Determining the relation between N-acetyltransferase-2 acetylator phenotype and antituberculosis drug induced hepatitis by molecular biologic tests

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ÖZET

N-asetiltransferaz-2 asetilatör fenotipi ile antitüberküloz ilaçlara bağlı hepatit arasındaki ilişkinin moleküler biyolojik yöntemler ile değerlendirilmesi

*Tüberküloz uzun süre kullanılması gereken ilaç kombinasyonlarıyla tedavi edilmekte ve izoniazid bu grup içerisindeki ana ilaçlar arasında yer almaktadır. Antitüberküloz ilaç kaynaklı hepatit, tüberküloz tedavisi sırasında karşılaşılan ciddi bir problemdir. N-asetiltransferaz-2 (NAT2), izoniazidi karaciğerde metabolize eden enzimdir ve bu yüzden hepatotoksisite gelişmesinde de etkisi olduğu düşünülmektedir. Ancak polimorfik NAT asetilasyon fenotipi ve antitüberküloz ilaç kaynaklı hepatit arasındaki ilişki hala tartışılmaktadır. Çalışmamızda, asetilatör durumunun antitüberküloz ilaç kaynaklı hepatit için risk faktörü olup olmadığının belirlenmesi amacıyla tüberküloz tanısı konan 100 olguda NAT2*5A, NAT2*6A, NAT2*7A/B ve NAT2*14A polimorfizmleri analiz edildi. Olguların 70'i tedavi sırasında hepatotoksisite gelişmeyen hastalardan seçildi ve kontrol grubu olarak hepatotoksisite gelişen olgulardan seçilen 30 hasta ise çalışma grubu olarak sınıflandırıldı. NAT2 polimorfizm sonuçlarına göre olgular üç fenotipik gruba ayrıldı. Kontrol grubunu oluşturan 70 olgunun 14 (%20)'ü hızlı, 37 (%52.9)'si orta, 19 (%27.10)'u yavaş; çalışma grubunu oluşturan 30 olgunun ise 3 (%10)'ü hızlı, 4 (%13.3)'ü orta ve 23 (%76.7)'ü yavaş asetilatör olarak belirlendi. Kontrol ve çalışma grupları asetilatör fenotiplerine göre karşılaştırıldığında aralarındaki farkın istatistiksel olarak anlamlı olduğu, yavaş asetilatör fenotipinin çalışma grubunda daha sık görüldüğü gözlendi. Sonuç olarak, moleküler biyolojik yöntemler kullanılarak NAT2 asetilatör fenotiplerinin analiz edilmesinin, tüberküloz tedavisi sırasında hepatotoksisite gelişebilecek olguların önceden saptanması ve tedavileri sırasında daha dikkatli takip edilmeleri konularında yarar sağlayacağı belirlendi.*

Anahtar Kelimeler: *N-asetiltransferaz-2, tüberküloz tedavisi, antitüberküloz ilaç kaynaklı hepatit, asetilatör fenotipi.*

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SUMMARY

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*Tuberculosis is treated with a group of drugs that need to be used over a long period of time and isoniazid is the major drug in this group. Antituberculosis drug-induced hepatitis is the most serious problem in tuberculosis treatment. The enzyme N-acetyltransferase-2 (NAT-2) metabolizes isoniazid in the liver so it is considered to cause hepatotoxicity. The association of polymorphic NAT acetylator status and antituberculosis drug-induced hepatitis is discussed. To determine whether acetylator status is a risk factor for antituberculosis drug-induced hepatitis, we genotyped NAT2*5A, NAT2*6A, NAT2*7A/B and NAT2*14A polymorphisms in 100 patients diagnosed with tuberculosis. 70 patients who did not develop hepatotoxicity were classified as the control group, and 30 patients who were diagnosed with antituberculosis drug-induced hepatitis were classified as the study group. NAT2 polymorphisms were divided into three phenotypic groups according to the analytical results obtained. Among the 70 patients constituting the control group; 14 (20%), 37 (52.9%), 19 (27.10%) patients were rapid, intermediate and slow acetylators respectively. In contrast, among the patients constituting the study group; 3 (10%), 4 (13.3%), 23 (76.7%) patients were rapid, intermediate and slow acetylators. The difference was statistically significant when the control and study groups were compared for their acetylator status. The proportion of slow acetylators was much higher in the study group. In conclusion, NAT2 acetylator phenotype analysis by molecular biology methods prior to medical treatment for tuberculosis, can be used both for determining the high-risk group of patients who may develop hepatotoxicity and for closer follow-up during treatment period.*

Key Words: *N - Acetyltransferase 2, tuberculosis treatment, anti-tuberculosis drug-induced hepatitis, acetylator phenotype.*

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. It is usually spread through the air (1). In the treatment of new smear positive patients isoniazid (H), rifampicin (R), pyrazinamid (Z) and ethambutol (E) or streptomicin (S) are used regularly for at least six months (2,3). Out of these drugs, H, R and Z have toxic effects on the liver and may cause development of hepatotoxicity. H is acetylated by N-acetyltransferase-2 (NAT2) and transformed to acetylisoniazid which is a urinary metabolite of H.

