Chronic Diseases Journal

DOI: 10.22122/cdj.v12i2.795
Published by Vesnu Publications

Investigations on mutations in katG gene and inhA promoter region associated with isoniazid resistance in the clinical isolates of mycobacterium tuberculosis from Tehran, Iran

Shahrzad Shafiei[1], Kambiz Davari[1], Sabah Hasani[2]

1 Department of Microbiology, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran
2 Lung Diseases and Allergy Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran

Original Article

Abstract

BACKGROUND: Isoniazid (INH) is one of the first-line drugs used for the treatment of Mycobacterium tuberculosis (Mtb). This study aimed to determine the mutations in katG and inhA promoter regions associated with INH resistance in clinical isolates of Mtb from Tehran, Iran.

METHODS: This descriptive cross-sectional study was conducted in the tuberculosis (TB) center of Tehran in 2020. 50 samples obtained from these patients were cultured on Löwenstein-Jensen medium (LJ), then, INH-resistant strains and their minimum inhibitory concentration (MIC) were determined using the proportional method. To determine the INH-resistance mutations, sequencing was performed following the amplification of both katG and inhA genes using real-time polymerase chain reaction (PCR). The genetic relationships were determined based on each strain's genetic pattern using the primers related to enterobacterial repetitive intergenic consensus PCR (ERIC-PCR), which is a proper tool used for typing Mtb strains. In this study, the phylogenetic tree was plotted using NTSYS software.

RESULTS: Five INH-resistant Mtb strains were isolated from 50 patients with TB in Tehran. All the studied resistant strains (100%) showed a mutation in codon 315 of the katG gene; none of them exhibited any mutation in the promoter. I335T mutation was observed in one INH-resistant strain (20%). The phylogenetic tree of the strains indicated seven clusters as well as 31 patterns in the strains. The strain with two mutations in 335 and 315 had MIC > 8.

CONCLUSION: KatG mutation could result in a high level of INH resistance. Therefore, routine identification of this mutation can help in determining the INH resistance, thereby preventing further propagation of these strains.

KEYWORDS: Mycobacterium Tuberculosis; Resistance; Isoniazid; Mutation; KatG; InhA

Date of submission: 10 Jan. 2023, ***Date of acceptance:*** 25 Feb. 2023

Citation: Shafiei S, Davari K, Hasani S. **Investigations on mutations in katG gene and inhA promoter region associated with isoniazid resistance in the clinical isolates of mycobacterium tuberculosis from Tehran, Iran.** Chron Dis J 2024; 12(2): 93-101.

Introduction

Mycobacterium tuberculosis (Mtb) is known as the primary cause of tuberculosis (TB) in human beings. Despite the considerable decline in infection and mortality rates of this disease in the last decade, TB has remained a significant health concern worldwide.[1] In the past 200 years, more than 1 billion people died due to TB. Nowadays, TB is regarded as the leading cause of death among human diseases. Moreover, it has been estimated that this disease annually infects 10.4 million people and causes 1.7 million deaths.[2] The results of surveys conducted in Iran in 2015 showed that the incidence of sputum-positive pulmonary TB in the whole country was 13 cases per hundred thousand people.[3]

Corresponding Author:
Kambiz Davari; Department of Microbiology, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran
Email: k.d.microbiology@gmail.com

This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 Unported License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Mtb is a spore-free acid-resistant (acid-fast) bacterium in both humans and animals.[4] Accordingly, this bacterium could affect some organs, including the lungs, central neural system, lymphatic system, bone tissue or joints, and blood circulation system. This bacterium infection depends on various factors such as genetic characteristics of the host, environmental factors, and genetic polymorphisms.[4] The intensive two-month treatment phase for the sensitive stains includes the administration of four drugs including rifampin (RIF), isoniazid (INH), pyrazinamide (PZA), and ethambutol (EMB). Afterward, RIF and INH are employed in a continuation phase to destroy the residual bacillus, which previously entered the hidden and mild phases.[5]

