N- acetyltransferase2 Genotype and its Association with Hepatitis among Filipino Children Treated with First Line Anti-Tuberculosis Drugs

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ABSTRACT

Rationale. Among the first line antituberculosis (anti-TB) drugs, the major drug incriminated in the development of hepatotoxicity is isoniazid (INH). The human N-acetyl transferase2 (NAT2) gene is mainly responsible for INH metabolism. This gene exhibits a hereditarily determined polymorphism. There is presently no study on the predominant NAT2 genotype among Filipinos. There are also no Filipino studies on the incidence of hepatitis and other adverse effects of first line anti-TB drugs.

Objectives. To determine the predominant NAT2 genotype and its association with the development of hepatitis among Filipino children given first line anti-TB drugs (INH, rifampicin and pyrazinamide) and to determine the incidence of hepatitis and other serious adverse reactions to these drugs.

Study Design. Prospective cohort study

Setting. Tertiary government hospital in Metro Manila

Study Population. Children one to 18 years old with pulmonary tuberculosis and normal liver function tests at baseline

Methods. Total bilirubin (TB), direct bilirubin (DB) and liver transaminases (AST and ALT) were checked routinely at baseline and at two, four, eight and 12 weeks after starting treatment. Within the first month of treatment, blood was also taken for NAT2 genotyping. The identification of the three NAT2 polymorphisms that are associated with a slow acetylator status—481C to T (NAT2*5), 590G to A (NAT2*6) and 857G to A (NAT2*7) was carried out by polymerase chain reaction-restriction fragment length polymorphism. All patients were followed up for a total of six months. The presence of any adverse effects like gastrointestinal symptoms, rash, hepatitis or drug fever was also monitored.

Results. A total of 24 children [mean age: 5 years; 11 males] were included. Majority (96%) were diagnosed by passive detection and mean Z score for growth was –1.38 (1 to –3). No patient developed hepatotoxicity or any side effects to anti-TB drugs. In 23 patients who had NAT2 genotyping, 39% and 22% were alleles homozygous for the NAT2*6 and NAT2*7, respectively. There was a combination of alleles in only three (13%) subjects.

Conclusion. NAT2*6 and NAT2*7 alleles associated with a slow acetylator status were detected among our patients although the presence of these variants did not lead to any hepatotoxicity nor any treatment-related side effects. A larger study with broader genotype analysis is needed to confirm the present findings.

Key Words: Isoniazid, hepatotoxicity, NAT2 genotype, Filipinos

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Introduction

Tuberculosis (TB) is a major health problem in the Philippines, being the sixth cause of morbidity and sixth cause of mortality. In 2007, the prevalence of radiographic pulmonary tuberculosis was 6.3% and bacteriologically confirmed PTB was 6.6 per 1000.[1]

As a result of the increased incidence of the disease, a greater number of patients are exposed to the risk of potentially serious hepatotoxic effects from antituberculosis drugs. Regimens containing isoniazid (INH), rifampicin (RMP) and pyrazinamide (PZA) are traditionally used as first-line treatment of hilar adenopathy and pulmonary disease caused by drug-susceptible *M. tuberculosis*. This three-drug regimen has been shown to have up to 95% success rate in the treatment of the disease.[2] The incidence of hepatotoxicity is typically from two to 36%[2,3] and the major drug incriminated is INH, although the risk increases when INH is combined with RMP and PZA.[4,5] Hepatitis due to intake of first-line antituberculosis drugs usually occurs in the first three months of treatment.[6] Other adverse effects of first-line antituberculosis drugs that may result in discontinuation of one or more drugs include gastrointestinal upset (nausea, vomiting, abdominal pain and anorexia), rash and drug fever.[6,7]

The development of hepatotoxicity from INH depends on whether the patient is a slow or fast (rapid) acetylator, regardless of the age of the patient.[8] There are two methods

of determining the INH acetylation status, either by phenotyping or genotyping. In a recently concluded study, the acetylator status was assessed based on the NAT2 genotype of 129 healthy Filipinos and 88% were rapid or intermediate acetylators (unpublished data). Similarly, 93% of Filipino TB patients were considered rapid acetylators based on an arbitrary INH plasma level <2.5 μg/ml.[9] Confounding factors that may affect INH levels, such as nutritional status, alcohol consumption, concomitant use of other hepatotoxic drugs and co-morbid illnesses were not considered. Determination therefore of acetylator status by genotyping of the human N-acetyltransferase2 (NAT2) gene may alleviate some of these concerns.

