UNIVERSITI TEKNOLOGI MARA

ASSOCIATION OF *N-ACETYLTRANSFERASE 2 (*NAT2*)* POLYMORPHISM WITH ANTITUBERCULOSIS DRUG INDUCED HEPATOTOXICITY AMONG PATIENTS IN MALAYSIA

NUR FARHANA BINTI MOHAMED NOOR

Thesis submitted in fulfillment
of the requirements for the degree of
Master of Science
(Clinical Pharmacy)

Faculty of Pharmacy

April 2022

ABSTRACT

Antituberculosis drug induced hepatotoxicity (ATDIH) is a serious adverse drug reaction. Factors that affect drug responses or increase the risks of adverse drug reactions include both endogenous and exogenous variables. Genetic variants of *N-acetyltransferase-2* (*NAT2*) have been associated with increased susceptibility to ATDIH. However, the findings of previous studies are inconclusive. Moreover, data about NAT2 polymorphism among patients in Malaysia is limited. This study aimed to determine the association of *NAT2* polymorphism with ATDIH. A systematic literature search and meta-analysis using RevMan5.3 were conducted to compute the pooled effect of the studies. The random-effect model was applied to estimate the pooled odd ratio (OR) and 95% confidence interval (CI). The Cochrane Q-statistic and I^2 statistics were used to assess and quantify heterogeneity. Then, a case-control study was conducted. Thirty-three (33) TB patients with ATDIH and 100 TB patients without ATDIH were recruited from tertiary hospitals in Klang Valley, Malaysia. Seven single nucleotide polymorphisms (SNPs) of the *NAT2* gene were determined through polymerase chain reaction and sequencing. The distribution of the genotypes and alleles frequencies between the two groups were compared and analysed using chi-square test. The odds ratio (OR) with 95% CI was used to evaluate the strength of the association between *NAT2* gene polymorphism and ATDIH. Risk factors associated with ATDIH were analysed using multiple regression analysis. A total of 12 studies, involving 580 cases and 3129 controls, were included in the meta-analysis. *NAT2* polymorphism was significantly associated with the risk of ATDIH with an odd ratio (OR) of 2.76 (1.86 – 4.10, 95% CI). Among the slow acetylator genotypes, *NAT2*5/*7* carries the highest risk associated with ATDIH. As for our case control study, the T allele of *NAT2*13A* (rs1041983), A allele of *NAT2*6B* (rs1799930) and *NAT2* slow acetylators (SA) were significantly associated with ATDIH with odd ratios (OR (95% CI) = 2.28 (1.25-4.16), 2.20 (1.25-3.86) and 3.39 (1.43-8.04), respectively. *NAT2*6A/*6A* is significantly associated with ATDIH among the *NAT2* diplotypes, with an OR of 2.67 (1.05-6.79, 95% CI). Underlying diabetes mellitus, high pre-treatment bilirubin, and being a NAT2 slow acetylator are independent risk factors for ATDIH with *p*-value of 0.041, 0.013 and 0.005, respectively. In conclusion, the meta-analysis show that *NAT2* polymorphism is associated with ATDIH. The significant role of *NAT2* polymorphism is further confirmed by present case-control study. Pre-genotyping of *NAT2* polymorphism in patients with high risk of ATDIH before prescribing antituberculosis treatment may reduce the incidence of ATDIH in Malaysia.

Keywords: NAT2, polymorphism, risk factor, antituberculosis, hepatotoxicity, Malaysia.

ACKNOWLEDGEMENT

My deepest praise to Allah S.W.T for giving me the opportunity, strength, patience, and good health to complete this challenging journey successfully and complete my thesis during the pandemic era.

With great sincerity I would like to acknowledge my supervisor, Professor Dr Teh Lay Kek as well as co-supervisors, Professor Dato' Dr Mohd Zaki Salleh and Dr Mohd Arif Mohd Zim. Thank you for your supervision, guidance, knowledge shared, patience and constant encouragement throughout this study. My gratitude also goes to my co-investigator, Dr Zamzurina Abu Bakar and En Muhammad Imran Lainlanor from Institut Perubatan Respiratori Kuala Lumpur; and Dr Noor Izyani Zakaria from Selayang Hospital. I would also like to thank all the medical staff from Institut Perubatan Respiratori Kuala Lumpur and Selayang Hospital for their kind help during samples and data collections.

My appreciation also goes to all the fellow researchers, staff and students from the Integrative Pharmacogenomic Institute (iPROMISE); Dr Fakhruzzaman Noorizhab, Dr Lim Wi Feng, Dr Mohd Salleh Rofiee, Ts Muhammad Azwat Abdullah, Pn Nur Madilawani, Muhammad Faris Fauzi Ismail, Nor Ain Othman, Nurul Ain Khoirudin, Norzuliana Zainal Abidin and Nur Amirah Asyikin Zaihuri. Thank you for the kind help, cooperation and support during the period of my study.

Finally, this thesis is dedicated to my dearest parents, my husband and my children, and other family members for their love and continuous moral support throughout my journey. A special thanks to all my friends for their continuous support.

TABLE OF CONTENTS

CHAPTER ONE
INTRODUCTION

1.1 Research Background

Tuberculosis (TB) infection is known to be caused by *Mycobacterium tuberculosis*, spreading from one person to another through droplets during sneezing and coughing. Globally, it is estimated that 9.9 million people fall ill with TB and 1.4 million of them die due to TB (World Health Organization, 2021). According to the Global TB Report 2020 by World Health Organization (WHO), 44% of the cases were reported from South-East Asian countries. The number of TB cases in Malaysia is relatively stable with 25,837 cases in 2018 and 26,352 cases in 2019. Even though Malaysia is categorized as having an intermediate TB burden, our neighbouring countries such as Indonesia and Thailand, still have a high burden of TB. Hence, the high influx of foreigners and workers from our neighbouring countries is relatively worrisome.

First line TB treatment consists of a combination of four drugs which are pyrazinamide, ethambutol, rifampicin and isoniazid (World Health Organization, 2010). Due to this multidrug regime, antituberculosis is associated with many adverse effects (Yee et al., 2003). Common and major adverse effects of antituberculosis treatment occur in the hepatic system, skin and gastrointestinal tract. These adverse effects have a negative impact on patient compliance, reduce the rate of successful treatment and may eventually cause treatment failure and relapse, and the emergence of drug resistance (Saukkonen et al., 2006).

Being a vital organ for drug metabolism, liver is at risk of injury, especially in multidrug regimes like in TB treatment. In Malaysia, the prevalence of antituberculosis drug induced hepatotoxicity (ATDIH) occurred between 7-9.7% (Cheah et al., 2018; Marzuki et al., 2008). Older individuals, having poor nutritional status or low body mass index (BMI), low albumin level, alcoholics, underlying liver disease, hepatitis B and C carriers, and *N-acetyltransferase-2* (*NAT2*) acetylator status are among the risk factors for ATDIH (Fauzi et al., 2004; Singla et al., 2010; Tostmann et al., 2008; Williams, Cegielski, & Dye, 2010). Patients of Asian ethnicity might be at higher risks of hepatotoxicity (Yee et al., 2003). In Malaysia, the common risk factors in developing