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MOLECULAR DETECTION OF embB MUTATION IN ETHAMBUTOL RESISTANT TUBERCULOSIS IN AL-SAMAWAH CITY /IRAQ

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Abstract

Background: There are many anti-tuberculosis drugs, which represent the first line of treatment, including ethambutol for short (EMB). Recent research has shown that TB is indeed EMB-resistant in many geographical regions in different parts of the world and is caused by embB gene mutations of EMB-resistant types of TB.

Objectives: In present study, we showed the embB mutation in isolates of M. tuberculosis using the real-time PCR method.

Methods: one hundred sputum samples suspected with M. tuberculosis revealed from the Center of Thoracic diseases in Al-Samawah city/ Iraq were characterized and diagnosis by conventional methods and molecular methods such as biochemical properties and real-time PCR. Primers were against embB mutation that was associated with EMB resistance.

Results A total of thirty five M. tuberculosis isolates from diagnosed Tuberculosis cases by conventional such as culture on L.J media and molecular method of PCR.

Out of 35 MTB isolates, 20 and 15 were resistant and susceptible to EMB drug, respectively. The data of study showed a difference between genders and ages according to embB mutation.

Keywords: Mycobacterium tuberculosis, Real-time-PCR, embB, Ethambutol.

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Introduction

MTB, the infection etiologic cause of tuberculosis (TB) originates wide-ranging morbidity and destruction especially, in growing places, and, has recorded a significant rise in its capacity to convert drug-resistant to a main general health emergency (Casali et al., 2014).. Mycobacterium tuberculosis (MTB) is characterized as long bacilli, neither gram-positive nor negative, non-motile, non-spore-forming bacilli, although it is stained with acid-resistant pigment. Hence, it is called acid-fast bacilli by acid alcohol decolonization then staying carbol-fuchsin stain (Levinson, 2010).

Tuberculosis remains one of the most significant health problems in the health field, with new cases of approximately 10 million tuberculosis and 2 million deaths annually, Where pulmonary tuberculosis is considered one of the most deadly and ancient diseases at the level of humanity, and this affects humans healthily and economically (WHO, 2012).

While the world has promised to almost stop the tuberculosis epidemic by 2030, the measures and activities do not match the political promises, and then we found that a quarter of the world's people have tuberculosis. The fact that ethambutol (EMB or E for ease) is an essential drug used to treat tuberculosis patients. It is recommended as an option for streptomycin as a fourth drug when the first steps of treatment until sensitivity becomes apparent but in the case that does not have genetic risk factors for drug resistance, the isoniazid (INH) resistance is approximately 4% (Hausler et al., 2001, Peloquin, 2002). The emb genes pointed to the (ABC) operon that carries out a role in ethambutol resistance. In case of resistant isolates, Mutations occurring in code 306 of embB Operon had high occurrence numbers (Telenti et al., (1997), Starks et al., (2009)).

The number and type of mutations that create resistance to M. tuberculosis help us to understand the molecular epidemiology of tuberculosis in different geographical areas.

There are many molecular procedures for determining sensitivity or resistance to antibiotics and studying the mutations that lead to bacterial resistance.

Most of the techniques or molecular methods that have been identified to demonstrate genetic mutations characteristic of drug resistance rely on point multiplication of the bacterial genome supported by product analysis. In fact, the number and number of mutations in the knowledge of the molecular resistance of pulmonary tuberculosis bacteria, as well as knowledge of the geographical reality of the different regions related to the epidemiology of tuberculosis infection. Also, there are several molecular procedures for identifying drug susceptibility and for diagnosing mutations that lead to antibiotic resistance. Most of the molecular techniques that have been made to identify genetic mutations characteristic of drug resistance rely on specific replication points of the bacteria genome supported by product analysis (Barun et al., (2006)).

