Catalase-Peroxidase Gene (Kat G) Deletion in Isoniazid Resistant Strains of Mycobacterium Tuberculosis

İZONİAZİD DİRENÇLİ M.TUBERCULOZİS SUŞLARINDA KATALAZ PEROKSİDAZ GEN (KAT G) DELESYONU

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Abstract

Objective: A recent study showed that some isoniazid-resistant *Mycobacterium tuberculosis* strains have a complete deletion of the gene (Kat G) encoding the catalase-peroxidase enzyme. We wished to examine what proportion of clinical isolates of isoniazid-resistant *M. tuberculosis* exhibit Kat G deletion in our Country.

Material and Methods: The relation between Kat G deletion and isoniazid-resistance was examined in 10 isoniazid-resistant and 26 isoniazid-sensitive, for a total of 36 isolates, using polymerase chain reaction (PCR) technique.

Results: Kat G deletion was observed in 70% of isoniazid-resistant and 7.7% isoniazid-sensitive isolates ($p<0.05$, x^2). The Kat G deletion ratio in isoniazid-resistant isolates were observed to be higher than in isoniazid-sensitive isolates [odds ratio, 28, (95% CI, 3.87 to 202.26)]. The specificity and sensitivity of the PCR technique, which revealed both Kat G deletion and isoniazid-resistance, were 92.3% and 70% , respectively.

Conclusion: The deletion of the Kat G gene may play an important role in resistance to isoniazid in most *M. tuberculosis* isolates in our Country.

Key Words: Catalase-Peroxidase Gene (KatG), isoniazid resistance, MDR-TB, mycobacterium tuberculosis

Özet

Amaç: Son zamanlarda yayınlanan çalışmalar izoniazid dirençli *M. tuberculosis* suşlarında katalaz-peroksidazı kodlayan KatG geninde total delesyonu göstermiştir. Bizde çalışmamızda izoniazid dirençli *M. tuberculosis* izolatlarında Kat G delesyonunun ülkemizdeki sıklığını araştırmayı amaçladık.

Gereç ve Yöntemler: Kat G delesyonu ile izoniazid direnci arasındaki ilişkiyi, PCR tekniği kullanarak 10 isoniazid dirençli ve 26 isoniazid hassas toplam 36 izolat üzerinde inceledik.

Bulgular: İzoniazid dirençli suşlarda % 70, isoniazid hassas suşlarda %7,7 oranında Kat G delesyonu saptandı ($p<0,05$, x^2). İsoniazid dirençli suşlarda Kat G delesyon oranı izoniazid hassas suşlara göre daha yüksek saptandı. Kat G delesyonu ile isoniazid direncini gösteren PCR tekniğinin spesifitesi %92,3 ve sensitivitesi %70 olarak bulundu.

Sonuç: Bu bulgular ülkemizde izoniazid dirençli çoğu *M. tuberculosis* izolatlarında direnç gelişiminde Kat G gen delesyonunun önemini vurgulamaktadır.

Anahtar Kelimeler: Katalaz -Peroksidaz Gen (KatG), izoniazid direnci, MDR-TB, m. tuberculozis

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M. tuberculosis is usually treated with only a limited number of antimicrobial agents, the most important ones being rifampin, isoniazid, streptomycin, and ethambutol.

Because of bactericidal effect and low cost, isoniazid is the most important drug used for the treatment of tuberculosis.[1] But the mechanism of its action and the development of resistance to the drug by *M. tuberculosis* has not been definitely established.[2] Recent advances in molecular biology have allowed identification of the genetic loci and biological mechanisms of resistance to various drugs. Previous studies have described resistance-

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associated mutations in *katG, inhA, kasA, ndh,* and the *oxy R- ahpC* intergenic region.[3-8] It is proposed that INH enters *M. tuberculosis* as a prodrug by passive diffusion and is activated by catalase-peroxidase, encoded by *katG.* Mycolic acid synthesis is the primary pathway inhibited by the action of isoniazid.[9] Two enzymes involved in the biosynthesis of mycolic acids have been suggested to be the targets of Kat G-activated isoniazid: the NADH-dependent enoly-acyl carrier protein reductase (*inhA)* and β- ketoacyl acyl carrier protein synthase (*KasA*). Certain promotor mutations of alkylhydroperoxidase reductase, encoded by *ahpC*, in INH- resistant isolates results in overexpression *of ahpC* as compensatory mechanism for the loss of catalase activity due to *KatG* mutations.[10] Recently, missence mutations were identified in *ndh,* a gene encoding NADH dehydrogenase, which is essential respiratory chain enzyme that regulates the NADH/ NAD^+ ratio in cells.[7] Molecular techniques can detect drug resistance in *M. tuberculosis*.

