

Evaluation of GeneXpert MTB/RIF for determination of rifampicin resistance among new tuberculosis cases in west and northwest Iran

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Abstract

Despite a *Mycobacterium tuberculosis* control programme and anti-tuberculosis drugs, drug-resistant tuberculosis (DR-TB) is one of the most serious public health issues worldwide. Rapid laboratory diagnosis of *M. tuberculosis* is needed for the diagnosis of multidrug-resistant (MDR) TB and to find the optimal treatment protocol. The purpose of this study was to detect resistance to rifampicin in new cases of TB using the GeneXpert MTB/RIF (*M. tuberculosis*/rifampicin) assay and the standard proportional method in west and northwest Iran. In this descriptive cross-sectional study, sputum samples were enrolled and screened for *M. tuberculosis* using Ziehl–Neelsen stain and mycobacterial culture. Samples from individuals with smear-positive TB were cultured on Lowenstein–Jensen medium; afterwards, the presence of resistance to rifampicin was examined by the GeneXpert MTB/RIF and standard proportional methods. A total of 400 new cases of suspected TB were collected, 162 (40.5%) of which were smear- and culture-positive for *M. tuberculosis*. The frequencies of rifampicin resistance in new smear-positive TB cases were 3.1% and 4.3% for GeneXpert and standard proportional method, respectively. Sensitivity and specificity of GeneXpert were 71% and 100%, respectively, compared with the proportional method. GeneXpert can be a quick and helpful method for the diagnosis of rifampicin-resistant TB in regions with high rates of DR-TB or MDR-TB. GeneXpert MTB-RIF assay must be used as an early diagnostic method whose results must be confirmed by the standard proportional method. The GeneXpert and proportional methods complement but do not replace each other.

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Introduction

Despite control programmes for *Mycobacterium tuberculosis* and anti-tuberculosis (TB) drugs, *M. tuberculosis* continues to be a major threat to global health; furthermore, it is the second leading cause of death worldwide [1,2]. According to the WHO, >9 million new cases of *M. tuberculosis* infection occur

each year, resulting in approximately 2 million deaths around the world [3]. Early diagnosis of diseased individuals can reduce the treatment period and transmission; and so it can decrease the burden of TB. Multidrug-resistant (MDR) TB is defined as *M. tuberculosis* that is resistant to at least rifampicin and isoniazid. Usually, >90% of rifampicin-resistant strains also show resistance to isoniazid [4–7].

Drug susceptibility testing is used widely and seems to be the most efficient method; however, the high costs prevent its widespread application [8]. The proportional method is the reference standard method that is widely used for *M. tuberculosis* diagnosis in Iran; however, this method takes 2–4 weeks to show results, and it must be performed by a laboratory specialist [9].

The rapid detection of *M. tuberculosis* in infected individuals is essential for disease management [5]. The GeneXpert MTB/RIF

(*M. tuberculosis*/rifampicin) assay is an automated, closed system and real-time PCR that is used in addition to Ziehl–Neelsen smears and mycobacterial culture [10]. The GeneXpert system has been approved by the WHO to be a widely used molecular diagnostic platform for the rapid detection of TB in several countries [11,12]. GeneXpert is capable of detecting rifampicin resistance in pulmonary and extrapulmonary specimens from clinical cases of TB. The GeneXpert can detect mutations in the *rpoB* gene and show the results in <2 hours [13,14].

Early diagnosis and treatment of TB and MDR-TB is important to control *M. tuberculosis*. GeneXpert cannot be used worldwide; but it is useful for regions with high rates of MDR-TB. Moreover, studies of GeneXpert have not been conducted in west and northwest Iran. The purpose of this study was to assess resistance to rifampicin in new cases of *M. tuberculosis* infection using the GeneXpert MTB/RIF assay and the standard proportional method.

Materials and methods

Bacterial isolates

The population under this cross-sectional study consisted of 400 sputum cases of suspected *M. tuberculosis* infection among new TB cases. Sputum samples were gathered from ten provinces of west and northwest Iran (East Azerbaijan, West Azerbaijan, Ardabil, Hamadan, Kurdistan, Zanjan, Ilam, Qazvin, Lorestan and Kermanshah) from March 2014 to November 2014, and moved to the TB reference laboratory of Kermanshah City, Iran. Sputum samples were screened for *M. tuberculosis* using Ziehl–Neelsen smear and mycobacterial culture [15]. New smear-positive TB cases were cultured on Lowenstein–Jensen medium and *M. tuberculosis* complex strains were identified on the basis of biochemical tests such as pigment production, niacin, nitrate reduction and the 68°C catalase test [16].

Antimicrobial susceptibility testing

Drug susceptibility testing against rifampicin (40 mg/L) and isoniazid (0.2 mg/L) in new smear-positive TB was performed by the proportional method on Lowenstein–Jensen medium. Drug resistance to rifampicin in TB was >1% in the rifampicin-containing media compared with the rifampicin-free media [15].

GeneXpert MTB/RIF

This method (Cepheid Xpert MTB/RIF assay G4 version 5) was performed on smear-positive sputum samples according to the manufacturer's recommendations. Briefly, after liquidation of sputum by shaking with the kit suspension 10–20 times, 2 mL of specimen was inserted into the GeneXpert MTB/RIF cartridge for PCR testing.