Material and Methods

Out of 100 patients with respiratory diseases, A complete of 35 patients suspected of tuberculosis after displaying medical manifestations of tuberculosis have been submitted for conventional laboratory prognosis and were advantageous for at least one of the diagnostic standards. The drug susceptibility

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checking out became accomplished through the percentage technique (with a critical concentration of 2 µg/ml for EMB) on L–J medium. Genomic DNA from every MTB subculture isolate.

Real-time PCR.

The method was conducted according to the manufacturer company, DNA extracted from culture isolate was used in real-time PCR for detection of anti- ethambutol resistance mutation, the primer used in this study, embBF (5'- CGACGCCGTGGTGATATTCG-3')and embBR (5'- CCACGCTGGGAATTCGCTTG-3').

The PCR response combination was DNA template 2mM, Nuclease-loose water 10mM, 2x SYBR green qPCR mix7mM, and overall primer 1mM. Amplification became finished in a Mastercycler Gradient (Eppendorf, Germany) using the subsequent software : preliminary denaturation at 95CO for 1 minute and forty cycles of denaturation at 94CO /20 seconds, then the next step was 60C annealing /30 seconds, extension step at 72CO/30 seconds, and the very last extension at 75Co/2 minutes.

Results

The present study found that 35 isolates of a total of 100 suspected samples with tuberculosis. All 35 isolates were positive biochemically properties and the results of the susceptibility testing method (standard L–J proportion method) indicated that 15 isolates (42.85%) were sensitive to EMB drug and the other 20 isolates (57.14 %) were resistive to anti-tuberculosis resistance drugs [Table 1]. In our study, the real-time PCR technique was used for the identification of embB gene mutations of TB isolates.

Figure (1) show that Ct values of embB genes were ranged between (14-35 cycles) of positive samples, real time PCR show also 28 and 7 were resistant and susceptible to EMB drug. The present study recorded a difference in age stages according to the response to EMB drugs that are resistant or sensitive. The mean of enrolled patients' ages was (38) years ranged between (17-65) years old, the 35 patients ages and sex match to control persons were enrolled in this study. There are 35 patients were enrolled in this study. The mean of the patients ages was (38) years ranging from (17-65) years old, as well as 35 ages and gender match control persons were enrolled in this study. there is no statistically significant difference between the age of patients and susceptibility to tuberculosis (P > 0.05, CI 95%) who explained that response to the infection of TB with no significant difference either young or elderly patients, this result is show in table (2).

Table (1): Drug susceptibility profile of TB isolates

Drug type	Samples	No. resistant isolates	No. sensitive isolates	Total of isolates
Rifampicin	100	35	0	35
Isoniazid	100	35	0	
Streptomycin	100	20	15	
Ethambutol	100	35	0	

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Table (2) Distribution of patients in present study according to gender.

Gender	(TB) No. (%)
Males	19 (54.2)
Females	16 (45.7)
Total	35

Many studies explained that males may get a chance of TB infection more than females and the reason that males are more in interact with outside environments and then increase the infection rates, especially in developing countries where infection there consider a stigma for females so we cannot get real data for females TB infection. In table (2) TB patients consist of 19 (54.2%) males and 16(45.7%) females.

