3.843	0.050#	22.50	1.001	505.846
rs1799930	GG	reference						
	GA	0.539	0.961	0.315	0.575	1.714	0.261	11.264
	AA	-18.718	13397.657	0	0.999	0	0	NA
rs1799931	GG	reference						
	GA	-0.965	1.153	0.701	0.402	0.381	0.040	3.648
	AA	-19.123	40192.97	0	1.00	0	0	NA

*Binary logistic regression for comparison between groups, $p < 0.05$ considered significant,

OR: odds ratio, C.I (95%): 95% confidence interval, #$p < 0.05$

4.5.2 Association of *NAT2* genotype of patients and incidence of AT-DILI between cases and controls

Association of *NAT2* genotypes and DILI was determined by binary logistic regression, and we did not find any significant association on the incidence of liver injury of patients (Table 28).

Table 28 Binary logistic regression analysis of the distribution of *NAT2* genotypes of patients between case and control

Variables		B	S.E	Wald	*p*-value*	OR	C.I 95% (lower-Upper)	
NAT2 genotype	*4/*4	reference						
	*4/*5B	0.00	29352.798	0	1.00	1.00	0	NA
	*4/*6A	18.9	17974.848	0	0.999	1.615×10^{8}	0	NA
	*4/*7B	0	21394.346	0	1.00	1.00	0	NA
	*5B/*5B	20.510	17974.848	0	0.999	8.1×10^{8}	0	NA
	*5B/*6A	20.797	17974.848	0	0.999	1.01×10^{9}	0	NA
	*5B/*7B	21.203	17974.848	0	0.999	1.6×10^{9}	0	NA
	*6A/*6A	0	22418.572	0	1.00	1.00	0	NA
	*6A/*7B	0	22913.522	0	1.00	1.00	0	NA
	*7B/*7B	-21.203	44029.195	0	1.00	1.00	0	NA

*Binary logistic regression for comparison between groups, $p < 0.05$ considered significant,

OR: odds ratio, C.I (95%): 95% confidence interval

4.5.3 Association of *NAT2* phenotype of patients and incidence of AT-DILI between cases and controls

Binary logistic regression also showed no association of *NAT2* phenotypes and the incidence of DILI of patients (Table 29).

Table 29 Binary logistic regression analysis of the distribution of *NAT2* phenotypes of patients between cases and controls

Variables		B	S.E	Wald	*p*-value*	OR	C.I 95% (lower-Upper)	
NAT2 phenotype	rapid	reference						
	intermediate	17.984	17974.841	0	0.999	6.4×10^{7}	0	NA
	slow	19.411	17974.841	0	0.999	2.7×10^{8}	0	NA

*Binary logistic regression for comparison between groups, $p < 0.05$ considered significant,

OR: odds ratio, C.I (95%): 95% confidence interval

4.6 Association of non-genetic characteristics of Myanmar TB patients and elevated liver enzymes during anti-TB treatment

The limitation of this study was a small number of cases, as only 5 patients with AT-DILI were included to this study. Thus, the association of NAT2 genotype and phenotype were not found in this study. However, it was noticeable that some patients in control groups showed increased liver enzyme levels, but it did not meet DILI criteria. Therefore, all patients (n=59) were stratified into two types by values of patients' liver enzymes: normal liver enzymes and elevated liver enzymes (based on normal values of laboratory liver function test). Then, the association between genetic or non-genetic factor and elevated liver enzyme were further analyzed.

The non-genetic characteristics of patients were compared between patients with and without elevated liver enzymes using Students T-test, and χ^2 or Fisher's exact test. The non-genetic characteristics of patients in two groups were not significant difference (Table 30). In the same way with the dose of anti-TB drugs, no significant association was observed between groups (Table 31).

Table 30 Association of non-genetic characteristics of patients and elevated liver enzyme ranges (n=59)

Variables	AST liver enzymes		ALT liver enzymes		ALP liver enzymes		*p*-value
	normal	elevated	normal	elevated	normal	elevated	
Age (mean)	31.39	31.50	31.25	32.12	30.63	35.67	>0.05[a]
Gender (n %)							>0.05[b]
Male	83.3	16.7	70.8	29.2	75	25	
Female	70.6	29.4	69.7	30.3	91.2	8.8	
BMI (mean)	20.10	18.58	20.06	19.05	19.83	19.08	>0.05[a]
Type of TB (n %)							>0.05[b]
P-TB	76.5	23.5	72	28	84.3	15.7	
EP-TB	71.4	28.6	57.1	42.9	85.7	14.3	
Sputum AFB (n %)							>0.05[b]
Positive	76.2	23.8	76.2	23.8	81	19	
Negative	75.7	24.3	66.7	33.3	86.5	13.5	

BMI: body mass index, P-TB: pulmonary TB, EP-TB: extra-pulmonary TB, INH: isoniazid, RIF: rifampicin, PZA: pyrazinamide, ETB: ethambutol

[a]Students T-test, [b]χ^2 or Fisher's exact test for comparison of variables between groups

Table 31 Association of doses of anti-TB drugs of patients and elevated liver enzyme ranges (n=59)

Variables	AST liver enzymes		ALT liver enzymes		ALP liver enzymes		*p*-value
	normal	elevated	normal	elevated	normal	elevated	
INH(mg/kg)	5.9091	6.3807	5.95	6.16	6.08	5.73	>0.05[a]
RIF(mg/kg)	10.3833	10.3812	10.40	10.36	10.5	9.75	>0.05[a]
PZA(mg/kg)	24.7407	23.9712	24.85	23.99	24.95	22.4	>0.05[a]
ETB(mg/kg)	18.2828	18.1033	18.40	17.89	18.45	17.11	>0.05[a]

BMI: body mass index, P-TB: pulmonary TB, EP-TB: extra-pulmonary TB, INH: isoniazid, RIF: rifampicin, PZA: pyrazinamide, ETB: ethambutol

[a]Students T-test for comparison of variables between groups

4.7 Association genetic characteristics of Myanmar TB patients and elevated liver enzymes during anti-TB treatment

4.7.1 Association of *NAT2* SNPs and elevated liver enzymes of patients

To differentiate the influence of genetic polymorphisms on liver enzymes of patients, the mean AST, ALT and ALP liver enzymes of patients between each *NAT2* SNPs were compared by one-way ANOVA test (Figure 5-8). *NAT2* rs1799929 has the impact on AST and ALT level as patients with CT variance had significantly higher AST levels and patients with TT variance has significantly higher AST and ALT levels (Figure 6).