INH [with the chemical name of isonicotinic acid hydrazide, and International Union of Pure and Applied Chemistry (IUPAC) name of pyridine-4-carbohydrazide] and its derivatives are N-containing heterocycles considered to be significantly important due to their diverse biological activities, including anti-mycobacterial, anti-bacterial, anti-viral, anti-fungal, anti-tumor, and anti-pain properties.[6] By inhibiting mycolic acid synthesis as well as destroying the cell wall integrity of the bacterium, INH can rapidly eliminate an active Mtb.[7] Moreover, INH is a water-soluble compound with low molecular weight, which can be rapidly absorbed by the digestive system. After its absorption, INH can promptly be distributed in all tissues and fluids of the body, including cerebrospinal fluid (CSF), saliva, peritoneum exudates, bronchus, and pulmonary alveoli.[8] Due to the long duration of the INH regimen, lack of patient tolerance, and risk of hepatotoxicity, its effect on preventing TB may be due to insufficient acceptance and incomplete treatment procedures. Moreover, non-treated TB can be attributed to the drug resistance property of the bacterium.[9]

In 2017, among 10 million TB cases, approximately 19% of recently cured cases and 43% of treated cases showed resistance to at least one of the first-line antibiotics administered to them.[10] Resistance to INH, which can be developed by treatment failure, recurrence, and progress in multidrug-resistant TB, is the most common type of resistance among patients with TB.[10] Of note, INH resistance is highly prevalent in Iran. Its prevalence rate was reported to be 13.58% and 7.93% in 2012 and 2014, respectively.[11,12] This phenomenon occurs due to the mutations in katG and inhA genes and their promoters (including ahpC, ndh, and furA). However, it is mainly attributed to the mutations in katG and inhA genes and their promoters.[13] Notably, the katG enzyme is a homodimer. Each one of these subunits consists of 740 amino acids, each of which contains a proto-haemin IX prosthetic group. The active site of heme is located in the second N-terminal of the enzyme, which is responsible for peroxidase activity and mutation in N-terminal INH. As well, it changes INH activation and consequently, results in INH resistance.[14] Although more than 300 different mutations have been identified in katG, the mutation in codon 315 of this gene was found to be more common compared to others (Ser315→Thr, S315T). On average, 64% of all the clinical INH-resistant isolations possess the katG 315 mutation.[15] In 2015, the results of the Torres et al. study showed that in 89.5% of INH-resistant strains, only two mutations (katG 315AGC-ACC and inhA promoter-15C-T) were identified.[16] In another study by Smaoui et al., it was found that 95.2% of the Mtb strains resistant to INH had mutations, of which katG S315T was the most common mutation (85.7%). In addition, two different point mutations in the inhA gene and its promoter region were identified as C-15T and G56A at a frequency equal to 14% and 10%, respectively.[17]

To reduce the death rate in patients infected

http://cdjournal.muk.ac.ir, 04 April

with resistant strains of Mtb, it seems necessary to identify these strains in a shorter time. The study of INH resistance mutations in Mtb isolates as the first line of TB treatment is so important that it should be studied to prevent the further spread of these strains in different cities and periods. Therefore, this study can be a suitable method to identify mutations and strains resistant to INH, and with its help, drug resistance can be minimized in the country. The present study determined mutations in the katG gene and inhA promoter region associated with INH resistance in the clinical isolates of Mtb from Tehran, Iran.

Methods

This descriptive cross-sectional study was conducted in the research laboratory of Pouya Gene Azma, Tehran. The required samples were selected from the suspected patients with TB referred to the TB center of Tehran in 2020 (January to November). In the present study, 50 samples were included in the study using the full count method. After obtaining their consent, their sputum samples were collected for performing further culturing and microscopic investigations. The included patients did not receive any treatment for TB before taking their sputum samples for testing in the current study.

Decontamination and preparation of microscopic sputum smear

At this stage, the obtained sputum samples were decontaminated using N-acetyl-L-cysteine (0.5%) and sodium hydroxide (NALC-NaOH) solutions in terms of the standard instruction. Moreover, sodium citrate was used to stabilize the acetyl-cysteine.[18] Thereafter, pellets were re-dissolved in 1.5 ml phosphate buffer (pH = 6.8). Afterward, samples were stained by the Ziehl-Neelsen method. Finally, they were washed and air-dried, and an optical microscope investigated the slides at the magnification of ×100.[19]

Maintenance of culture from clinical isolates of Mtb

After preparing the sediments for culturing, a mycobacteria growth indicator tube (MGIT) vial was prepared, which contained polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin. After using a sterile pipette, a 0.5 ml sample was added to the MGIT vials. The tubes were tightly capped and then shaken; later, MGIT vials were transferred to the BACTEC device and kept at 37° C.[19]

Sensitivity to INH

At this stage, the drug sensitivity test to INH (Merck, Germany) (0.2 µg/ml INH) was conducted on the Löwenstein-Jensen (LJ) medium containing Mtb colonies using the proportional method in terms of the Center for Disease Control and Prevention (CDC) standards.[20] To check the drug stability in the LJ medium, we used the H37Rv (ATCC27294) standard strain. At the defined standard dose, no colony of the above-mentioned strain was observed.