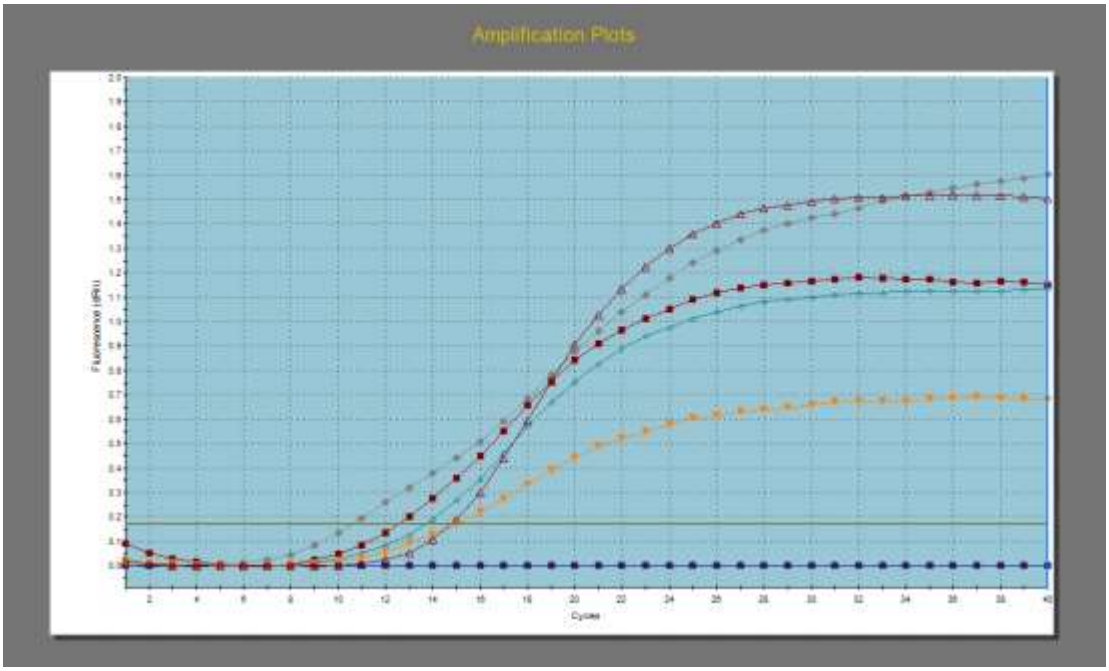

Figure (1): Real time PCR amplification plot for embB of MTB strains mutation.

Discussion

The mutations that occur in MTB could be associated with many gene loci and embB loci mutation is EMB resistance that elevates the number of ethambutol resistant isolates of TB, especially in developing countries of the world, this study used real-time PCR to determine mutation in embB. The study was conducted by using samples of 35 TB isolates from the center of thoracic diseases in Al-Samawah city in Iraq, the proportion method was used to check the isolates for EMB sensitivity (Esfahani et al., 2016) at first.

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The results showed that the concentrations of 2, 5, and 10 µg/ml represented 20 (57%) of the isolates were resistant to (EMB), and 15 (42.85%) of the isolates were susceptible to EMB by the real-time PCR method, the cycles amplification of the DNA templates were monitored by detection of the level of fluorescence continuously measures. For samples as well as for the positive control, the fluorescent signals started to rise at many cycles, ranging between 14–35. Esfahani, 2017 studied embB mutation by using the PCR-SSCP method and reported that Mutations in MTB are a locus of embB that are determined in EMB resistant bacteria and their sequence in the operon in genetically distinct sensitive and resistant bacteria (Esfahani et al., (2016), Sreevatsan et al., (1997)).

On the other hand, Starks AM, et al. 2008 used the MIC technique to detect spontaneous mutants of wild-type embB, and his data indicated that embB 306 mutations are capable of allowing ethambutol resistance, and investigation of these mutations should also be considered when developing rapid molecular detection tests (Angela, et al., (2009) , Rezaei et al., (2016)).

Our results appeared the ratio of males was higher than that of females; these findings are in line with the previous study (Al-Jubouri, et al. (2017).

WHO explained that the male ratio was higher than the female. Globally, men suffered more than women who have TB infections. This is due to the risk factors with men such as smoking, drug abuse alcoholism; furthermore, biological procedures such as the effect of hormones on sex are responsible for the significant difference between sex susceptibility to tuberculosis (Nhamoyebonde and Leslie (2014).

Conclusion

Through our experience, we found the real-time PCR technique a good tool to detect embB mutation. Furthermore, the study explained the difference between the genders and ages according to embB mutations. The findings can be applied for guiding the therapy.

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