The human NAT2 gene, which is mainly responsible for INH metabolism, has an open reading frame of 870 bp, and exhibits several polymorphisms.[10] The NAT2*4 wild type allele codes for fast acetylation while several polymorphic variants including NAT2*5, NAT2*6 and NAT2*7 have been demonstrated in slow acetylators.[11] Among Japanese patients, six of 102 (6%) patients who developed adverse drug reactions to INH had the NAT2*5, NAT2*6 and NAT2*7 alleles.[10,12] These findings suggest that knowledge of the NAT2 genotype of a population is important to monitor and optimize treatment with INH.

The NAT2 genotype among Filipinos has not been investigated. Furthermore, although it is generally accepted that Filipinos are fast acetylators based on the INH phenotype study by Maramba et al.,[9] there is presently no Filipino study on the incidence of hepatotoxicity from first-line antituberculosis drugs. It is estimated that 10-15% of patients on first-line antituberculosis drugs in the Philippines develop hepatotoxicity based on aminotransferase levels elevated to at least three times normal levels with or without symptoms of jaundice, malaise, anorexia or fever.

This study determined the predominant NAT2 genotype and its association with the development of hepatitis among Filipino children with pulmonary tuberculosis in whom INH, RMP and PZA were prescribed. It also determined the incidence of hepatitis and other serious adverse effects with the use of first-line anti-TB drugs.

Methods

This was a prospective cohort study that was conducted at the Department of Pediatrics of the University of the Philippines College of Medicine Philippine General Hospital and the Institute of Human Genetics of the National Institutes of Health, University of the Philippines Manila. The approval of the ethics committee of the Institution was obtained.

Inclusion and exclusion criteria. Included were children one to 18 years old diagnosed to have pulmonary tuberculosis and with normal baseline total and direct bilirubin, AST and ALT levels. The diagnosis of pulmonary tuberculosis was based on the presence of three or more of the following[13]: (a) exposure to a patient with tuberculosis; (b) positive tuberculin skin test using 5 TU defined as an induration of ≥10mm regardless of BCG status or an induration of ≥5 mm in the presence of close contact with a known case of TB or chest x-ray findings or clinical findings suggestive of TB; (c) chest radiograph suggestive of pulmonary tuberculosis; (d) signs or symptoms suggestive of PTB including: cough or wheezing >2 weeks, unexplained fever >2 weeks, loss of weight or failure to gain weight or weight faltering after a viral infection or exanthema. The biological demonstration of TB bacilli in the smear/culture makes a confirmatory diagnosis of TB in children regardless of other criteria.

Children with extrapulmonary or disseminated tuberculosis were excluded as they may be on other drugs that are also potentially hepatotoxic. Patients with signs of chronic liver disease (hepatomegaly, splenomegaly, facial telangiectasia, palmar erythema and clubbing) and those with a previous history of allergy or adverse drug reactions to anti-TB drugs were also excluded.

Conduct of investigation. After informed consent was obtained, the antituberculosis drugs were given as follows: INH at 10-15 mg/kg/day (maximum of 300 mg), RMP at 10-15 mg/kg/day (maximum of 600 mg/day) and PZA at 20-30 mg/kg/day (maximum 2 gms/day). The patients were advised to take the drugs 30 minutes before meals. INH, RMP and PZA were given for two months during the intensive phase of the disease followed by INH and RMP for another four months as maintenance, for a total of six months duration.

Total bilirubin, direct bilirubin, and liver transaminases (AST and ALT) were checked at baseline and routinely at two, four, eight and 12 weeks after starting treatment. Within the first month after treatment, blood was also taken for NAT2 gene genotype.

All patients were followed up for a total of six months. On the day of follow-up, patients were asked to take the drug in front of the research assistant. During each weekly follow-up, the empty bottles of anti-tuberculosis drug were inspected and a patient was judged as compliant if >80% of the recommended doses have been given.