Minimum inhibitory concentrations (MIC) determination

The proportional method was employed to determine the MIC of the INH-resistant samples. For this purpose, various concentrations of INH (as 0.12, 0.25, 0.5, 1, 2, 4, and 8 µg ml^{-1}) were assessed, and the concentration resulting in 99% inhibition of Mtb cells was finally selected as the MIC by macrodilution method.[21]

Deoxyribonucleic acid (DNA) extraction by cetyltrimethylammonium bromide (CTAB)

Firstly, the bacteria grown on the LJ medium were collected; after being inactivated for 20 minutes at 80° C, the CTAB method was employed to extract the DNA. During a typical process, firstly, 70 µl lysozyme (10 mg ml^{-1}) was added and then transferred to a bain-marie at 37° C. Next, 75 µl sodium dodecyl sulfate (SDS)/proteinase K (10%) was added, and the obtained mixture was then incubated in a bain-marie at 65° C. Afterward, 100 µl of sodium chloride (NaCl) (5M) and 100 µl of CTAB/NaCl (which had been placed at 65° C) were added to the mixture, and it was incubated for 10 minutes

http://cdjournal.muk.ac.ir, 04 April

at 65° C. Subsequently, 750 µl chloroform/isoamyl alcohol (1:25) was added, and the mixture was well-mixed. The samples were centrifuged at 11000 g for 8 minutes, and then three phases were performed. The supernatant was transferred to a new tube, and 450 µl of cold isopropanol was added to that. At this phase, the mixture was placed at -20° C for 30 minutes. It was finally centrifuged at 11000 g for 15 minutes. Finally, after removing the supernatant, the residual sediment, which was genomic DNA, was washed with ethanol 70% twice, and distilled water was added to that.[22]

Determination of the mutations related to INH resistance

In the present study, the primers mentioned in table 1 as well as real-time polymerase chain reaction (PCR) were employed to amplify the katG gene and inhA promoter. To do this, 12.5 µl RealQ Plus 2x Master Mix, 0.5 µl forward primer and 0.5 µl reverse primer, 2 µl DNA, and 9.5 µl distilled water were all mixed together and then placed in a PCR device. Thereafter, the real-time PCR was conducted using an AB Applied Biosystems device (Invitrogen, California, USA) under the following conditions: initial denaturation for 3 minutes at 95° C, 30 denaturation cycles for 30 seconds at 95° C, primer linkage for 30 seconds at 60° C for inhA gene and at 59° C for katG, extension for 30 seconds at 72° C, and the final extension for 5 minutes at 72° C. Next, the reaction product was electrophoresed in agarose 1% gel to ensure amplification. The real-time PCR product was finally sent to Gene Fanavaran Company in Tehran for sequencing. Moreover, the mutations were determined with bringing the isolated sequences into line with the wild sequence using MEGA software (version 7.0).

Isolates typing using enterobacterial repetitive intergenic consensus (ERIC) method

The collected isolates were identified using the ERIC-PCR method, which was first used by Sechi et al. in 1998.[24] The reaction mixture in 25 µl of the final volume contained 12.5 µl of master mix, 2 µl of both forward [oligonucleotide sequence (5'→3'): ATGTAAGCTCCTGGGGATTCAC] and reverse (AAGTAAGTGACTGGGGTGAGCG) primers,[24] 3 µl of DNA sample, and 7.5 µl of distilled water. The reaction mixture was kept at 95° C for 3 minutes followed by 30-35 seconds cycles at 95° C, 1 minute at 52° C, and 2 minutes at 72° C. As well, the final extension was done for 5 minutes at 72° C in a thermocycler (BioRad, California, USA). The reaction products were electrophoresed at 90 V for 90 minutes in a 1% agarose gel and then checked. To determine the isolates' phylogenetic tree, their dendrograms were plotted via NTSYS software (version 2.2) using the unweighted pair group method with arithmetic mean (UPGMA) method.