The polymorphisms in the NAT2 gene are associated with rapid, intermediate, and slow acetylation by causing changes in the structure of the enzyme (4,5). In this study we aimed to evaluate whether NAT2 polymorphisms are a risk factor for hepatotoxicity due to antituberculosis drugs in the patients under tuberculosis chemotherapy.

MATERIALS and METHODS

Patients diagnosed with tuberculosis at the Department of Chest Diseases, Izmir Training and Research Hospital for Chest Diseases and Thoracic Surgery, between October 2003 and May 2004 were included in our study. Patients were received H 5 mg/kg (max. 300 mg/day), R 10 mg/kg (max. 600 mg/day), Z 25 mg/kg (max. 2000 mg/day), E 15-25 mg/kg (max. 1500 mg/day) and end of the treatment all of the patients were cured.

Only patients with serum levels before initiation of treatment within the following ranges were

included in the study: alanine aminotransferase (ALT): 0-40 U/L, aspartate aminotransferase (AST): 5-45 U/L, and total bilirubin: 0.2-1.6 mg/dL. In addition patients with positive serum hepatitis B surface antigen (HBsAg), hepatitis C antibody (anti-HCV) or hepatitis A immunglobulin M antibody (anti-HAV IgM), and patients with alcoholic liver disease or any hepatic or systemic disease that could cause liver function disorder, were excluded from the study.

A total of 100 Turkish patients (24 women and 66 men) who fit the criteria were included in our study. Of these, 70 patients who did not have drug-induced hepatitis constituted the control group and 30 patients who had hepatotoxicity constituted the study group. Our study protocol was approved by Ege University Medical Faculty Ethic Committee on 20.02.2004 with the number 03-12/2M-73.

Liver function tests were repeated when hepatotoxicity symptoms (nausea, vomiting, abdominal pain, malaise, jaundice) appeared during the treatment. Drug-induced hepatitis criteria were defined as follows:

1. An increase in AST and ALT levels of more than 3-fold above normal or more than 5-fold above starting level or,

2. A greater than normal increase in ALT and AST levels together with hepatitis symptoms or,

3. A high bilirubin level.

When hepatotoxicity developed, patients' drugs were stopped. Anti-HAV IgM, HBsAg, anti-HCV tests were retested and abdominal USG was taken to exclude viral hepatitis. When the ALT, AST levels returned to normal, the same drugs were started at the same dosages.

Genomic DNA was extracted from the patients' blood samples using High Pure PCR Template Preparation Kit (Roche Applied Science, Germany). NAT2 polymorphisms G→A 191 NAT2*14A, C→T 481 NAT2*5A, G→A 590 NAT2*6A, G→A 857 NAT2*7A/B were analyzed with a NAT2 Mutation Detection Kit (Roche Applied Science, Germany).

RESULTS

The average age of the 30 patients (15 men and 15 women) in the study group is 39.80 ± 14.70 and the average age of the 70 patients (51 men and 19 women) in the control group is 37.30 ± 14.01. There were no significant differences when the control and study groups were compared in terms of age (x^2= 31.034, p> 0.05), use of cigarettes and alcohol (x^2= 1.515, p> 0.05 and x^2= 0.983, p> 0.05) respectively, DM (x^2= 0.529, p> 0.05).

There were no statistical differences between the control and study groups for NAT2*5A and NAT2*7A/B polymorphisms. (x^2= 3.181, p> 0.05 and x^2= 3.570, p> 0.05) respectively. NAT2*14A wild type allele was found in all patients.

For NAT2*6A polymorphism the proportion of heterozygote and homozygote genotypes was higher in the study group than in the control group (x^2= 10.037, p= 0.007). The results of NAT2 genotyping are shown in Table 1.

The combined haplotypes with all three mutant alleles for NAT2*5A, NAT2*6A and NAT2*7A/B polymorphisms) were statistically higher in the study group than that of the control group (x^2= 21.28, p< 0.001) (Table 2).