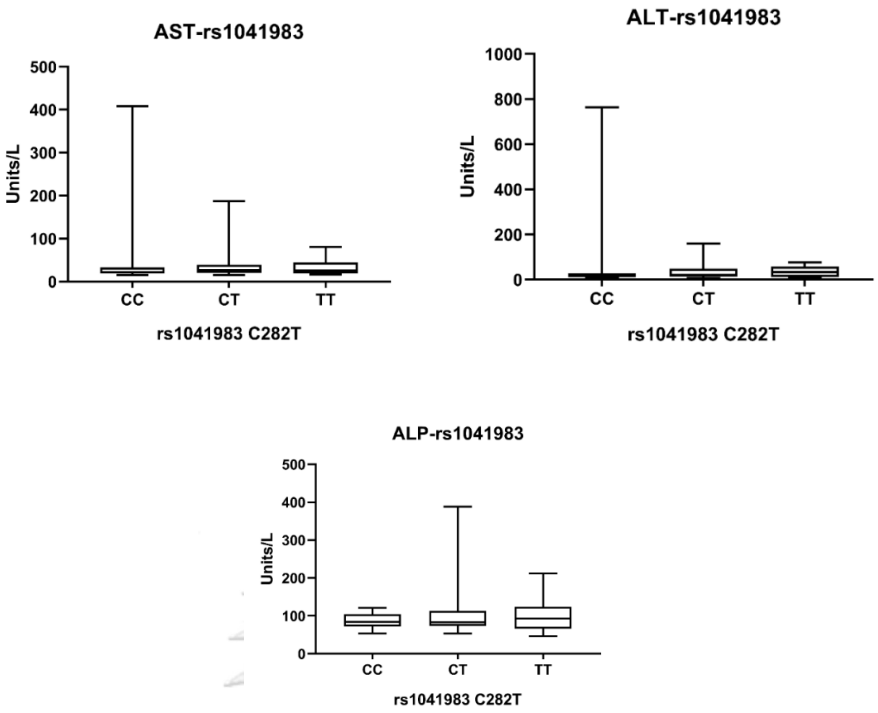


Figure 5 Mean AST, ALT and ALP liver enzymes of *NAT2* rs1041983 genotypes, CC (n=11), CT (n=30) and TT (n=18)

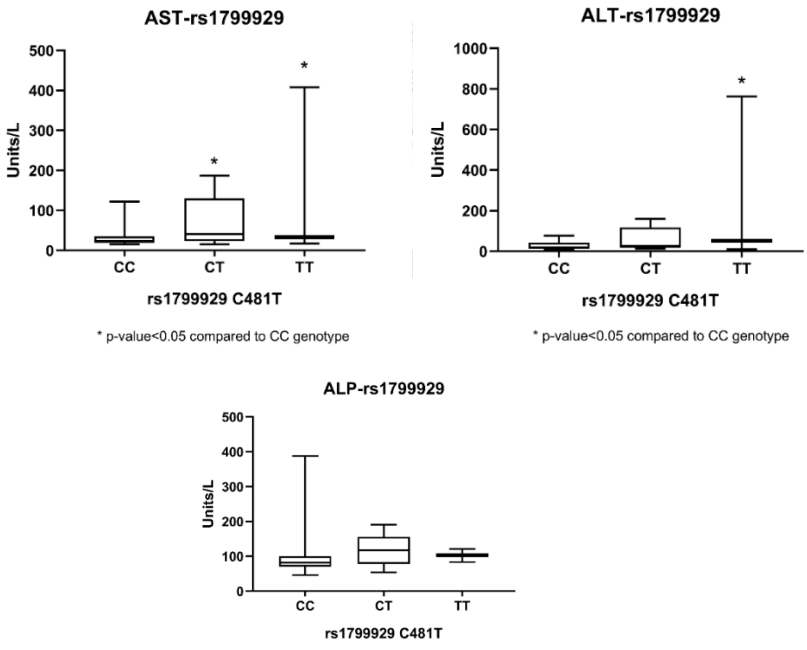


Figure 6 Mean AST, ALT and ALP liver enzymes of *NAT2* rs1799929 genotypes, CC (n=46), CT (n=10) and TT (n=3)

*p<0.05 compared to CC genotype

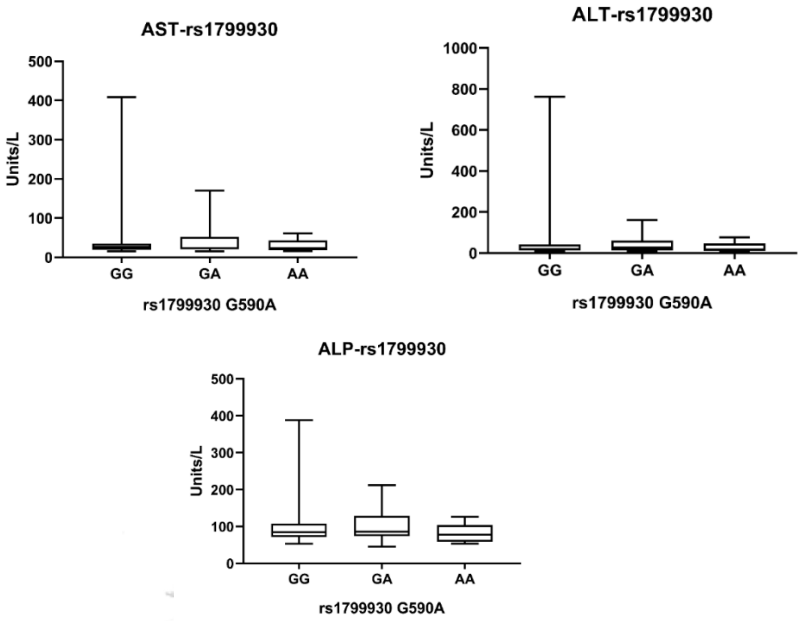


Figure 7 Mean AST, ALT and ALP liver enzymes of *NAT2* rs1799930 genotypes, GG (n=26), GA (n=24) and AA (n=9)

