VNU Journal of Science: Medical and Pharmaceutical Sciences, Vol. 35, No. 1 (2019) 81-87

VNU Journal of Science: Medical and Pharmaceutical Sciences

Journal homepage: https://js.vnu.edu.vn/MPS

Original Article

# Drug Resistance and Distribution of *NAT2* Variants in Newly Diagnosed and Recurrent Vietnamese Pulmonary Tuberculosis Patients

Vu Thi Thom[1,*], Le Thi Luyen[1], Le Anh Tuan[1], Pham Thi Hong Nhung[1], Nguyen Thi Thu Ha[2]

[1]*VNU School of Medicine and Pharmacy, 144 Xuan Thuy, Cau Giay, Hanoi, Vietnam*

[2]*National Hospital of Tropical Diseases, 78 Giai Phong, Dong Da, Hanoi, Vietnam*

Received 03 May 2019
Revised 09 May 2019; Accepted 21 June 2019

**Abstract:** Drug resistant TB is currently a global challenge causing high risk of death and expanding the disease. This study explores the prevalence of drug resistance in newly diagnosed and recurrent TB patients and identifies the association between *NAT2* gene polymorphism distribution and acetylator phenotype of *NAT2* gene and the two study groups. The study results show that the newly diagnosed TB had l lower male ratio and younger age in comparison to the recurrent TB. Newly diagnosed group was more sensitive to first line TB drugs. However, both groups had significant resistance ratio in relation to INH and SM. Finally, the allele and acetylator phenotype frequency of *NAT2* showed the significant association with TB status. The study concludes that the newly diagnosed and recurrent TB patients expressed differently in their profiles concerning patient's background, drug resistance and *NAT2* allele distribution.

*Keywords:* Drug resistance, INH, *NAT2* polymorphism, newly diagnosed TB, recurrent TB1.

## 1. Introduction

Tuberculosis (TB) is a highly contagious infectious disease, complex clinical diagnosis and prolonged treatment period. According to WHO statistics (2018), with the continuous efforts of the world, between 2000 and 2018 it is estimated that about 53 million people have been discovered and treated for TB to help reduce the death rate due to falling to 37%. Despite its focus on control, tuberculosis is still one of the top 10

* Corresponding author.
*Email address:* thomtbk5@gmail.com

https://doi.org/10.25073/2588-1132/vnumps.4166

causes of death and the number one cause of death due to infectious disease (in HIV). Vietnam is one of the hot spots of tuberculosis, ranking 14th out of 30 countries with high TB burden (WHO, 2018) [1]. In 2017, the total number of TB cases reported in Vietnam was 105,733 of which about 80% were newly infected and re-infected patients. Although worldwide, the incidence of tuberculosis is decreasing by about 2% per year but so far TB is still a challenge due to the development and spread of drug-resistant TB. Patients with drug-resistant TB often have long, expensive treatment periods. In Vietnam, the average cost of a multidrug-resistant tuberculosis is about $400, much higher than the cost of more than $150 for regular TB patients. According to the latest WHO statistics, 3.5% of new TB cases and 18% of new TB cases are resistant to rifampicin or multiple resistance to rifampicin and isoniazid (MDR/ RR-TB) globally. In Vietnam, this figure is estimated at 4.1% and 17% respectively. However, this is only an estimate because only about 32% - 67% are assessed for resistance to rifampicin (WHO, 2018) [1]. In the first-line anti-TB drugs, in addition to rifampicin and isoniazid, the two most important drugs in TB treatment regimens are streptomycin, ethambutol, pyrazinamide. In recent years, the situation of drug resistance with these three drugs has received little attention. Focusing only on the two most important drugs, rifampicin and isoniazid, can ignore the noticeable changes in drug resistance of these three drugs.