Patients with wildtype for all of the NAT2 polymorphisms were phenotyped as rapid acetylators (RA), patients heterozygous for one of them were phenotyped as intermediate acetylators (IA) and patients who were homozygous mutant or heterozygous for more than one of the polymorphisms were phenotyped as slow acetylators (SA). Thus in the control group 14 (20.00%) patients had RA, 37 (52.90%) patients had IA and 19 (27.10%) patients had SA phenotype; in the study group 3 (10.00%) patients had RA, 4 (13.30%) patients had IA and 23 (76.70%) patients had SA phenotype (Table 3, Figure 1).

When the RA, IA and SA phenotypes in the study and control groups were compared, a statistically meaningful difference was observed (x^2= 21.499, p< 0.001). The proportion of patients with slow acetylator phenotype is significantly higher in the study group.

Table 1. Genotype and allele frequencies of the NAT2 polymorphisms among cases and controls and their associations with risk of hepatotoxicity [number (percent)].

Polymorphisms	Genotypes/ haplotype	Control group (n= 70)	Study group (n= 30)	p	OR	(95% CI)
NAT2*5A (C481T)	CC	39 (55.70%)	12 (40.00%)		1	
	CT	24 (34.30%)	16 (53.30%)	> 0.05	2.1	0.88-5.36
	TT	7 (10.00%)	2 (6.70%)		0.93	0.17-5.08
	C	102 (72.85%)	40 (66.66%)	> 0.05	1.34	0.70-2.58
	T	38 (27.15%)	20 (33.33%)			
NAT2*6A (G590A)	GG	41 (58.60%)	9 (30.00%)		1	
	GA	26 (37.10%)	15 (50.00%)	0.007	2.63	1.00-6.87
	AA	3 (4.30%)	6 (20.00%)		9.11	1.91-43.47
	G	108 (77.14%)	33 (55.00%)	0.002	2.76	1.45-5.26
	A	32 (22.86%)	27 (45.00%)			
NAT2*7A/B (G857A)	GG	65 (92.90%)	24 (80.00%)		1	
	GA	4 (5.70%)	5 (16.70%)	> 0.05	3.39	0.84-13.67
	AA	1 (1.40%)	1 (3.30%)		2.71	0.16-45.03
	G	134 (95.71%)	53 (88.34%)	> 0.05	2.95	0.95-9.19
	A	6 (4.29%)	7 (11.66%)			
NAT2*14A (G191A)	GG	70 (100.0%)	30 (100.0%)		1	
	GA	-	-	> 0.05		
	AA	-	-			
	G	140 (100.0%)	60 (100.0%)	> 0.05		
	A	-	-			

Table 2. Combined haplotype frequency distributions of NAT2*5A, NAT2*6A and NAT2*7A/B polymorphisms in the study and control group.

Haplotype			Study group		Control group	
NAT2*5A (C481T)	NAT2*6A (G590A)	NAT2*7A/B (G857A)	N	Combined haplotype frequency	N	Combined haplotype frequency
C	G	G	19	0.3167	75	0.5357
C	G	A	3	0.0500	4	0.0287
C	A	G	16	0.2667	22	0.1572
C	A	A	2	0.0333	1	0.0071
T	G	G	10	0.1667	28	0.2000
T	G	A	1	0.0167	1	0.0071
T	A	G	8	0.1333	8	0.0571
T	A	A	1	0.0167	1	0.0071
Total			**60**	**1.0000**	**140**	**1.0000**

Table 3. Acetylator phenotypes according to gender in the control and study groups.

Gender	Group	Rapid acetylator	Intermediate acetylator	Slow acetylator	p
Female	Control (n= 19)	1 (5.30%)	10 (52.60%)	8 (42.10%)	0.014
	Study (n= 15)	3 (20.00%)	1 (6.70%)	11 (73.30%)	
Male	Control (n= 51)	13 (25.50%)	27 (52.90%)	11 (21.60%)	< 0.001
	Study (n= 15)	-	3 (20.00%)	12 (80.60%)	
Total	Control (n= 70)	14 (20.00%)	37 (52.90%)	19 (27.10%)	< 0.001
	Study (n= 15)	3 (10.00%)	4 (13.30%)	23 (76.70%)	