The study procedure was explained to all included patients whose samples were collected for the study, and consent forms were filled out by them. The names of the subjects were not included in any of the study outputs, including the article. Furthermore, all the experiments and reports were in terms of the guidelines of the Declaration of Helsinki and the Ethics Committee of Islamic Azad University of Sanandaj, Iran (Ethical code: IR.IAU.SDJ.REC.1401.081)

Table 1. The primers used in real-time polymerase chain reaction (PCR)

Gene	Primer sequence (5′ → 3′)	Reference
KatG-forward	5′-GAAGAGCTCGTATGGCACCGGAAC-3′	This study
KatG-reverse	5′-AGAGGTCAGTGGCCAGCA-3′	This study
InhA-forward	5′-CCTCGCTGCCCAGAAAGGGA-3′	23
InhA-reverse	5′-GTAACCAGGACTGAACGGGATA-3′	23

Results

Demographic and clinical properties of the studied participants: Out of 50 smear-positive patients with TB, 33 cases were men (66%), and 17 cases were women (34%). The ages of the studied patients ranged from 12 to 85 years old.

INH resistance and MIC determination: At this stage, five INH-resistant isolates were found. Accordingly, one of them had a high-level resistance (> 8 µg/ml). The MIC of the two isolates was 4 µg/ml, while the other isolates showed lower MIC values (Table 2).

Table 1. Minimum inhibitory concentrations (MIC) of the resistant strains

MIC (µg/ml)	Number of isolates/total numbers of INH-resistant isolates	%
1	1/5	20
1-2	1/5	20
4	2/5	40
8 <	1/5	20

MIC: Minimum inhibitory concentration; INH: Isoniazid

Determination of resistance mutations in both katG and inhA genes: The studied genes were amplified using real-time PCR (Figure 1). All the obtained isolates (100%) showed a point mutation in codon 315 of the katG gene, and only one of them (20%) exhibited a mutation in codon 335 (Table 3 and Figure 2). Of note, no mutation was observed in the promoter gene of inhA in any isolate (Table 3).

Phylogenetic tree: Based on figure 3, some isolates, such as isolates 1 and 25, were 100% similar. As well, the isolates 11, 39, 41, 47, and 50 were resistant to INH. According to ERIC-PCR, all the isolates were categorized into 7 clusters. Accordingly, cluster C4 possessed one isolate.

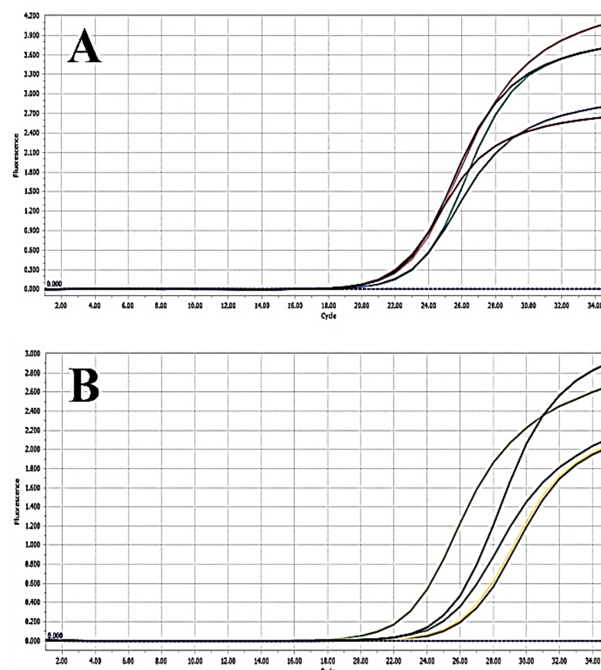

Figure 1. The amplification of (A) katG and B) inhA genes in the isoniazid (INH)-resistant isolates

The highest number of isolates was in cluster C2 (containing 29 isolates).

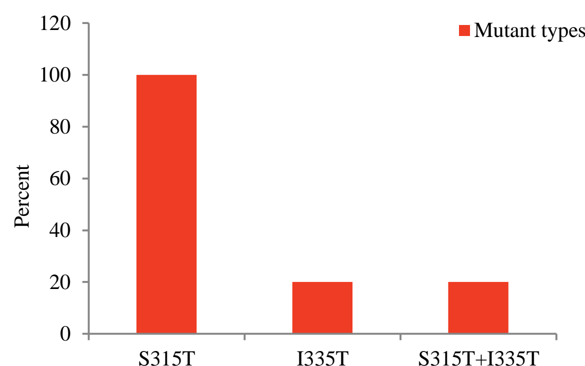

Figure 2. Frequency of S315T and I335T mutations in the studied isolates

Table 3. The point mutation in both katG and inhA genes, minimum inhibitory concentrations (MIC) of the 5 isoniazid (INH)-resistant isolates (→ indicates the mutation)