Currently, isoniazid is used in most TB patients by age, sex, in regimens for treatment of new TB patients, tuberculosis treatment and including preventive treatment [2]. Isoniazid is metabolized by *NAT2* enzyme in the liver. Genetic polymorphism of *NAT2* gene is known to be closely related to response to isoniazid in patients with TB. Research in South Africa showed that although more than 98% of patients adhere to treatment, but the situation of drug resistance still ocurs and another study also confirmed that resistance is not only due to non-compliance but also due to other specific pharmacokinetics of drugs between individuals. On the other hand, some studies have shown that a patient's genetic factor may also be one of the risk factors for TB infection [3-6].

## 2. Materials and methods

### *2.1. Study objects*

This study enrolled 125 TB patients with 69 newly diagnosis and 56 recurrent TB patients from 3 hospitals including Vietnam National Lung Hospital, Hanoi Lung Hospital and National 74-Hospital from 2017 to 2018. This process was approved by IRB of School of Medicine and Pharmacy, Vietnam National University Hanoi.

### *2.2. Methods*

#### *2.2.1. Data collection and sampling*

Patient samples and data was collected by the guideline of Ministry of Health, Vietnam for TB. For gene analysis, venous blood was drawn into EDTA containing tubes, frozened and stored at -20$^{o}$C.

#### *2.2.2. NAT2 gene analysis*

DNA from each patient was obtained from venous total blood samples by using E.Z.N.A blood DNA Mini Kit (Omega-Biotek Inc., USA). PCR-RFLP and Sanger's sequencing were applied to determine NAT2 genotype by using a pair of specific primers (5'-GGA ACA AAT TGG ACT TGG-3' and 5'-TCT AGC ATG AAT CAC TCT GC-3'). PCR mixture was composed of 20 ng/µl DNA template, 0.5 µM of each primer (Phusa biochem Inc., Vietnam), Kapa 2G ™ Robust HotStart ReadyMix 2x (Kapa Biosystems Inc., USA). PCR program settings included preheating at 95°C for 3 min, 35 cycle of 95°C for 10s, 57°C for 15s, 72°C for 60s, and then extension at 72°C for 10 min.

#### *2.2.3. Data analysis*

Sequence analysis was performed by a BLAST search in the GenBank database and BioEdit version 7.1.9 software. Data analysis

was performed with SPSS 20.0. Statistical properability p<0.05 was considered as significant difference.

## 3. Results

### *3.1. General data of study population*

Table 3.1 showed that the general data newly diagnosis TB and recurrent TB groups. In study population, male number in comparison to female number was nearly two times in newly diagnosis TB group and approximately four times in recurrent TB group. The age of patients in recurrent TB was higher than that of newly diagnosis TB.

Table 3.1. General information of study population

| **Criteria** | **Pulmonary Tuberculosis Groups** | | |
|---|---|---|---|
| ($\bar{X} \pm SD$) *or (%)* | Newly diagnosis TB (n=69) | Recurrent TB (n=56) | **p** |
| **Gender (male/female)** | 63.8/36.2 | 87.5/12.5 | 0.002 |
| **Age (years)** | 41.11±14.70 | 48.79±13.58 | 0.003 |
| **BMI** | 18.91±*2.25* | 18.65±*2.40* | 0.539 |
| **Bacteria culture period (hours)** | 210.35±10.37 | 250.61±13.65 | 0.018 |
| **Growth unit** | 2285.0±658.57 | 1915.3±748.70 | 0.711 |

Note: BMI: Body Mass Index; $\bar{X} \pm$ SD: mean ± standard deviation

### *3.2. Drug resistance of TB in study population*

In newly diagnosis TB, the ratio of case sensitive to all drugs (71.1%) was two times higher than that of recurrent TB group (39.3%) whereas the ratio of resistant drug in recurrent TB group (60.7%) was nearly three times higher than the newly diagnosis TB group (21.7%), significantly (p<0.05). The results were presented in table 3.2. In aspect to each first line TB drug, the resistance ratio of two groups newly diagnosis and recurrent TB were only significant difference with isoniazid and streptomycin treatment with p=0.001 and p=0.000, respectively.