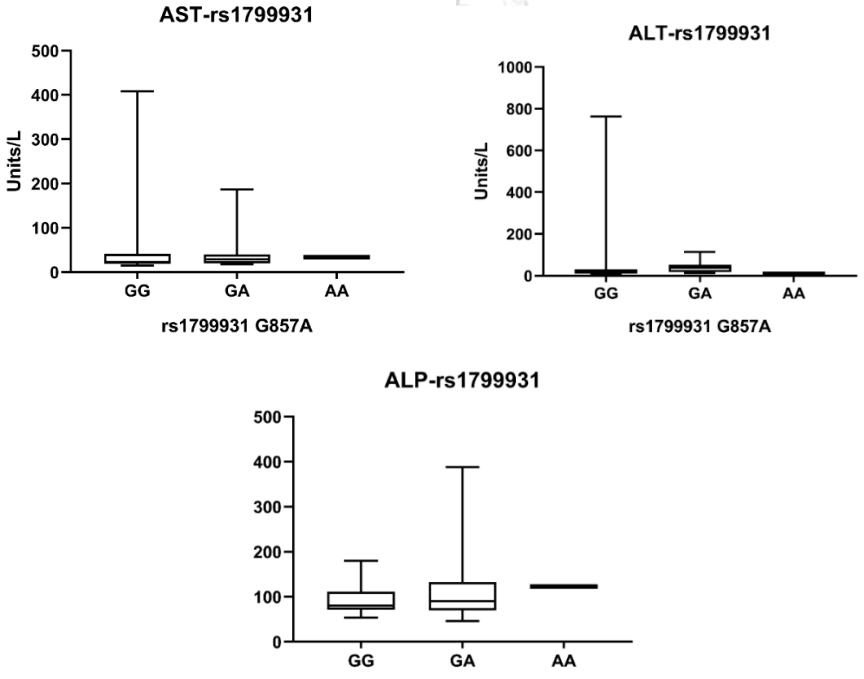


Figure 8 Mean AST, ALT and ALP liver enzymes of *NAT2* rs1799931 genotypes, GG (n=36), GA (n=22) and AA (n=1)

The association of *NAT2* SNPs and AST, ALT and ALP liver enzyme levels was determined using binary logistic regression. The result demonstrated the association of *NAT2* rs1799929 CT genotype with the AST liver enzymes ranges of patients by an odds ratio of 4.635 (95% C.I =1.078 – 19.840, p = 0.039) (Table 32). However, there was no association between *NAT2* SNPs distribution and elevated ALT and ALP liver enzyme ranges of patients (Table 33 and 34). The results suggested that patients who had CT genotype of *NAT2* rs1799929 increased AST liver enzymes than patients with other *NAT2* SNPs.

Table 32 Binary logistic regression analysis of the distribution of *NAT2* SNPs allele of patients between patients with normal AST levels and patients with elevated AST levels (n=59)

SNP	Geno-type	B	S.E	Wald	*p*-value*	OR	C.I 95% (lower-Upper)	
rs1040983	CC	reference						
	CT	0.160	0.906	0.031	0.860	1.174	0.199	6.935
	TT	0.811	0.928	0.764	0.382	2.250	0.365	13.870
rs1799929	CC	reference						
	CT	1.531	0.743	4.249	0.039#	4.625	1.078	19.840
	TT	0.833	1.285	0.425	0.514	2.312	0.186	28.717
rs1799930	GG	reference						
	GA	0.878	0.708	1.540	0.215	2.406	0.691	9.632
	AA	1.012	0.892	1.286	0.257	2.750	0.479	15.794
rs1799931	GG	reference						
	GA	-0.163	0.639	0.065	0.799	0.850	0.243	2.973
	AA	-20.142	40192.97	0	1.00	0.00	0.00	NA

*Binary logistic regression for comparison between groups, $p < 0.05$ considered significant,

OR: odds ratio, C.I (95%): 95% confidence interval, #$p < 0.05$

Table 33 Binary logistic regression analysis of the distribution of *NAT2* SNPs allele of patients between patients with normal ALT levels and patients with elevated ALT levels (n=59)

SNP	Geno -type	B	S.E	Wald	*p*-value*	OR	C.I 95% (lower –Upper)	
rs1040983	CC	reference						
	CT	0.588	0.887	0.439	0.507	1.800	0.317	10.232
	TT	1.052	0.919	1.310	0.252	2.864	0.473	17.351
rs1799929	CC	reference						
	CT	0.693	0.733	0.893	0.345	2.00	0.475	8.420
	TT	1.792	1.273	1.980	0.159	6.00	0.495	72.771
rs1799930	GG	reference						
	GA	0.711	0.634	1.257	0.262	2.036	0.588	7.052
	AA	-0.100	0.929	0.012	0.914	0.905	0.147	5.583
rs1799931	GG	reference						
	GA	0.929	0.597	2.420	0.120	2.531	0.785	8.157
	AA	-19.986	40192.970	0.00	1.00	0.00	0.00	NA

*Binary logistic regression for comparison between groups, $p < 0.05$ considered significant,

OR: odds ratio, CI (95%): 95% confidence interval

Table 34 Binary logistic regression analysis of the distribution of *NAT2* SNPs allele of patients between patients with normal ALP levels and patients with elevated ALP levels (n=59)

SNP	Geno -type	B	S.E	Wald	*p*-value*	OR	CI 95% (Lower –Upper)	
rs1040983	CC	reference						
	CT	19.859	12118.633	0.00	0.999	4.2×10^{8}	0.00	NA
	TT	19.593	12118.633	0.00	0.999	3.2×10^{8}	0.00	NA
rs1799929	CC	reference						
	CT	1.025	0.818	1.570	0.210	2.786	0.561	13.832
	TT	-19.331	23205.422	0.00	0.999	0.00	0.00	NA
rs1799930	GG	reference						
	GA	0.995	0.776	1.645	0.200	2.706	0.591	12.385
	AA	-19.166	13397.657	0.00	0.999	0.00	0.00	NA
rs1799931	GG	reference						
	GA	1.386	0.771	3.237	0.072	4.00	0.883	18.112
	AA	-18.836	40192.970	0.00	1.00	0.00	0.00	NA

*Binary logistic regression for comparison between groups, $p < 0.05$ considered significant,

OR: odds ratio, CI (95%): 95% confidence interval

4.7.2 Association of *NAT2* genotypes and elevated liver enzymes of patients

To determine the effect of *NAT2* genotypes on the liver enzymes, one-way ANOVA was used to compare AST, ALT and ALP levels of patients with each *NAT2* genotype. We found the highest liver AST and ALT in *NAT2**5B/*5B genotype and the highest ALP in *NAT2**5B/*6A genotype (Figure 9). In addition to this, the AST and ALT liver enzymes between *NAT2* genotypes showed the difference (Table 35).