The presence of any adverse effects like jaundice, hepatitis (≥3 times elevation in the AST/ALT levels), gastrointestinal symptoms (anorexia, nausea, vomiting and abdominal pain), rash, or drug fever was monitored.

Liver function tests. All determinations of total bilirubin (TB), direct bilirubin (DB), aspartate transaminase (AST) and alanine transaminase (ALT) were done at the Department of Laboratories of the Philippine General Hospital using standard techniques.

NAT2 genotype. Peripheral blood from patients was collected in an EDTA tube and was immediately submitted to the Institute of Human Genetics for genomic DNA

extraction. Polymerase chain reaction-restriction fragment length polymorphism was used to identify substitutions at C481T (NAT2*5), G590A (NAT2*6) and G857A (NAT2*7). PCR reaction was carried out in a 50 µl volume containing 300 ng of genomic DNA, 200 µM each of NTP, 100 nM each of forward primer (5'AGATGTGGCAGCCTCTAGAA-3') and reverse primer (5'-ATTAGTGAGTTGGGTGATAC-3'), 10 mM Tris HCl (pH 8.3), 1.5 mM $MgCl_2$ and 2.5 U AmpliTaq DNA. The PCR cycling condition consisted of an initial denaturation step of 5 min at 95°C, 35 cycles of 1 min 94°C, 1 min 56°C and 1 min 72°C followed by final extension period of 7 min 72°C.

Sample size estimate. In a previous study, NAT2*5, NAT2*6 and NAT2*7 genotypes were associated with a 6% incidence of adverse drug reactions with INH intake.[10] Using this estimate, based on a level of significance of alpha=0.05 and a desirable width difference of 0.05, the estimated sample size is at least 87 patients. If we assume a 10% withdrawal or lost to follow up, the total number of patients that have to be recruited is 96 patients.

Results

Twenty four patients were investigated with a mean age of five years that ranged from one to 11 years. Eleven were boys. Majority (96%) of them were detected because they were already symptomatic. All except one patient had normal z scores for nutritional status of the weight for height with a mean z score of –1.38 (+1 to –3). The patient with severe malnutrition had a BMI z score = –3. One patient was lost to follow-up from the second week of the study.

Table 1 shows the baseline total and direct bilirubin and transaminase levels. The values were normal at baseline and remained within normal during the subsequent monitoring from the second to 12th week when patients were taking the anti-TB meds. No patient developed hepatotoxicity or any adverse effects from the drug. Improvement in signs and symptoms were also noted in all patients after three months of drug intake.

Table 1. Mean total and direct bilirubin and transaminase levels at baseline and follow-up

	Baseline (n=24)	2nd week (n=23)	4th week (n=23)	8th week (n=23)	12th week (n=23)
Total bilirubin (µmol/L)	0.23	0.23	0.30	0.30	0.30
Direct bilirubin (µmol/L)	0.06	0.06	0.10	0.10	0.10
Alanine transaminase (IU/L)	35.9	38.8	39.5	40.0	39.4
Aspartate transaminase (IU/L)	32.0	31.0	35.2	34.9	35.0

Normal values: *Total bilirubin: 0.2-1.2; direct bilirubin: 0.05-0.5; Alanine transaminase: 0-41; Aspartate transaminase: 0-38*

Of the 23 patients who had NAT2 genotyping (Table 2), results showed nucleotide change from guanine to adenine at position 590 of NAT2*6 and at position 857 of NAT2*7 in 39% and 22% of cases, respectively. There was a combination of these alleles in only three (13%) subjects.

Table 2. NAT2 genotype in 23 Filipino children treated with first line anti-tuberculosis drugs, n(%)

C481T		G590A		G857A	
C/C	23 (100)	G/G	8 (35)	A/A	1 (4)
C/T	0	A/G	9 (39)	A/G	5 (22)
T/T	0	A/A	6 (26)	G/G	17 (74)

Discussion

The present pilot study showed that majority of Filipino children had the slow acetylator alleles for isoniazid (INH) metabolism. This was demonstrated by the presence of nucleotide changes from guanine to adenine at position 590 of NAT2*6 and at position 857 of NAT2*7. However, the presence of these alleles, leading to a slow acetylator status, did not cause any hepatotoxicity or any adverse reactions to INH.