Table 3.2. Drug resistant ratio in newly diagnosis and recurrent TB

| **Drug resistance (n, %)** | | **Pulmonary Tuberculosis Groups** | | **Statistic tests** |
|---|---|---|---|---|
| | | Newly diagnosis TB (n=69) | Recurrent TB (n=69) | |
| **Sensitive to all drugs** | | 54 (71.1%) | 22 (39.3%) | **$\chi$2=20.931** |
| **Resistant to at least one drug** | | 15 (21.7%) | 34 (60.7%) | p=0.000 |
| **INH** | Sensitive | 58 (84.1%) | 32 (57.1%) | **$\chi$2=11.108** |
| | Resistant | 11 (15.9%) | 24 (42.9%) | p=0.001 |
| **RIF** | Sensitive | 68 (98.6%) | 51 (91.1%) | $\chi$2=3.784 |
| | Resistant | 1 (1.4%) | 5 (8.9%) | p=0.089 |
| **SM** | Sensitive | 60 (87.0%) | 31 (72.8%) | **$\chi$2=15.588** |
| | Resistant | 9 (13.0%) | 25 (44.6%) | p=0.000 |
| **EMB** | Sensitive | 68 (98.6%) | 51 (91.1) | $\chi$2=3.784 |
| | Resistant | 1 (1.4%) | 5 (8.9%) | p=0.089 |
| **PZA** | Sensitive | 68 (98.6%) | 52 (92.9%) | $\chi$2=2.610 |
| | Resistant | 1 (1.4%) | 4 (7.1%) | p=0.172 |
| **MDR(*)** | Sensitive | 68 (98.6%) | 51(91.1%) | $\chi$2=3.784 |
| | Resistant | 1 (1.4%) | 5 (8.9%) | p=0.089 |

Note: INH: isoniazid; RIF: rifampicin; SM: streptomycin; EMB: ethambutol, PZA: pyrazinamide; MDR(*): Resistant to at least RIF and INH

### *3.3. NAT2 polymorphism and acetylation genotype in newly diagnosis and recurrent TB groups*

Table 3.3. *NAT2* allele frequency in newly diagnosis and recurrent TB groups

| ***NAT2* allele** | **Allele frequency** | | |
|---|---|---|---|
| | **Total (n=125*2)** | Newly diagnosis TB (n=69*2) | **Recurrent TB (n=56*2)** |
| ***4** | 116 (0.464) | 73 (0.529) | 43 (0.384) |
| ***5** | 16 (0.064) | 10 (0.072) | 6 (0.054) |
| ***6** | 78 (0.312) | 38 (0.276) | 40 (0.357) |
| ***7** | 40 (0.160) | 17 (0.123) | 23 (0.205) |
| | Statistic test | P=0.069 | |
| | **Total** | Newly diagnosis TB | **Recurrent TB** |
| **Wildtype allele** | 116 (0.464) | 73 (0.529) | 43 (0.384) |
| **Variant allele** | 134 (0.536) | 65 (0.471) | 69 (0.616) |
| | Statistic test | P=0.022 | |

Table 3.4. *NAT2* genotype frequency in newly diagnosis and recurrent TB groups

| ***NAT2* genotype** | **Genotype frequency** | | |
|---|---|---|---|
| | **Total (n=125)** | Newly diagnosis TB (n=69) | **Recurrent TB (n=56)** |
| ***4*4** | 34 (27.2%) | 25 (36.2%) | 9 (16.1%) |
| ***4*5** | 7 (5.6%) | 5 (7.2%) | 2 (3.6%) |
| ***4*6** | 28 (22.4%) | 12 (17.4%) | 16 (28.6%) |
| ***4*7** | 13 (10.4%) | 6 (8.7%) | 7 (12.5%) |
| ***5*5** | 1 (1.4%) | 0 (0%) | 1 (0.8%) |
| ***6*6** | 15 (12%) | 8 (11.6%) | 7 (12.5%) |
| ***7*7** | 5 (4.0%) | 1 (1.4%) | 4 (7.1%) |
| ***5*6** | 5 (4.0%) | 2 (2.9%) | 3 (5.4%) |
| ***5*7** | 2 (1.6%) | 1 (1.4%) | 1 (1.8%) |
| ***6*7** | 15 (12.0%) | 8 (11.6%) | 7 (12.5%) |
| | **Statistic test** | P=0.206 | |

When observed the significant difference in allele frequency between two groups, we performed the *NAT2* acetylation phenotype analysis. In general, recurrent TB group contained most of intermediate and slow *NAT2* acetylator (83.9%). Whereas, in newly diagnosis TB group, the *NAT2* rapid acetylator for INH was more abundant with 36.2% while in recurrent TB group it was only 16.1%. This reached the statistically significant difference presented in table 3.5 (p=0.042).