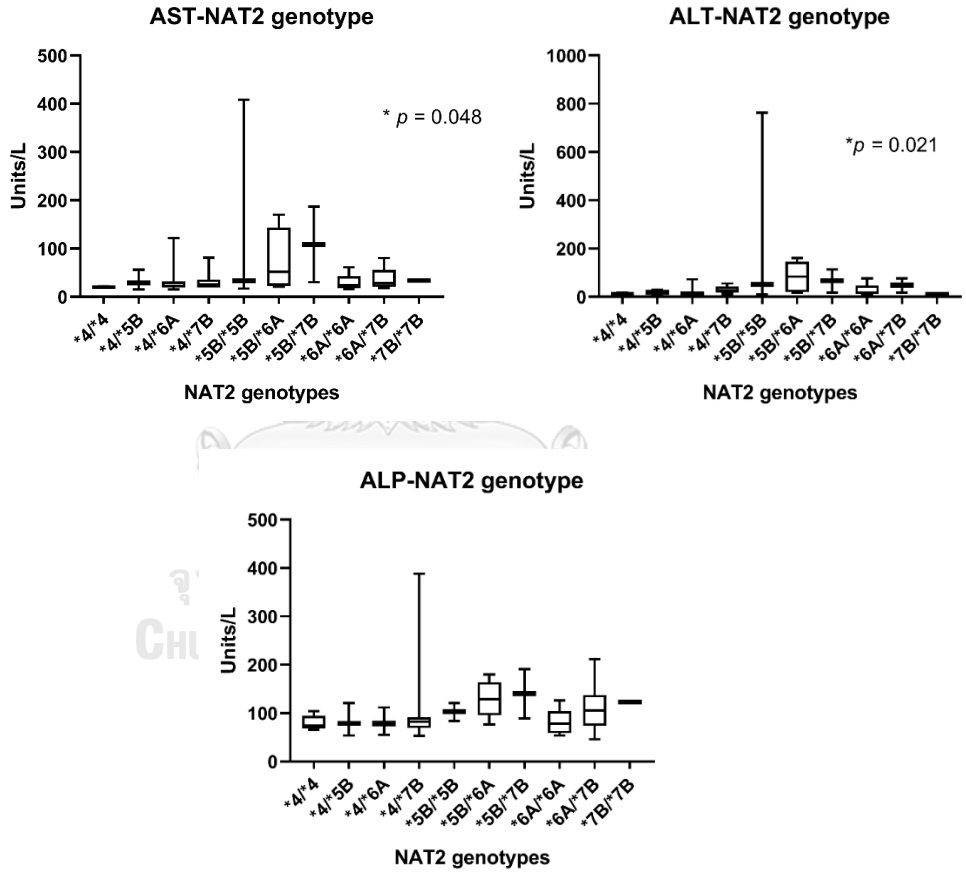


Figure 9 Mean AST, ALT and ALP liver enzymes of *NAT2* genotypes

Table 35 Distribution of the *NAT2* genotype, *NAT2* phenotype and the respective liver enzymes in Myanmar patients (n=59)

Phenotype	Genotype	n (%)	AST (mean ± SD)	ALT (mean ± SD)	ALP (mean ± SD)
Rapid acetylator	*4/*4	5(8.5)	20.20 ± 1.30	14.40 ± 2.88	80.20 ± 14.97
Intermediate acetylator	*4/*5B	3(5.1)	33.33 ± 20.84	19.67 ± 8.62	84.67 ± 33.86
	*4/*6A	11(18.6)	34.10 ± 31.66	19.50 ± 19.432	79 ± 14.414
	*4/*7B	12(20.3)	31.25 ± 17.24	30.27 ± 14.92	111.75 ± 92.90
Slow acetylator	*5B/*5B	3(5.1)	152.67 ± 221.27	274.67 ± 423.406	102.67 ± 18.502
	*5B/*6A	5(8.5)	76.80 ± 64.844	82.60 ± 63.669	129.60 ± 38.371
	*5B/*7B	2(3.4)	108.50 ± 11.016	66.00 ± 67.882	140 ± 72.125
	*6A/*6A	9(15.3)	30.11 ± 15.34	27.78 ± 24.75	82.44 ± 25.26
	*6A/*7B	8(13.6)	38.00 ± 21.99	46.75 ± 17.59	112.38 ± 50.86
	*7B/*7B	1(1.7)	34	10	123
p-value[a]			0.048[#]	0.02[#]	0.597

[a]ANOVA for comparison between groups, $p < 0.05$ considered significant, [#]$p < 0.05$

4.7.3 Association of *NAT2* phenotypes and elevated liver enzymes of patients

To determine the influence of *NAT2* phenotypes on liver enzyme levels, patients were grouped into three types based on their *NAT2* phenotypes; slow acetylators, intermediate acetylators and rapid acetylators. Baseline characteristics and anti-TB doses were presented according to patients' *NAT2* phenotypes (Table 36 and 37). All groups of patients had no different demographic characteristics and anti-TB doses. But, the liver enzymes between each *NAT2* phenotype showed significant results (Table 38). Patients with slow *NAT2* phenotypes had the highest liver enzymes among the three groups (Figure 10). Additionally, the rapid acetylator phenotypes did not show elevated liver enzymes when compared with Fishers' exact test (Table 39).

Table 36 Characteristics of patients between *NAT2* phenotypes (n=59)

Characteristics	Slow (n =28)	Intermediate(n=26)	Rapid(n=5)	*p*-value
Age (mean ± S.D)	32.93 ± 8.472	28.69 ± 7.024	34.6 ± 8.385	0.093[a]
BMI	19.55 ± 3.38	20.22 ± 3.18	20.08 ±1.5	0.732[a]
Gender (n, %)				
Male	12	12	0	0.309[b]
Female	16	14	5	
Type of TB (n, %)				
P-TB	26 (51%)	20 (39.2%)	5 (9.8%)	0.236[b]
EP-TB	2 (25%)	6 (75%)	0	

BMI: body mass index, P-TB: pulmonary TB, EP-TB: extra-pulmonary TB

[a]ANOVA, [b]χ^2 or Fisher's exact tests for comparison between groups

Table 37 Doses of anti-TB drugs of patients between *NAT2* phenotypes (n=59)

Anti-TB drugs	Slow (n =28)	Intermediate (n=26)	Rapid (n=5)	*p*-value
INH (mg/kg) (mean ± S.D)	5.91 ± 0.89	6.05 ± 0.99	6.24 ± 0.37	0.681[a]
RIF (mg/kg) (mean ± S.D)	10.39 ± 1.14	10.29 ± 0.99	10.54 ± 1.16	0.867[a]
PZA (mg/kg) (mean ± S.D)	24.72 ± 3.86	24.06 ± 3.39	25.83 ± 4.09	0.576[a]
ETB (mg/kg) (mean ± S.D)	18.22 ± 1.95	18.26 ± 1.36	18.22 ± 1.38	0.995[a]

INH: isoniazid, RIF: rifampicin, PZA: pyrazinamide, ETB: ethambutol, [a]ANOVA for comparison

Table 38 Liver enzymes of Myanmar TB patients between *NAT2* phenotypes (n=59)