Strain No.	MIC (µg/ml)	katG mutation		inhA mutation		
		315 codon	335 codon	-15	-17	-47
11	4	→ACC	ATC	C	C	G
39	> 8	→ACC	→ACC	C	C	G
41	4	→ACC	ATC	C	C	G
47	1-2	→ACC	ATC	C	C	G
50	1	→ACC	ATC	C	C	G

MIC: Minimum inhibitory concentration

http://cdjournal.muk.ac.ir, 04 April

Clustering was performed based on a similarity of more than 50%. The isolates had 31 patterns (isolates with 100% similarity were considered as one pattern). The INH-resistant isolates were distributed into 3 clusters (namely C2, C3, and C7) (Figure 3).

Discussion

This study aimed to determine mutations in the katG gene and inhA promoter region associated with INH resistance in the clinical isolates of Mtb from Tehran, and the results of the present study showed that five INH-resistant Mtb strains were isolated from 50 patients with TB in Tehran. All of the studied resistant strains (100%) showed a mutation in codon 315 of the katG gene; none of them exhibited any mutation in the promoter. The I335T mutation was observed in one INH-resistant strain (20%). The phylogenetic tree of the strains indicated seven clusters as well as 31 patterns in the strains. The strain with two mutations in 335 and 315 had MIC > 8.

A wide frequency range has been reported for the resistance mutations, from 39.4% to 91.3% for katG and from 4.3% to 34.4% for inhA.[25] In the present study, the mutation in katG was observed to have the highest value, which is in line with the previous reports. In a previous study conducted in Iran, the frequency of mutation in codon 315 of the katG gene using the restriction fragment length polymorphism PCR (RFLP-PCR) method (Ser→Thr) was reported as 71.9%.[25]

By comparing INH MIC data with genomic mutation, this study indicates that the high resistance of Mtb to INH could be predicted by the genomic mutation determination. Our study also shows that the mutations in both codons 315 and 335 of the katG gene could result in a high INH resistance in Mtb (MIC > 8). Furthermore, this study confirmed the relationship between the known resistance mutations and the phenotype level of the intended resistance.[26,27]

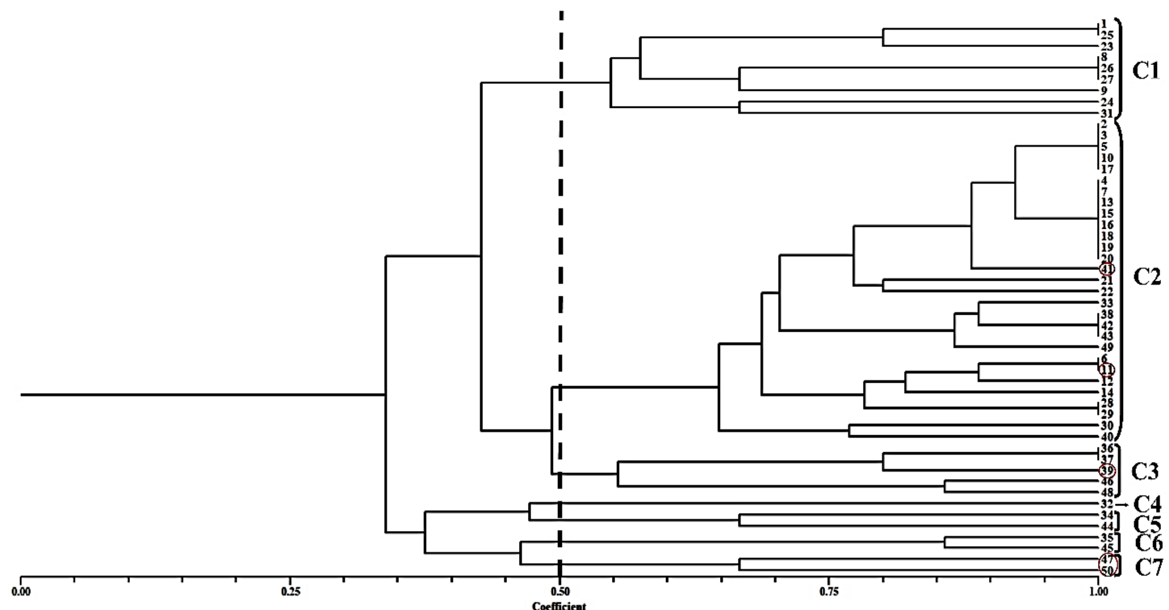

Figure 3. Dendrogram of enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) patterns of 50 Mycobacterium tuberculosis (Mtb) strains determined using unweighted pair group method with arithmetic mean (UPGMA) method and Dice coefficient [dashed line: 50% similarity; C1-C7: Clusters (the isolates with similarity more than 50% were placed in one cluster); red circles: INH-resistant isolates]