We wished to examine the proportion of Kat G deletion, which we think the important molecular mechanism for isoniazid resistant strains of *M. tuberculosis* in Turkey. We examined the frequency of deletion in the kat G gene in isoniazid sensitive and resistant *M. tuberculosis* strains by using a PCR procedure for the first time in Turkey.

Material and Methods

Bacterial isolates: Isoniazid resistant and drug susceptible 36 *M. tuberculosis* strains isolated in randomly selected months were obtained from the Clinical Microbiology Laboratory in our hospital. All strains used in this study were grown on Lowenstein-Jensen media. Susceptibility testing of isolates was performed by using the proportion count method. Isoniazid was tested at 0.2 μgr/ml. All isolated strains were transported in Lowenstein-Jensen culture tubes to Departments Microbiology and Pediatric Moleculer Pathology in an other University.

PCR Procedure: The investigators doing the PCR procedure were blinded to the drug susceptibility information of the isolates. *M. tuberculosis* DNA was extracted by boiling a loopful of the bacterial growth from Lowenstein-Jensen culture slants in 100 μL of distilled water. These samples were put into sterile tubes which contains 5 ml distilled water and glass bead. Afterwards bacteria suspensions were obtained by shaking tubes in vortex.Samples were then analyzed by PCR. The PCR reaction mixture contained 200 *μM* dNTP (Pharmacia Biotechnology, Piscataway, NJ), 50mM TRIS-HCl (pH 8,8), 50m*M* KCl, 2,5m*M* $MgCl_2$, 0,1% Triton X-100, and 0,25 units of Taq polymerase (Perkin-Elmer Cetus, Norwalk, CT). The sequences of the oligonucleotide primers (TB 1 and TB 2) used to amplify the M. tuberculosis catalase gene segment are as follows:

TB 1F.5'- GAG ATC GAG CTG CTG GAG GAG CC- 3'

TB 2R.5'- AGC TGC AGC CCA AAG GTG TT –3'

The sequences of oligonucleotide primers (Kat1 and Kat2) used to amplify the *M.tuberculosis* catalase gene segment are follows (Figure 1):

Kat 1F.5'-GCG ATCA CAT CAT CCG

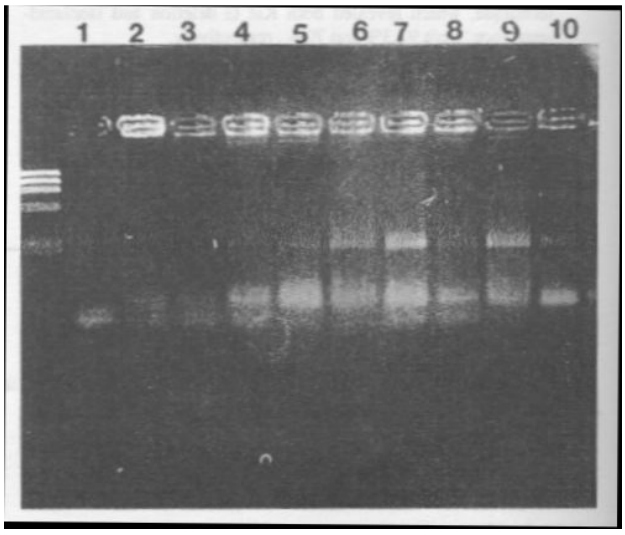

Figure 1. Analysis of KatG sequences by polymerase chain reaction (PCR)
Position of 282-bp Kat G- amplified DNA product indicated by arrow. Lanes 2- 4 strains showing absence of Kat G DNA sequences, Lanes 5- 11 strains showing presence of KatG DNA sequences

TGAT CACA-3'

Kat-2R.5'-GTCA GGCG TCA AGTC GAC TG-3'

282-bp KatG amplified DNA products were controlled by using 2% agarose gel. All samples that yielded a negative reaction were tested at least twice and were also subjected to additional reactions with other sets of primers known to amplify other regions of M. tuberculosis DNA.