Table 3.5. Association between *NAT2* phenotype and INH resistance in TB groups

| ***NAT2* acetylation phenotype** | **TB group** | | **Total** |
|---|---|---|---|
| | Newly diagnosis TB (n=69) | **Recurrent TB (n=56)** | |
| **Rapid** | 25 (36.2%) | 9 (16.1%) | **34 (27.2%)** |
| **Intermediate** | 23 (33.3%) | 25 (44.6%) | **48 (38.4%)** |
| **Slow** | 21 (30.4%) | 22 (39.3%) | 43 (34.4%) |
| **Statistic test** | **$\chi$2=6.353; p=0.042** | | |

## 4. Discussion

According to the WHO report (2018), around 6 million men have tuberculosis worldwide, while only about 3.2 million women have TB. In Vietnam, in 2006-2007, the National Tuberculosis Program conducted a nationwide tuberculosis investigation revealed that the prevalence of male TB is 4-5 times higher than for women [7]. In our study, the ratio of men/women was 2.9, significantly higher in some other domestic studies at 2.4 times [8]. This can be explained by the fact that the study is limited to patients with pulmonary tuberculosis, the number of studies is not large enough and because of recent years the development of socio-economic conditions - people's awareness increases muscle Women's access to health systems. The male/female disparity in the new tuberculosis group is 1.76, approximately equal to 1.92 in the study of Hoang Thi Phuong (2009) [2]. The ratio of male to female in the pulmonary tuberculosis re-treatment group of the study was 7.0 higher than in the Hoang Ha study (4.0) (2009) [7]. The reason may be that men often do not have the patience to follow the treatment process, along with high risk factors such as smoking, alcoholism so often leads to relapse.

The average age in the study of the pulmonary tuberculosis re-treatment group (48.79 years) was higher than that of the new pulmonary tuberculosis group (41.11 years). The difference in average age between the two groups was statistically significant ($p < 0.05$). This reflects the relationship between age and new TB status lower than remission. The difference between the two groups may be because in older people the general resistance status of the body is inferior to that of young people.

For anti-tuberculosis drugs, isoniazid, rifampicin, streptomycin, ethambutol, and pyrazinamide after more than half a century have been used, the most resistant strains of bacteria are found in different levels. Since 1997, the National Tuberculosis Program has conducted four surveys of the national rate of anti-tuberculosis resistance. In 2005-2006, the third national drug resistance survey, general drug resistance was 30.9%; in re-treatment TB, the rate of drug-resistant TB was 58.9%; multi-drug resistance is 19.3%; resistance to isoniazid 43.5% and streptomycin is 50.7%; and multi-drug resistance 2.7% in new TB [9]. According to research by Hoang Thi Phuong (2009), there are 31.6% of patients resistant to any drug (56/177). Thus, the overall drug resistance rate of the study is 39.2%, which tends to be higher than the national rate [2]. This may be because the patients in this study were patients at three hospitals in Hanoi area, where the population is concentrated, and are the facilities that treat patients with drug-resistant TB. This is also a noticeable sign in the national tuberculosis prevention action program, to take measures to minimize the spread of tuberculosis, especially drug-resistant TB.

The fourth drug resistance survey of the national tuberculosis prevention program (2011-2012), the overall rate of multi-drug resistance is 4%, the re-treatment group is 23.3% [9]. And according to the latest WHO report (2018) in 2017, the estimated multi-drug resistance rate in Vietnam in new patients is 4.1% and relapse is 17%. Thus, the multidrug resistance rate in the re-treatment TB group in this study is much lower than the rate of the fourth national drug resistance survey as well as the latest WHO report (2018) and some studies in other countries [1].