Of note, the mutation in inhA promoter was not accompanied by undesirable treatment outcomes (no cure or death). However, the mutation in inhA was found to have the capability of increasing the risk of recurrence in patients treated with INH and EMB only. In contrast, katG mutations were shown to be accompanied by some undesirable treatment outcomes.[28] In our study, no mutation was observed in the inhA promoter, while mutations in katG resulted in a high INH resistance.

Recently, Ogari et al. in their study investigated various mutations in TB samples. As a result, they found that the katG gene with numerous mutations in its codon 315 (90%) was the most common region for the development of INH resistance. Furthermore, they reported that the mutation in S315T was accompanied by INH resistance. Notably, they observed no mild drug resistance caused by the mutation in the inhA gene promoter.[19] In the present study, 100% of the INH-resistant strains had S315T mutation. However, no mutation was observed in the inhA promoter gene. Therefore, the high similarity between these two studies could be due to similar drug analogs in the studied regions.

To answer the question of whether katG mutation could result in treatment failure after high-dose INH, further studies should be performed. Since most of the MICs related to katG S315T strains were found to be under the World Health Organization (WHO)-recommended levels (between 15 and 20 mg/kg), INH could still exhibit relatively significant activity and then it could be applied in the medication regimen.[15] In the current research, the mutations in both codons 315 and 335 of the katG gene resulted in higher MICs. However, the higher dosage of INH may be responsive.

In Tunisia, Smaoui et al. conducted a study. Their results showed that katG S315T was the most common mutation (85.7%). Among the 21 strains examined, only one strain had no mutation.[17] In the comparison of these two studies, it can be seen that the prevalence of mutations in codon 315 of the katG gene was somewhat higher in Tehran than in Tunisia. The higher prevalence of resistance in one study may be due to differences in sample size, poor management of TB cases, irregular supply of anti-TB drugs, and non-adherence to treatment.[17]

In other studies in Iran, it was observed that the prevalence of mutations in codon 315 of the katG gene was higher than that obtained in the present study. For example, Dinmohammadi et al. in their study investigated the presence of mutations in specific regions of the katG and inhA genes in 90 positive culture samples of patients with lung disease. 34.5% of INH-resistant samples had the Thr315 phenotype. Moreover, 65.5% of resistant samples had the Ser315.[25] The Thr315 phenotype is a mutated phenotype, which was 34.5% in their study and 10% in the present study.

In the present study, isolate 39 exhibited high-level resistance to INH (MIC > 8 µg/ml). In the case of INH, low-level resistance is defined by MIC ranging from 0.5 to 8 µg/ml, and the strain is considered as a high-level resistance above 8 µg/ml.[22] Strain 39 possessed the mutation simultaneously in both codons 315 and 335 (Table 3), explaining its high resistance to INH. Accordingly, this strain is placed in the C3 cluster in the plotted dendrogram, which has five strains. Heym et al. in their study expressed that both S315T and I335T mutations could result in a high-level INH-resistance.[29] Isolate 39 possessed both of these mutations simultaneously, which could be troubling.

ERIC-PCR is a proper tool used for typing Mtb strains. As well, it has been introduced as a proper alternative for pulsed-field gel electrophoresis (PFGE). Therefore, in the present study, we employed this straightforward, cost-effective method.[30] Thereafter, the mutated strains were placed in

http://cdjournal.muk.ac.ir, 04 April

C2 (11 and 41), C3 (39), and C7 (47 and 50) clusters (Figure 3). According to the obtained results from ERIC-PCR, the sample No. 6 was 100% similar to the sample No. 11; however, only the sample No. 6 possessed a mutation in its katG gene.

The sample No. 11 had no mutation and also exhibited no INH resistance. This result indicates that these two strains had a common clonal origin; however, strain 6 acquired an S315T mutation. It is noteworthy that selective pressure due to exposure to insufficient dosages can result in mutation.