The PCR products were purified according to the manufacturer's instructions by E.Z.N.A. Cycle-Pure Kit (Omega Bio-tek, Doraville-USA) prior to sequencing.

Results

Thirty-six (10 isoniazid-resistant and 26 isoniazid-sensitive) isolates of *M. tuberculosis* were obtained. DNA was extracted from all isolates and we confirmed that all of them were M.tuberculosis. The catalase primers amplified a 282-bp DNA segment of 3 (30%) of isoniazid-resistant and 24 (92.3%) of isoniazid-sensitive isolates ($p< 0.05$, x^2 test) (Table 1). The catalase primers repeatedly failed to amplify DNA extracted from 7 (70%) isoniazid-resistant and 2 (7.7%) isoniazid-sensitive isolates. Kat G deletion ratio in isoniazid resistant isolates were observed higher than isoniazid sensitive isolates [odds ratio, 28, (95% CI, 3.87 to 202,26)]. For katG deletion, the specificity, sensitivity, positive and negative predictive values of the assay compared with conventional methods were 92.3%, 70%, 77.8% and 88.8%, respectively.

Discussion

The incidence of tuberculosis is increasing in many countries, and control of the disease is further threatened by the emergence of drug resistance. Recent advances in molecular biology have allowed identification of the genetic loci and biological mechanisms of resistance to various drugs.[2-4,11-18] For the first time, 1992, the relationship between isoniazid-resistance and loss of catalase activity were explained by the identification of the katG gene, encoding the catalase-peroxidase enzyme of *M. tuberculosis* which is indispensable for isoniazid susceptibility. Zhang et al. found that total deletion of the katG gene occurs in ~20% of isoniazid-resistant isolates.[3] Furthermore, they showed that 2 of 3 resistant strains of M.tuberculosis strains completely lack of the catalase gene. They concluded that in a subset of isoniazid resistant isolates, the lack of catalase activity is due to the complete loss of the Kat G gene.[13] Initially, resistance was believed to be caused by complete deletion of the Kat G gene, but with the use of PCR and PCR-SSCP, it was shown that both deletion and mutation of the Kat G gene could lead to resistance.[19]

Stoecle et al found that 31 (76%) of 41 isoniazid-resistant isolates in New York City contained Kat G sequences. These findings suggest that the potential diagnostic application of Kat G sequence as a marker for isoniazid resistance would yield a test of low sensitivity and specifisity.[20]

In the present study, we found that 3 (30%) of isoniazid resistant and 24 (92,3%) of isoniazid sensitive isolates contained Kat G sequences ($p < 0,05$, x^2). A significantly higher katG deletion was found in isoniazid-resistant strains compared with isoniazid-sensitive strains.

Table 1. Evaluation of Kat G deletion in isoniazid resistant and sensitive patients

	İsoniazid- resistant strains of *M. tuberculosis,*n (%)	isoniasid-sensitive strains of *M. tuberculosis*, n (%)	Total, n
Kat G deletion positive	7 (70)	2 (7.7)	9
Kat G deletion negative	3 (30)	24 (92.3)	27
Total, n	10	26	36

x^2 , $p < 0.05$

When compared our findings with Stoeckle et al., we found similar Kat G deletion rates in isoniasid sensitive strains but significant was found in isoniazid- resistant strains (24% versus 70%). This could be explained by the difference in geographical origins.In the study of Abal et al[21] from Kuwait ,the mutation S315T was found in 46 (69%) of the 67 resistant strains. The prevalence of this mutation was highest (80%) in isolates recovered from patients of South Asian origin and lowest in isolates from patients of Middle Eastern origin (44%). These results point to a varying prevalence of the mutation within the Kat G gene in clinical *M.tuberculosis* isolates recovered from patients of different ethnic groups within the same country.

The specificity and sensitivity of PCR technique, which reveals katG deletion and isoniazid-resistance, is 92.3% and 70%, respectively. These findings support the importance of katG deletion in the development of isoniazid-resistance in Turkey.

Finally, resistance to isoniazid in most *M. tuberculosis* isolates is due to the mutations on KatG gene in Turkey. But there is a further need to identify KatG varations in *M.tuberculosis* isolates.

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