Among the first five anti-tuberculosis drugs, the highest rate of drug resistance was streptomycin (44.6%) and followed by isoniazid (42.9%) on the recurrent tuberculosis group. From 1996-1997, when Vietnam conducted the first nationwide drug resistance survey, the rate of isoniazid resistance was 20%; streptomycin resistance is 24%. By the fourth survey in 2011-2012, this rate in re-treatment patients was 43.5% of isoniazid and 50.7% of streptomycin resistance [9]. Thus, the rate of streptomycin and isoniazid resistance in the re-treatment patients of the study is lower than the recent drug resistance survey and some other domestic

studies (streptomycin resistance is 79.5%, isoniazid resistance is 82.2%). [10] but higher than foreign authors [11]. The rate of isoniazid in the pulmonary tuberculosis re-treatment group of the study is lower than many other domestic studies [7]. This may be the result of efforts to reduce drug resistance in the national TB program. But the rate of resistance of isoniazid is still higher and higher than that of the world [11], and there is a much higher rate between re-tuberculosis and new pulmonary tuberculosis. Therefore, it is necessary to have strict monitoring measures in the use of isoniazid treatment, to ensure that isoniazid is still a good source of medicine for TB treatment and TB prevention.

The distribution frequency of a combination of 10 genotypes between the new TB group and the re-treatment TB was not different (p = 0.206). The proportion of wild type homozygous genotypes accounts for the largest proportion, followed by alleles in the combination of alleles * 4 and alleles * 6. The frequency of allele distribution and the proportion of *NAT2* genotypes is also studied in many human populations around the world. Many studies have shown that, in Europeans, Europeans, Indians, Omanis, Moroccans, the frequency of allele *NAT2* * 5 is large, alleles * 4, * 6 and * 7 occupy small percentage [3]. For example, in the Moroccan population in the study of Guaoua et al. (2014), the allele distribution frequency * 5 accounted for 53% and genotype combinations of * 5 such as * 5 * 5, * 4 * 5, * 5 * 6 accounts for nearly 70%, this is completely different from the allele distribution and *NAT2* genotype in the Vietnamese patient population in this study [3]. The study of Toure et al (2016) on Senegan tuberculosis patient populations (Africa) shows the presence of polymorphs * 4, * 5, * 6, * 7, * 12, * 14 in which frequency Allele distribution * 5, * 6 accounted for the highest proportion (26.6%) [5]. Studies on Asian populations such as Korean and Japanese show a much lower ratio * 5, * 6, * 7 more. However, in Japanese mainly * 6, * 7 accounts for a very low rate, while in Korean people the allele ratio of * 6 and * 7 are similar to our results.

We see the acetylation of *NAT2* phenotype in populations of Europe, Africa and Asia in general are similar [5-6] In these populations, mainly isoniazid metabolic phenotypes slow and medium, fast metabolic phenotype accounts for less than 20%. In the Americas, the average metabolic phenotype is the majority, the rate of metabolism is fast and the same is similar. In contrast, in the North Asian population, the rate of rapid and average metabolic phenotypes accounts for over 80%, few have slow metabolic phenotype [8]. A study on Thai patients with anti-tuberculosis resistance and liver damage showed that 71.7% had a slow metabolic phenotype, 22.6% had a moderate metabolic phenotype and 5.7% had a transfer pattern. rapid metabolism, while slow, medium, and fast *NAT2* metabolic phenotype in the control group without liver damage was 22.4%; 62.4% and 15.3% [8]. Similar to the study of Thailand, the phenylation phenotype in the Vietnamese TB population in this study is mainly the medium and slow metabolic phenotype (> 70%), below 30% with the rate rapid metabolic phenotype. In the new tuberculosis group, there was a uniform distribution between the acetylated *NAT2* phenotypes, but in the tuberculosis regimen, the rapid acetylation phenotype was significantly lower than the other two groups (16%) and similar to the distribution in Asians in general and lower than North Asian populations (40%) [3].