The most important limitations found in the current study were the small number of positive samples in the province to conduct the study and the high cost of preparing laboratory kits.

It is suggested that in future studies, the prevalence of mutations in inhA, katG, and rpoB genes in other provinces of the country should be investigated to achieve a national pattern.

Conclusion

S315T mutation was observed to be highly prevalent in the katG gene, which can result in high-level INH resistance along with I335T mutation. No mutation was observed in the inhA promoter. Therefore, it is preferable to conduct diagnostic tests, to identify a mutation in the katG gene in the country's TB centers. By performing the routine identification of these mutations, besides determining the INH-resistant strains in clinical samples, further propagations of the resistant strains can be avoided as well.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgments

The authors wish to express their gratitude to the Islamic Azad University, Sanandaj Branch, for its support.

Financials support and sponsorship

This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1. Kiran D, Podell BK, Chambers M, Basaraba RJ. Host-directed therapy targeting the Mycobacterium tuberculosis granuloma: a review. Semin Immunopathol. 2016; 38(2): 167-83.
2. Gagneux S. Ecology and evolution of Mycobacterium tuberculosis. Nat Rev Microbiol. 2018; 16(4): 202-13.
3. Metanat M, Sharifi-Mood B, Alavi-Naini R, Aminianfar M. The epidemiology of tuberculosis in recent years: Reviewing the status in south-eastern Iran. Zahedan J Res Med Sci. 2012; 13(9): e93704.
4. Gemechu A, Giday M, Worku A, Ameni G. In vitro anti-mycobacterial activity of selected medicinal plants against Mycobacterium tuberculosis and Mycobacterium bovis strains. BMC Complement Altern Med. 2013; 13: 291.
5. Hoagland DT, Liu J, Lee RB, Lee RE. New agents for the treatment of drug-resistant Mycobacterium tuberculosis. Adv Drug Deliv Rev. 2016; 102: 55-72.
6. Hu YQ, Zhang S, Zhao F, Gao C, Feng LS, Lv ZS, et al. Isoniazid derivatives and their anti-tubercular activity. Eur J Med Chem. 2017; 133: 255-67.
7. Kandler JL, Mercante AD, Dalton TL, Ezewudo MN, Cowan LS, Burns SP, et al. Validation of novel mycobacterium tuberculosis isoniazid resistance mutations not detectable by common molecular tests. Antimicrob Agents Chemother. 2018; 62(10): e00974-18.
8. Wang P, Pradhan K, Zhong XB, Ma X. Isoniazid metabolism and hepatotoxicity. Acta Pharm Sin B. 2016; 6(5): 384-92.
9. Njie GJ, Morris SB, Woodruff RY, Moro RN, Vernon AA, Borisov AS. Isoniazid-rifapentine for latent tuberculosis infection: A systematic review and meta-analysis. Am J Prev Med. 2018; 55(2): 244-52.
10. Flentie K, Harrison GA, Tukenmez H, Livny J, Good JAD, Sarkar S, et al. Chemical disarming of isoniazid resistance in Mycobacterium tuberculosis. Proc Natl Acad Sci U S A. 2019; 116(21): 10510-7.
11. Nasiri MJ, Rezaei F, Zamani S, Darban-Sarokhalil D, Fooladi AA, Shojaei H, et al. Drug resistance pattern of Mycobacterium tuberculosis isolates from patients of five provinces of Iran. Asian Pac J Trop Med. 2014; 7(3): 193-6.
12. Marjani M, Baghaei P, Tabarsi P, Shamaei M, Mansouri D, Masjedi MR, et al. Drug resistance

