Letter to the Editor

Minor Contribution of *inhA-15* Mutations to the Rapid Detection of Isoniazid Resistance in *Mycobacterium Tuberculosis* Isolates

Dear Editor,

In the present work, the necessity for the use of *inhA-15* testing in detecting isoniazid resistance was challenged for the first time. We found that the molecular detection of *inhA-15* C to T alterations in the fast detection of isoniazid-resistant tuberculosis (TB) is not necessary and should not be recommended for routine work.

In this descriptive study, out of 98 clinical isolates of *Mycobacterium tuberculosis* at The Tuberculosis Research Center in the Iranian city of Arak, 64 isolates that had already been determined to be phenotypically resistant or susceptible to isoniazid were analyzed for mutations in the regulatory region of *inhA-15* with 2 allele-specific polymerase chain reaction (AS-PCR) and sequence methods. The H37Rv strain was used as a negative control.

The mutations in *inhA-15* were determined by using 3 primers, namely TB92, TB93, and Rmut, via AS-PCR. The primer sequences were as: TB92: 3-CCTCGCTGCCCAGAAAGGGA-5, TB93: 3-ATCCCCCGGTTTCCTCCGGT-5, Rmut: 5-AGTCACCCCGACAACCTATTA-5.

The detection of mutations in codon 315 of the *katG* gene was performed in accordance with a previous study.[2] In order to prove whether or not the mutations occurred in the *inhA-15* point and to confirm the methodology of AS-PCR, we employed the sequence method (the gold standard of molecular methods). Therefore, 248 bp bands containing the promoter region of *inhA-15* were amplified with primers TB92 and TB93 by PCR. The PCR products were purified and sequenced using an ABI apparatus.

The strains harboring mutation in *inhA-15* generated a band at 174 bp. Primers TB92 and TB93 created 248 bp bands in all the strains. Molecular Evolutionary Genetics Analysis (MEGA) software determined that TB92 bound to the nucleotide -169 upstream of the *inhA* gene and to the inside of the *fabG1* gene (or *mabA*) and that it was a forward primer in PCR. TB93 bound to the nucleotide +79 of the *inhA* gene, and it was a reverse primer making a 248 bp band with TB92.

The Rmut primer is a reverse primer that binds to the nucleotide +5 to -15 *inhA* promoter region in mutant strains and creates a 174 bp band with TB92. This primer is designed for mutant strains, and strains without mutations in this region do not show this band.

Figure 1 depicts a view of the situation of the primers and AS-PCR-resulted products in a strain harboring mutation at *inhA-15*.

The AS-PCR product was electrophoresed on 1.5% agarose gel. In this context, the mutant strains in *inhA-15* showed 2 bands of 248 bp and 174 bp, and the strains with no mutations in this region were only 248 bp band, where there was 98% phenotypic compliance.

As a result, of the 42 strains resistant to isoniazid, 5 strains had mutations in *inhA-15*. Also, of the 22 strains susceptible to isoniazid, 1 strain (901H) had a mutation in that area. In other words, this strain was phenotypically susceptible to isoniazid, and while there was no mutation in *katG315* (which is perfectly logical), both AS-PCR and sequence methods showed mutations in *inhA-15*.

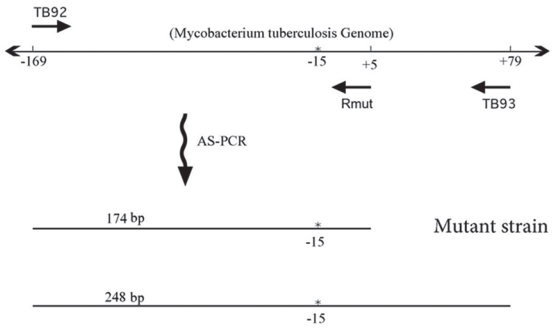

Figure 1: Schematic view of the binding site primers and the AS-PCR method. AS-PCR, allele-specific polymerase chain reaction

To assess the accuracy of the results of AS-PCR, we utilized the sequence method as the gold standard of molecular parts. Complete conformity (100%) between the results of the sequence method and AS-PCR, indicating the accuracy of the method used, is a molecular perspective.

The majority of the previous investigations has determined the mutation in *inhA-15* just in total strains, including the number of the strains susceptible or resistant to isoniazid, and did not specifically determine mutations in *katG315* and mutation in *inhA-15* separately. The distributions of *inhA-15* mutations were varied: 2.7%, 8%, 20%, 22.9%, 31.6%, and 37.8%. Three separate studies by Sajduda (2004), Yang (2005), and Feuerriegel (2009) examined the association between mutations in *inhA-15* and *katG315* and isoniazid resistance.

In the present study, from the 42 strains resistant to isoniazid, 5 (11.9%) strains had mutations in *inhA-15.* All 5 strains simultaneously had mutations in *inhA-15* and *katG315*.

It has been previously proven that mutations in the *inhA* promoter are associated with low-level resistance to isoniazid. Thus, in the discussion of the epidemiology and prevalence of isoniazid, resistance is not very important, while mutations in *katG315* are of great significance regarding high-level resistance to isoniazid.[1]

Interestingly, we examined 106 strains resistant to and 62 strains susceptible to isoniazid and obtained results similar to those reported previously by other investigators.[1,3,4] Nonetheless, Wu et al.[5] succeeded in proving *inhA-15* mutation in 3 (4.8%) strains susceptible to isoniazid. In the present study, chiming in with the results reported by Wu et al.,[5] we detected a case of isoniazid-susceptible phenotype, albeit with mutations in *inhA-15*.

According to the literature, in phenotypic resistance with no mutations in *katG315*, resistance can be caused by mutations in *inhA-15*. The drug-susceptible strain can still be considered sensitive even with harbored mutation at *inhA-15* (Wu et al., 2006).[5] Consequently, phenotypic drug susceptibility testing is more valuable than is the molecular testing of *inhA-15*.

Given the findings of the current study and those reported by Wu et al.[5] apropos isoniazid-susceptible strains harboring mutation in *inhA-15*, we conclude that *inhA-15* testing cannot provide valuable results in comparison with the fast and highly valuable test of *katG315*.

Clinical practice requires expedited detection, increased accuracy, decreased costs, and reduced number of samples per day and, thus, reduced workload. Accordingly, it is of scientific and strategic significance that unnecessary work such as that in the evaluation of molecular resistance to isoniazid be avoided in routine practice.

The detection of mutations in *KatG315* with or without the results of mutations in *inhA-15* is sufficient for the prediction of resistance to isoniazid. Mutations in *inhA-15* cannot be valuable as a complementary test for the rapid detection of resistance to isoniazid in the clinical isolates of TB in routine work.

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