## 5. Conclusion

The overall rate of drug resistance for first-line anti-TB drugs is relatively high at 39.2%. The rate of Streptomycin and Isoniazid resistance is high, especially in the recurrent pulmonary group. *NAT2* allele distribution and *NAT2* acetylator phenotype in newly diagnosis and recurrent TB were significantly difference.

## Acknowledgements

This study was supported by a grant from the Vietnam Ministry of Science and Technology,

and Newton Fund Vietnam (Grant Number HNQT/SPĐP/01.06).

## References

[1] WHO, Global Tuberculossi report, https://www.who.int/tb/publications/global_report/en/, 2018 (accessed 16 April 2019).

[2] Hoàng Thị Phượng, Nghiên cứu đặc điểm lâm sàng, cận lâm sàng, tính kháng thuốc của vi khuẩn ở bệnh nhân lao phổi mới kết hợp bệnh đái tháo đường, Luận văn tiến sĩ Y học, trường Đại học Y Hà Nội, 2009.

[3] S. Guaoua, I. Ratbi, F.Z. Laarabi, S.A. Elalaoui, IC. Jaouad, A. Barkat, A. Sefiani, Distribution of allelic and genotypic frequencies of NAT2 and CYP2E1 variants in Moroccan population, BMC Genet. 15 (2014) 156.

[4] A. Toure, M. Cabral, A. Niang, C. Diop, A. Garat, L. Humbert, M. Fall, A. Diouf, F. Broly, M. Lhermitte, D. Allorge, Prevention of isoniazid toxicity by NAT2 genotyping in Senegalese tuberculosis patients, Toxicol Rep. 3 (2016) 826-831.

[5] M. Majumder, N. Sikdar, S. Ghosh, B. Roy, Polymorphisms at XPD and XRCC1 DNA repair loci and increased risk of oral leukoplakia and cancer among NAT2 slow acetylators, Int J Cancer. 120(10) (2007) 2148-2156.

[6] S. Morita, M. Yano, T. Tsujinaka, Y. Akiyama, M. Taniguchi, K. Kaneko, H. Miki, T. Fujii, K. Yoshino, H. Kusuoka, M. Monden, Genetic polymorphisms of drug-metabolizing enzymes and susceptibility to head-and-neck squamous-cell carcinoma, Int J Cancer. 80(5) (1999) 685-688.

[7] Hoàng Hà, Nghiên cứu một số đặc điểm lâm sàng, cận lâm sàng, sinh học của vi khuẩn ở bệnh nhân lao phổi điều trị lại, Luận án tiến sỹ Y học, Trường Đại học Y Hà Nội, 2009.

[8] S. Wattanapokayakit, T. Mushiroda, H. Yanai, N. Wichukchinda, C. Chuchottawon, S. Nedsuwan, A. Rojanawiwat, S. Denjanta, T. Kantima, J. Wongyai, W. Suwankesawong, W. Rungapiromnan, R. Kidkeukarun, W. Bamrungram, A. Chaiwong, S. Suvichapanich, S. Mahasirimongkol, K. Tokunaga, NAT2 slow acetylator associated with anti-tuberculosis drug-induced liver injury in Thai patients, Int J Tuberc Lung Dis. 20(10) (2016) 1364-1369.

[9] Đinh Ngọc Sỹ, Chiến lược quản lý bệnh lao đa kháng thuốc tại Việt Nam, Tạp chí khoa học Hội Phổi Pháp - Việt. 2(3) (2011) 40-42.

[10] Nguyễn Thu Hà, Trần Văn Sáng, Đinh Ngọc Sỹ, Lâm sàng, cận lâm sàng và tính kháng thuốc của vi khuẩn lao ở bệnh nhân lao phổi tái phát, JFran Viet Pneu. 2(3) (2011) 63-67.

[11] D. Tu, L. Zhang, J. Su, Resistance and efficacy of treatment in relapse pulmonary tuberculosis, Zhonghua Jie He He Hu Xi Za Zhi. 23 (11) (2000) 666-668.