pattern and outcome of treatment in recurrent episodes of tuberculosis. East Mediterr Health J. 2012; 18(9): 957-61.
13. Nguyen QH, Contamin L, Nguyen TVA, Banuls AL. Insights into the processes that drive the evolution of drug resistance in Mycobacterium tuberculosis. Evol Appl. 2018; 11(9): 1498-511.
14. Rabha A, Singh A, Grover S, Kumari A, Pandey B, Grover A. Structural basis for isoniazid resistance in KatG double mutants of Mycobacterium tuberculosis. Microb Pathog. 2019; 129: 152-60.
15. Lempens P, Meehan CJ, Vandelannoote K, Fissette K, de Rijk P, Van Deun A, et al. Isoniazid resistance levels of Mycobacterium tuberculosis can largely be predicted by high-confidence resistance-conferring mutations. Sci Rep. 2018; 8(1): 3246.
16. Torres JN, Paul LV, Rodwell TC, Victor TC, Amallraja AM, Elghraoui A, et al. Novel katG mutations causing isoniazid resistance in clinical M. tuberculosis isolates. Emerg Microbes Infect. 2015; 4(7): e42.
17. Smaoui S, Siala M, Hadj FS, Kammoun S, Marouane C, Hachicha S, et al. Molecular characterization of Mycobacterium tuberculosis strains resistant to isoniazid. Int J Mycobacteriol. 2016; 5(Suppl 1): S151.
18. Sharma M, Misra RN, Gandham NR, Jadhav SV, Angadi K, Wilson V. Comparison of modified Petroff's and N-acetyl-L-cysteine-sodium hydroxide methods for sputum decontamination in tertiary care hospital in India. Medical Journal of Dr D Y Patil University. 2012; 5(2): 97-100.
19. Ogari CO, Nyamache AK, Nonoh J, Amukoye E. Prevalence and detection of drug resistant mutations in Mycobacterium tuberculosis among drug naive patients in Nairobi, Kenya. BMC Infect Dis. 2019; 19(1): 279.
20. Davis GL, Ray NA, Lahiri R, Gillis TP, Krahenbuhl JL, Williams DL, et al. Molecular assays for determining Mycobacterium leprae viability in tissues of experimentally infected mice. PLoS Negl Trop Dis. 2013; 7(8): e2404.
21. Schonfeld N, Bergmann T, Vesenbeckh S, Mauch H, Bettermann G, Bauer TT, et al. Minimal inhibitory concentrations of first-line drugs of multidrug-resistant tuberculosis isolates. Lung India. 2012; 29(4): 309-12.
22. Babamahmoodi F, Mahdavi MR, Jalali H, Talebi B, Roshan P, Mahdavi M. Evaluation of gene mutations involved in drug resistance in mycobacterium tuberculosis strains derived from tuberculosis patients in mazandaran, iran, 2013. Int J Mol Cell Med. 2014; 3(3): 190-5.
23. Abkar R, Zolfaghari MR, Arjomandzadegan M, Ahmadi A, Sadrnia M, Kahbazi M, et al. Minor Contribution of inhA-15 Mutations to the Rapid Detection of Isoniazid Resistance in Mycobacterium Tuberculosis Isolates. Iran J Med Sci. 2016; 41(2): 161-3.
24. Sechi LA, Zanetti S, Dupre I, Delogu G, Fadda G. Enterobacterial repetitive intergenic consensus sequences as molecular targets for typing of Mycobacterium tuberculosis strains. J Clin Microbiol. 1998; 36(1): 128-32.
25. Dinmohammadi F, Farnia P, Biglari A, Kazempoor M, Ramazanzadeh R, Masjedi MR, et al. Identification of the mutations related to resistance of mycobacterium tuberculosis to isoniazid by use of PCR-RFLP in TB patients. Sci J Kurdistan Univ Med Sci. 2010; 14(4): 1-9.
26. Ramaswamy SV, Reich R, Dou SJ, Jasperse L, Pan X, Wanger A, et al. Single nucleotide polymorphisms in genes associated with isoniazid resistance in Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2003; 47(4): 1241-50.
27. Kambli P, Ajbani K, Sadani M, Nikam C, Shetty A, Udwadia Z, et al. Defining multidrug-resistant tuberculosis: Correlating GenoType MTBDRplus assay results with minimum inhibitory concentrations. Diagn Microbiol Infect Dis. 2015; 82(1): 49-53.
28. Huyen MN, Cobelens FG, Buu TN, Lan NT, Dung NH, Kremer K, et al. Epidemiology of isoniazid resistance mutations and their effect on tuberculosis treatment outcomes. Antimicrob Agents Chemother. 2013; 57(8): 3620-7.
29. Heym B, Saint-Joanis B, Cole ST. The molecular basis of isoniazid resistance in Mycobacterium tuberculosis. Tuber Lung Dis. 1999; 79(4): 267-71.
30. Sampaio JL, Viana-Niero C, de Freitas D, Hofling-Lima AL, Leao SC. Enterobacterial repetitive intergenic consensus PCR is a useful tool for typing Mycobacterium chelonae and Mycobacterium abscessus isolates. Diagn Microbiol Infect Dis. 2006; 55(2): 107-18.