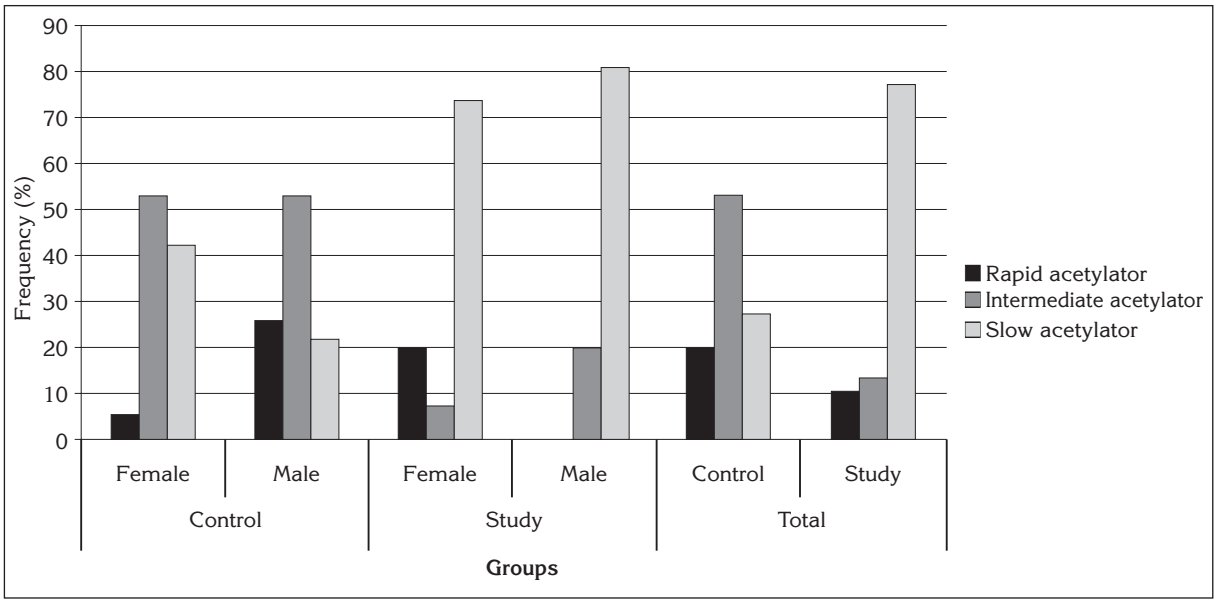

Figure 1. Frequencies of acetylator phenotypes according to control and study groups.

DISCUSSION

Hepatotoxicity due to long term concomitant use of more than one antituberculosis drug is a serious problem in the treatment of tuberculosis. Several studies have shown that acetylator phenotypes, age, sex, weight, alcohol consumption, severity of the disease and hepatitis B infection are involved in drug-induced liver injury (6-9). The most important one among these factors is the acetylator phenotype and its effect has been discussed for many years (7,8,10-12).

The concept of slow acetylation phenotype as a risk factor for hepatotoxicity in relation to acetylator phenotype which has been reported in previous studies is supported by the fact that NAT2 enzymes with a slow acetylator phenotype cause very slow acetylation of monoacetylhydrazine and result in an accumulation of toxic metabolites (8).

Huang et al. evaluated the association of polymorphic NAT acetylator status and antituberculosis drug-induced hepatitis, and found that 26.4% of SA and 11.1% of RA developed hepatotoxicity (p= 0.013) (13). In another study hepatotoxicity was found to be 4.0% (95% CI 1.94-6.06) and 28.0% (95% CI 26.0-30.0) in IA and SA's respectively (14). Hiratsuka et al. reported that using the standard dosage of H (300 mg/day) in SA carries a higher risk of developing serious adverse drug reactions (12). Hepa-

totoxicity levels in the slow acetylator phenotypes have been found to be higher compared to the other phenotypes as observed in this study (SA: 83.3%, IA: 2.4%, RA: 0%).

We found that slow acetylators have higher risk of developing hepatotoxicity than intermediate and rapid acetylators ($p < 0.001$) (SA: 76.7%, IA: 13.3%, RA: 10.0%). Although slow acetylators had higher risk, there were 19 (27.10%) slow acetylator patients had not develop hepatotoxicity. Contradictory finding may be due to the presence of other polymorphisms in the NAT2 gene. Studying other polymorphisms, such as NAT2*12 and NAT2*13, may help to clarify these conflicting results.

In previous studies the effect of combined heterozygous genotype on development of hepatotoxicity has not been discussed. In our study 7 (10.00%) cases in the control group and 12 cases (40.00%) in the study group were heterozygous for both NAT2*5A and NAT2*6A polymorphisms, therefore it can be speculated that combined heterozygous genotype predisposes individuals to a risk of developing hepatotoxicity. Nevertheless further studies with larger study groups are needed to evaluate the effect of other combined heterozygous genotypes in developing hepatotoxicity.

In the present study development of hepatotoxicity in patients on antituberculosis drugs was associated with slow acetylator phenotype. Our study shows that analysis of NAT2 polymorphisms could be applied in the clinic before tuberculosis treatment, in order to determine patients with a risk of developing hepatotoxicity.

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