Liver enzymes	Slow (n =28)	Intermediate (n=26)	Rapid (n=5)	*p*-value
AST(median ± S.D)	31.50 ± 80.919	24.50 ± 23.46	20.20 ± 1.30	0.080[c]
ALT(median ± S.D)	37.00± 141.006	18.50 ± 16.76	16.00 ± 2.88	0.022[c#]
ALP(median ± S.D)	99.50 ± 41.135	79.50 ± 66.26	74.00 ± 14.973	0.057[c]

[c] Kruskal-Wallis H test for comparison between groups, [#] $p < 0.05$

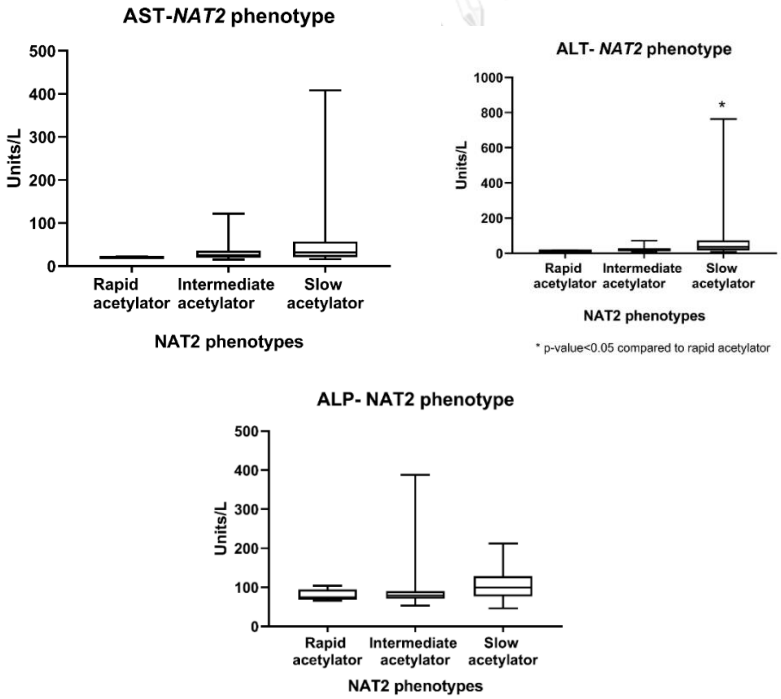


Figure 10 Mean AST, ALT and ALP liver enzymes of *NAT2* phenotypes, rapid (n=5), intermediate (n=26) and slow (n=28)

***p<0.05 compared to *NAT2* rapid acetylator**

Table 39 Liver enzyme ranges of Myanmar TB patients between *NAT2* phenotypes (n=59)

Liver enzymes		% of patients within liver enzymes range, (n)			χ^2	*p*-value*
		Slow (n=28)	Inter (n=26)	Rapid (n=5)		
AST	normal	37.8% (17)	51.1% (23)	11.1% (5)	6.269	0.035#
	elevated	78.6% (11)	21.4% (3)	0		
ALT	normal	35.7% (15)	52.4% (22)	11.9% (5)	7.097	0.023#
	elevated	76.5% (13)	23.5%(4)	0		
ALP	normal	44% (22)	46% (23)	10% (5)	1.305	0.472
	elevated	66.7% (6)	33.3 (3)	0		

*Fisher's exact test for comparison between groups, #$p < 0.05$

4.8 Determination of relationship between non-genetic and genetic characteristics and liver enzymes of patients by regression model

4.8.1 Determination of relationship between non-genetic and genetic characteristics and AST liver enzymes of patients by regression model

We would like to determine the relationship between non-genetic and genetic characteristics and liver enzymes of patients and how these characteristics influence on liver enzymes of patients, we analyzed the factors that had potential to effect on liver enzymes of patients by simple linear regression. With AST liver enzymes of patients, we discovered age, BMI, rs1799929 (*NAT2* C481T), and *NAT2* phenotype of patients showed *p*- value of less than 0.15 and then, added these variables to multiple linear regression with stepwise method (Table 40).

The rs1799929 (*NAT2* C481T), isoniazid doses, and age of patients showed a linkage with AST liver enzymes of patients (B = 49.334, $p < 0.001$, B = 22.241, $p = 0.007$

and B = 1.991, *p*=0.025 respectively) (Table 41). And, this regression model explained 35.6% of the variation of AST liver enzymes of patients (R^2=0.356). And, age, INH doses, *NAT2* phenotype also showed an association with AST liver enzymes of patients (B = 2.581, *p* = 0.008, B = 22.003, *p* = 0.016 and B = 23.781, *p*=0.038 respectively) (Table 42). And, this regression model also explained 22.2% of the variation of AST liver enzymes of patients (R^2=0.222). According to this regression model, patients with old age, patients with *NAT2* rs1799927 and patients with *NAT2* slow acetylator phenotype had increased AST liver enzymes than the other patients.

Table 40 Simple linear regression analysis between non-genetic and genetic characteristics and AST liver enzymes of patients (n=59)

Variable	B	S.E	Beta	t	*p*-value*	95% C.I		R^2	Adj R^2
						Lower	Upper		
Age	2.168	0.959	0.289	2.260	0.02#	0.246	4.090	0.084	0.067
Gender	5.662	16.027	0.047	0.353	0.725	-26.445	37.768	0.002	-0.016
B.wt	-1.037	1.019	-0.169	-1.282	0.205	-3.348	0.735	0.029	0.011
Height	0.273	1.049	0.035	0.260	0.796	-1.829	2.375	0.001	-0.017
BMI	-4.112	2.748	-0.196	-1.497	0.140#	-9.617	1.392	0.038	0.021
TB types	-1.134	24.258	-0.006	-0.047	0.963	-49.728	47.459	0	-0.018
INH doses	13.380	9.269	0.189	1.444	0.154	-5.187	31.947	0.036	0.019
RIF doses	-2.591	7.547	-0.046	-0.343	0.733	-17.710	12.527	0.002	-0.016
PZA doses	-1.865	2.159	-0.115	-0.864	0.391	-6.190	2.461	0.013	-0.004
ETB doses	-2.041	4.793	-0.057	-0.426	0.672	-11.642	7.56	0.003	-0.015
rs1041983	-12.854	11.211	-0.151	-1.147	0.256	-35.316	9.602	0.023	0.005
rs1799929	51.398	12.601	0.479	4.079	0.000#	26.155	76.641	0.229	0.215
rs1799930	-8.539	10.925	-0.104	-0.782	0.438	-30.425	13.347	0.011	-0.007
rs1799931	-6.575	14.988	-0.059	-0.439	0.663	-36.599	23.449	0.003	-0.014
NAT2 genotype	1.789	2.986	0.080	0.598	0.552	-4.195	7.770	0.006	-0.011
NAT2 phenotype	22.694	11.937	0.246	1.901	0.062#	-1.218	46.606	0.061	0.044