The development of hepatotoxicity from INH depends on whether the patient is a slow or fast (rapid) acetylator, regardless of the age of the patient.[8] INH is first acetylated by hepatic N-acetyltransferase (NAT) to produce a metabolite, acetylisoniazid, which is rapidly hydrolyzed to monoacetylhydrazine. The latter is oxidized by cytochrome P4502E1 to form intermediate hepatotoxic metabolites, such as acetyldiazine, acetylonium ions, acetylradical and ketone. Monoacetylhydrazine further undergoes acetylation by NAT to form a non-toxic metabolite, diacetylhydrazine. In patients who are slow acetylators, the rate of acetylation is slower not only with the parent component but also with monoacetylhydrazine, the precursor to the toxic intermediates.

It is important to know the predominant NAT2 genotype for INH metabolism among Filipinos, considering that discrepancies between phenotyping and genotyping exist. Several studies have shown differences in NAT2 activity within each genotypic group and possible variability in NAT2 activity may exist when phenotyping is performed. In the only study conducted in a referral hospital for tuberculosis in the Philippines, the INH phenotype was determined after an initial INH dose of 10 mg/kg/day. The INH plasma level on the sixth hour was measured by spectrophotometry and 93% were considered rapid acetylators on the basis of INH plasma levels. It has been shown, however, that there is no significant association between adverse reactions and plasma concentrations of INH, as the final chemical structure(s) causing hepatotoxicity is (are) not INH. Confounding factors that may affect INH levels, such as nutritional status, alcohol

consumption, concomitant use of other hepatotoxic drugs and co-morbid illnesses were also not considered in this study. Neither was there any statement regarding standardization of the INH phenotyping method, calibration of the equipment utilized and presence, if any, of an inter- or intra-observer variability in the interpretation of results. Day-to-day intra-individual variation in acetylation activity may also affect the reliability of the phenotype.

For screening, we used several polymorphisms for NAT2, namely, NAT2*5, NAT2*6 and NAT2*7. These alleles have been reported in slow acetylators among Asians and have been associated with the development of isoniazid (400 mg/day) and rifampicin (450 mg/day) induced hepatotoxicity.[11] Ohno et al.[12] reported that out of 77 Japanese patients who were screened, seven with a combination of these polymorphic variants developed an elevation of the serum ALT or AST levels, defined as more than 1.5 times the upper limit of normal. Three of the seven stopped their INH intake while four continued the regimen, with one having transaminase levels elevated to more than five times the upper limit of normal. In another study, among 102 Japanese patients on INH (300 mg/day) for tuberculosis, five of six with NAT2*6 and NAT2*7 alleles developed adverse reactions while on intake of the drug twice per week for more than two weeks. Adverse reactions including nausea/vomiting, fever (temperature >38ºC), visual impairment, peripheral neuritis and elevated serum amino transaminase activity following initiation of INH therapy were observed, which disappeared after INH discontinuation. Similarly, the NAT2*5A, NAT2*5B and NAT2*6A were demonstrated among healthy Caucasian volunteers to be associated with decreased activity of the NAT2 enzyme and a higher plasma concentration of the INH.

It is interesting to note that despite the presence of the NAT2*6 and NAT2*7 polymorphic alleles in 39% and 22% of our patients respectively, we did not observe any hepatotoxicity nor any adverse reactions to INH. It is possible that our patients also possess highly active NAT2 alleles, such as the NAT2*4 that offsets the slow acetylator. Carriers of NAT2*4/4 genotype (wild type) have been reported to have higher acetylisoniazid to isoniazid ratios than those with other NAT2 genotypes; thus, their elimination half lives of the drug is shorter and they have lower serum INH concentrations. The NAT2 enzyme is also dependent on the concentration of the substrate and it is likely that at 10-15 mg/kg/day, the standard dose given to Filipino children, we are prescribing the optimum INH therapy that will provide maximum benefit with minimal risk.

The small number of patients that were investigated and the limited genotype analysis that we were able to perform does not allow us to make a definitive conclusion. The sample size should be increased and further researches should be done to include the reported variants for rapid acetylators, such as the NAT2*4 and NAT2*11. Other genetic polymorphism that confers the acetylator status should also be explored. An analysis of the acetylhydrazine and INH plasma levels should also be simultaneously performed so that the genotype–phenotype association among Filipinos could be established.

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