B – Linear coefficient, S.E – Standard Error, C.I – Confidence interval, AST – Aspartate aminotransferase, **p*-value was calculated by simple linear regression analysis, #$p < 0.15$

Table 41 Multiple linear regression analysis between non-genetic and genetic characteristics and AST liver enzymes of patients (n=59)

Model		Unstandardized Coefficients		Standardized Coefficients	t	*p*-value*	95% Confidence Interval for B	
		B	S. E	Beta			Lower	Upper
1	Constant	-165.545	60.794		-2.723	0.009	-287.429	-43.661
	rs1799929	49.334	12.066	0.459	4.089	< 0.001#	25.143	73.525
	Isoniazid dose	22.241	7.992	0.315	2.783	0.007#	6.219	38.264
	Age	1.991	0.863	0.265	2.306	0.025#	0.260	3.722
R^2=0.356, Adj R^2= 0.320								

B – Linear coefficient, S.E – Standard Error, C.I – Confidence interval, AST – Aspartate aminotransferase Age, BMI, INH doses, rs1041983, rs1799929, rs1799930 and rs1799931 to multiple linear regression,

**p*-value was calculated by stepwise linear regression analysis

Table 42 Multiple linear regression analysis between non-genetic and genetic characteristics and AST liver enzymes of patients (n=59)

Model		Unstandardized Coefficients		Standardized Coefficients	t	*p*-value*	95% C.I for B	
		B	S.E	Beta			Lower	Upper
1	Constant	-226.037	73.839		-3.061	0.003	-374.076	-77.998
	NAT2 phenotype	23.781	11.187	0.258	2.126	0.038#	1.352	46.210
	Isoniazid dose	22.003	8.823	0.311	2.494	0.016#	4.314	39.692
	Age	2.581	0.932	0.344	2.771	0.008#	0.713	4.449
R^2=0.222, Adj R^2= 0.179								

B – Linear coefficient, S.E – Standard Error, C.I – Confidence interval, AST – Aspartate aminotransferase Age, INH doses, *NAT2* phenotype to multiple linear regression, rapid acetylator as reference phenotype

**p*-value was calculated by stepwise linear regression analysis

4.8.2 Determination of relationship between non-genetic and genetic characteristics and ALT liver enzymes of patients by regression model

To explore the effect of non-genetic and genetic characteristics of patients on ALT liver enzymes of patients, we performed the same simple linear regression method with ALT liver enzymes of patients (Table 43). Then, we also made multiple linear regression with rs1799929 (*NAT2* C481T), *NAT2* phenotypes, INH doses and age of the patients. We detected an association of rs1799929 (*NAT2* C481T) and ALT liver enzymes of patients (B = 85.944, $p < 0.001$) and this regression explained 22.4% ($R^2 = 0.224$) of the variation in ALT levels of patients (Table 44). Likewise, age of the patients showed a relationship with the ALT levels of patients (B = 3.382, $p = 0.045$) and described 7.1% ($R^2 = 0.071$) of the variations (Table 45). We got the consistent relationship of age and *NAT2* rs1799929 on ALT liver enzymes of patients.

Table 43 Simple linear regression analysis between non-genetic and genetic characteristics and ALT liver enzymes of patients (n=59)

Variable	B	S.E	Beta	t	p-value*	95% C.I		R^2	Adj R^2
						Lower	Upper		
Age	3.382	1.652	0.266	2.048	0.045#	0.072	6.693	0.071	0.054
Gender	11.072	27.453	0.054	0.403	0.688	-43.946	66.090	0.003	-0.015
B.wt	-1.378	1.759	-0.015	-0.784	0.437	-4.903	2.147	0.011	-0.007
Height	-0.005	1.790	0.00	-0.003	0.998	-3.592	3.583	-0.018	0.998
BMI	-3.828	4.785	-0.107	-0.800	0.427	-13.418	5.762	0.012	-0.006
TB types	-14.480	41.313	0.047	-0.350	0.727	-97.272	68.312	0.002	-0.016
INH doses	11.788	16.080	-0.098	0.733	0.467	-20.437	44.013	0.010	-0.008
RIF doses	-10.092	12.808	-0.106	-0.788	-0.434	-35.760	15.577	0.011	-0.007
PZA doses	-4.137	3.673	-0.150	-1.126	0.265	-11.497	3.223	0.023	0.005
ETB doses	-7.030	8.125	-0.116	-0.865	0.391	-23.313	9.254	0.013	-0.005
rs1041983	-23.024	19.069	-0.161	-1.207	0.232	-61.238	15.190	0.026	0.008
rs1799929	85.944	21.578	0.473	3.983	<0.001#	42.701	129.186	0.224	0.210
rs1799930	-14.666	18.762	-0.105	-0.782	0.438	-52.267	22.935	-0.011	-0.007
rs1799931	-13.686	25.783	-0.071	-0.531	0.598	-65.357	37.984	0.005	-0.013
NAT2 genotype	2.930	5.096	0.077	0.575	0.568	-7.283	13.142	0.006	-0.012
NAT2 phenotype	36.125	20.472	0.231	1.765	0.083#	-4.901	77.151	0.054	0.036

B – Linear coefficient, S.E – Standard Error, C.I – Confidence interval, ALT – Alanine aminotransferase, **p*-value was calculated by simple linear regression analysis, #$p < 0.15$

Table 44 Multiple linear regression analysis between non-genetic and genetic characteristics and ALT liver enzymes of patients (n=59)

Model		Unstandardized Coefficients		Standardized Coefficients	t	*p*-value*	95% C.I for B	
		B	S. E	Beta			Lower	Upper
1	Constant	22.577	13.405		1.684	0.098	-4.288	49.442
	rs1799929	85.944	21.578	0.473	3.983	<0.001#	42.701	129.186
R^2=0.224, Adj R^2= 0.210								

B – Linear coefficient, S.E – Standard Error, C.I – Confidence interval, ALT – Alanine aminotransferase,

INH doses, rs104983, rs1799929, rs1799930 and rs1799930 to multiple linear regression,

**p*-value was calculated by stepwise linear regression analysis

Table 45 Multiple linear regression analysis between non-genetic and genetic characteristics and ALT liver enzymes of patients (n=59)

Model		Unstandardized Coefficients		Standardized Coefficients	t	*p*-value*	95.0% C.I for B	
		B	S. E	Beta			Lower	Upper
1	Constant	-59.877	53.670		-1.116	0.269	-167.435	47.681
	Age	3.382	1.652	0.266	2.048	0.045	0.072	6.693
R^2=0.071, Adj R^2= 0.054								

B – Linear coefficient, S.E – Standard Error, C.I – Confidence interval

ALT – Alanine aminotransferase, rapid acetylator as reference phenotype

Age, BMI, INH doses and *NAT2* phenotype to multiple linear regression

**p*-value was calculated by stepwise linear regression analysis

Chapter V

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The aim of this study was to identify the influence of *NAT2* polymorphisms on AT-DILI in Myanmar TB patients. Previous studies in various ethnic groups showed the association of *NAT2* polymorphism and AT-DILI and slow acetylators had greater risk to develop DILI during anti-TB treatment (8, 24, 47, 74). Four *NAT2* SNPs; rs1041983, rs1799929, rs1799930, and rs1799931, were determined in this study as these SNPs are commonly found in Asia and Southeast Asia population (23). In addition, previous study suggested that four-SNP genotype panel is effective to determine *NAT2* phenotypes (75). Fifty-nine Myanmar TB patients from Samut Sakorn Hospital; 54 controls and 5 cases, were enrolled in this study. All patients were newly diagnosed, fist-time treatment with a standard 6-month TB regimen and had received the recommended doses of anti-tuberculosis treatment (76). Baseline characteristics of cases and controls were not significantly different and non-genetic factors were not associated with AT-DILI.

The distribution of *NAT2* polymorphisms in Myanmar population has been reported for the first time in this study. *NAT2* rs1041983 was highly found in all patients (n=59), together with the most common allele, *NAT2**6A. This finding is in agreement with other Southeast Asian populations; Thai and Indonesian (23, 73). In contrast, studies in Japan and Greenland reported that *NAT2**4 was the most common (71, 72).

Considering genotype distribution, the most commonly found genotype is *NAT2**4/*7B. In addition, 47% of patients (n=28) were the slow acetylators and 44% (n=26) were intermediate acetylators. *NAT2* phenotype distribution of Myanmar population in this study is similar to previous studies in the South East Asia population (73, 74, 77) showing higher distribution of slow and intermediate acetylators than rapid acetylators.

Most of *NAT2* SNPs, genotype and phenotype distribution were in Hardy-Weinberg Equilibrium. A deviation from Hardy-Weinberg Equilibrium was found in *NAT2* rs1799929. A small sample size together with seven sub-ethnic groups may explain this deviation. Furthermore, the distribution of *NAT2* rs1799929 and *NAT2**5B were higher in cases than controls, which might contribute to AT-DILI.

Binary logistic regression analysis revealed that patients with CT and TT genotypes of *NAT2* rs1799929 had significantly higher risk of DILI than CC genotype. Although CT and TT genotypes of *NAT2* rs1799929 are linked to *NAT2**5, a slow *NAT2* allele, there was no association between *NAT2* genotype and phenotype and AT-DILI in this study. The small number of cases (n=5) might interfere the regression analysis. However, the prominent association of *NAT2* SNPs rs1799929 and DILI had been found. In addition, previous study in India population also reported that *NAT2**5 allele with *NAT2* SNPs rs1799929 was highly presented in AT-DILI patients (54). Future study with larger group of Myanmar patients might suggest the association of *NAT2* genotype and phenotype and AT-DILI.

Elevated liver enzyme levels had been noted in control patients after 2-week anti-TB treatment. Because these levels did not meet the DILI criteria, the patients did not classify as case (AT-DILI). However, to consider the risk of anti-TB treatment-induced elevated liver enzyme, association between *NAT2* polymorphisms and liver enzyme; AST, ALT and ALP levels, has been analyzed in this study. All patients (n=59) were stratified into two groups: normal liver enzymes and elevated liver enzymes. It is shown that CT genotype of *NAT2* rs1799929 was highly found in patients with elevated AST levels. In addition, one-way ANOVA revealed the significant effects of *NAT2* genotypes on AST and ALT levels, and the highest AST and ALT levels were presented in slow acetylators with *NAT2**5B/*5B. Taken together, *NAT2* rs1799929 is associated with elevated liver enzymes and the incidence of DILI in this study. Moreover, slow acetylators, which are mostly found in Myanmar population, are associated with the risk of elevated liver enzyme.

Finally, multiple linear regression analysis suggested the significant association of *NAT2* SNPs rs1799929, isoniazid dose and age of the patients and AST levels. This analysis model described 35% of the variation of the AST levels. Furthermore, *NAT2* phenotype, isoniazid dose and age of the patients also explained 22% of the variation of the AST levels. Based on these two models analysis, patients with *NAT2* slow allele, *NAT2* slow acetylator phenotype had more chance to get the elevated AST levels during anti-TB treatment. In the same way, *NAT2* SNPs rs1799929 also exhibited the 22% of the variation of the ALT levels. These regression model suggested *NAT2* SNPs

rs1799929 was the genetic determiner of elevated liver enzymes during anti-TB treatment. This is in agreement with previous study by Tostmann et al (2007) showing that advanced age, slow acetylators status were the risk factors for AT-DILI (12).

5.2 LIMITATION

In this study, the case group contained only five patients with DILI because of time limitation of the study. Moreover, it was difficult to retrieve some Myanmar migrant workers, patients with DILI. A small number of patients with DILI may interfere the analysis for association between *NAT2* genotypes and phenotypes and AT-DILI. Nevertheless, the association of *NAT2* SNPs rs1799929 and DILI has been found. Moreover, *NAT2* SNPs rs1799929 is an important factor to predict elevated AST and ALT levels. Future study with larger Myanmar patients is required for a stronger association and prediction.

5.3 BENEFIT

According to the pharmacogenetic based anti-tuberculosis study in Japanese patients (78), researchers suggested TB patients with rapid acetylation phenotype, which is commonly found in Japanese population, to use the higher INH dose to increase therapeutic outcome. On the other hand, as Thai patients are normally intermediate and slow acetylators, Thai TB treatment guidelines suggest patients with AT-DILI to undergo *NAT2* genotyping to guide INH dose adjustment. This study showed *NAT2* polymorphisms of Myanmar TB patients were similar to Thai population. Thus, Thai TB treatment guideline can be used in ADR management of Myanmar migrant

workers to promote anti-TB drug compliance, and consequently reduce the epidemic of TB infection in Thailand. Moreover, these findings support great benefit in establishing TB treatment guidelines of the Myanmar population and support the appropriate pharmaceutical care to reduce the adverse reaction, increase drug compliance and promote successful treatment of TB patients in Myanmar.

5.4 FUTURE STUDY

The present study is the first study to determine the genetic polymorphism of the Myanmar population. Further study is needed to extend in a large population to confirm the association of *NAT2* polymorphism and DILI. Moreover, additional genetic polymorphisms in *CYP2E1* and *GST* gene together with *NAT2* polymorphisms should also be studied.

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controlled trial for pharmacogenetics-based therapy. Eur J Clin Pharmacol. 2013;69(5):1091-1101.10.1007/s00228-012-1429-9

APPENDIX

APPENDIX I

Certificate of Ethics Approval

AF 02-12

The Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University
Jamjuree 1 Building, 2nd Floor, Phyathai Rd., Patumwan district, Bangkok 10330, Thailand,
Tel/Fax: 0-2218-3202 E-mail: eccu@chula.ac.th

COA No. 267/2018

Certificate of Approval

Study Title No. 227.1/61 : ASSOCIATION BETWEEN *NAT2* POLYMORPHISMS AND ANTI-TUBERCULOSIS DRUG-INDUCED LIVER INJURY IN MYANMAR PATIENTS IN THAILAND

Principal Investigator : MISS KHIN SANDI THAW

Place of Proposed Study/Institution : Faculty of Pharmaceutical Sciences, Chulalongkorn University

The Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University, Thailand, has approved constituted in accordance with the International Conference on Harmonization – Good Clinical Practice (ICH-GCP).

Signature: Prida Tasanapradit ..... Signature: Nuntaree Chaichanawongsaroj .....

(Associate Professor Prida Tasanapradit, M.D.) Chairman

(Assistant Professor Nuntaree Chaichanawongsaroj, Ph.D.) Secretary

Date of Approval : 19 November 2018 Approval Expire date : 18 November 2019

The approval documents including

1) Research proposal
2) Patient/Participant Information Sheet and Informed Consent Form
3) Researcher
4) Questionnaire

Protocol No. 227.1/61
Date of Approval 19 NOV 2018
Approval Expire Date 18 NOV 2019

The approved investigator must comply with the following conditions:

1. *The research/project activities must end on the approval expired date of the Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University (RECCU). In case the research/project is unable to complete within that date, the project extension can be applied one month prior to the RECCU approval expired date.*
2. *Strictly conduct the research/project activities as written in the proposal.*
3. *Using only the documents that bearing the RECCU's seal of approval with the subjects/volunteers (including subject information sheet, consent form, invitation letter for project/research participation (if available).*
4. *Report to the RECCU for any serious adverse events within 5 working days*
5. *Report to the RECCU for any change of the research/project activities prior to conduct the activities.*
6. *Final report (AF 03-12) and abstract is required for a one year (or less) research/project and report within 30 days after the completion of the research/project. For thesis, abstract is required and report within 30 days after the completion of the research/project.*
7. *Annual progress report is needed for a two- year (or more) research/project and submit the progress report before the expire date of certificate. After the completion of the research/project processes as No. 6.*

APPENDIX II

Informed Consent Form

Informed Consent Form

Address .....
Date .....

Code number of participant .....

I who have signed here below agree to participate in this research project **Title** "Association between *NAT2* polymorphisms and anti-tuberculosis drug-induced liver injury in Myanmar patients in Thailand"

Principle researcher's name – Ms. Khin Sandi Thaw
Contact address - Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok
Telephone – 099-4211-486, 064-00-51424

I have **(read or been informed)** about rationale and objective(s) of the project, what I will be engaged with in details, risk/harm and benefit of this project. The researcher has explained to me and I **clearly understand with satisfaction.**

I willingly **agree** to participate in this project and *agree to access my medical record from the hospital, then,* consent the researcher to collect venous blood sample for 5 milliliters (mL) or 1 teaspoonful for genotyping procedure. *I willingly agree to give about 10-15 minutes for interview.* All information especially DNA genotyping are confidential.

I have **the right** to withdraw from this research project at any time as I wish with no need to **give any reason**. This withdrawal **will not have any negative impact upon me (*e.g. still receive the usual services*).**

Researcher has guaranteed that procedure(s) acted upon me would be exactly the same as indicated in the information. Any of my personal information will be **kept confidential.** Results of the study will be reported as total picture. Any of personal information which could be able to identify me will not appear in the report.

If I am not treated as indicated in the information sheet, I can report to the Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University (RECCU). Jamjuree 1 Bldg., 2nd Fl., 254 Phyathai Rd., Patumwan district, Bangkok 10330, Thailand, Tel./Fax. 0-2218-3202 E-mail: eccu@chula.ac.th.

I also have received a copy of information sheet and informed consent form.

Sign.....
Researcher

Sign.....
Participant

Sign.....
Witness

Protocol No. 227.1167
Date of Approval 19 NOV 2018
Approval Expire Date 18 NOV 2019

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Informed Consent Form
For parent or guardian

Address.....

Date.....

Code number of participant .....
I who have signed here below is (indicate: father/mother/legal guardian) of (name of participant) ..... agree to participate in this research project
Title "Association between *NAT2* polymorphisms and anti-tuberculosis drug-induced liver injury in Myanmar patients in Thailand"

Principle researcher's name – Ms. Khin Sandi Thaw

Contact address - Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok
Telephone – 099-4211-486, 064-00-51424

I and person under my care have been informed about rational and objective(s) of the project, and what will be done in details upon the person under my care, risk/harm and benefit of this project. I have read details in the information sheet and **clearly understand with satisfaction.**

I willingly **agree** to let the person under my care participate in this project and *agree to access my medical record from the hospital, then,* consent the researcher to collect venous blood sample for 5 milliliters (mL) or 1 teaspoonful for genotyping procedure. *I willingly agree to give about 10-15 minutes for interview.* All information especially DNA genotyping are confidential.

Either the person under my care or I have **the right** to withdraw from this research project at any time as wished, with no need **to give any reason.** This withdrawal **will not have any negative impact upon person under my care or me (e.g.: receive the same usual services).**

Researcher has guaranteed that procedure(s) which will be acted upon the person under my care would be exactly the same as indicated in the information. Any personal information of person under my care will be **kept confidential.** Results of the study will be reported as total picture. Any personal information which could be able to identify person under my care and myself will not appear in the report.

If the person under my care **is not treated as indicated in the information sheet,** I can report to the Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University (RECCU). Jamjuree 1 Bldg., 2nd Fl., 254 Phyathai Rd., Patumwan district, Bangkok 10330, Thailand, Tel./Fax. 0-2218-3202 E-mail: eccu@chula.ac.th.

Protocol No. 227.1/61
Date of Approval 19 NOV 2018
Approval Expire Date 18 NOV 2019

I also have received a copy of information sheet and informed consent form.

Sign .....
Researcher

Sign .....
Participant

Sign .....
Parents or guardian of participant

Sign .....
Witness

Protocol No. 227.1761
Date of Approval 19 NOV 2018
Approval Expire Date 18 NOV 2019

VITA

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