Statistical analysis

Sensitivity and specificity of the proportional method and GeneXpert MTB/RIF assay in the diagnosis of drug resistance to rifampicin was calculated using the following formula:

$$(\text{Sensitivity} = a/a + b, \text{Specificity} = d/c + d)$$

where a, true positive; b, false positive; c, false negative; d, true negative.

Results

A total of 400 non-duplicated new cases of suspected TB were collected in west and northwest Iran, 162 (40.5%) new TB cases were smear- and culture-positive for *M. tuberculosis*. Kermanshah and East Azerbaijan provinces had the highest incidence of new smear-positive TB—19% and 17.3%, respectively.

Mean age (\pm SD) of the individuals with new cases of smear-positive TB was 51.6 \pm 20.3 years with minimum and maximum ages of 15 and 89 years, respectively. Seventy-six were women (46%) with a mean age of 51 years and 86 were men (54%) with a mean age of 52 years. All smear- and culture-positive cases were tested for detection of antibiotic susceptibility using two tests, proportional method and GeneXpert MTB/RIF assay. The frequency of isoniazid resistance in new TB cases was 3.1% by the proportional method (Table 1).

Overall, both tests were positive in five individuals (3.1%). Thirteen of the smear-positive cases were positive for resistance to rifampicin using the proportional method. Comparison of the proportional method and GeneXpert revealed that among the eight samples that were found to be discordant, six cases of mixed *M. tuberculosis* and non-tuberculous mycobacterial infection were observed while in two of the eight cases, GeneXpert was not capable of diagnosing rifampicin-resistant TB. In total, seven (4.3%) of the new TB cases were true-positive for resistance to rifampicin using the proportional method.

Multidrug resistance was found in 0.6 of new TB cases (1/162). Sensitivity and specificity of GeneXpert compared with that of the proportional method were 71% and 100%, respectively.

Discussion

Drug-resistant TB is one of the most serious public health issues worldwide [2,7]. The major reasons for the increasing number of DR-TB are that some techniques need special equipment, and many techniques are expensive and may need time-consuming, proper diagnosis in high-prevalence countries [7,17].

TABLE 1. Prevalence of *Mycobacterium tuberculosis* and resistance to rifampicin and isoniazid by GeneXpert and proportional method in this study

Provinces of Iran	Samples	Positive smear (%)	Resistance (%)		
			GeneXpert <i>M.tuberculosis</i> /Rifampicin		Proportional method Rifampicin/ Isoniazid
			Rifampicin	Rifampicin	Isoniazid
Kermanshah	84	31 (19.1%)	2	2	1
East Azerbaijan	78	28 (17.3%)	1	1	0
Lorestan	59	27 (16.7%)	1	1	0
Kurdistan	43	23 (14.2%)	1	2	0
Ardabil	30	14 (8.6%)	0	0	2
West Azerbaijan	36	12 (7.4%)	1	1	0
Hamadan	23	11 (6.8%)	0	0	1
Ilam	22	6 (3.7%)	0	0	1
Qazvin	17	5 (3.1%)	0	0	0
Zanjan	8	5 (3.1%)	0	0	0
Total	400	162	5 (3.1%)	7 (4.3%)	5 (3.1%)

In the current study, 162 (40.5%) sputum smear-positive TB cases were identified. In our study, there were inconsistent results between the GeneXpert assay and the proportional method. In the comparison of the proportional method and GeneXpert, eight controversial cases were observed that were diagnosed by the proportional method but not using the GeneXpert method. There was evidence of mixed infections with *M. tuberculosis* and non-tuberculous mycobacteria in six of the eight discordant samples in which resistance to rifampicin was detected by the proportional method. Other studies have reported co-infection with *M. tuberculosis* and non-tuberculous mycobacteria [18–20]. In total, seven (4.3%) of the new TB cases were true positive for resistance to rifampicin by the proportional method and in five cases both tests were positive.

The proportional method is unable to check the colony morphology of the growing bacteria. It is clear that the proportional method had low specificity as it was only able to diagnose resistance to rifampicin but not rifampicin-resistant *M. tuberculosis*. Hence it only identified rifampicin resistance not specifically rifampicin-resistant *M. tuberculosis*, hence its low specificity. In contrast, GeneXpert was not capable of diagnosing mixed *M. tuberculosis* and non-tuberculous mycobacteria infections and their resistance to rifampicin.

Of the eight inconsistencies between the two methods, the proportional method was able to detect rifampicin-resistant TB in two cases whereas GeneXpert was unable to do so.

These findings demonstrated that GeneXpert can detect a large number of *rpoB* mutations, but not all mutations that cause rifampicin resistance.

The results of some studies were different in some regions. For example, the sensitivity and specificity of the GeneXpert assay in *M. tuberculosis* samples from Africa, South Africa and Turkey have been reported to be 92.7%–100% and 96.3%–100%, respectively [21–25]. Whereas sensitivity of the GeneXpert assay in Vietnam and Malaysia was reported to be

59% and 53%, respectively [24,26]. Sensitivity and specificity of GeneXpert in our population were 71% and 100%. Moreover, the WHO endorsed the use of GeneXpert MTB/RIF assay for the detection of *M. tuberculosis* with rifampicin resistance, which is the most useful assay for regions with high rates of human immunodeficiency virus–*M. tuberculosis* co-infection or MDR-TB [21,27]. The variations of the specificity and sensitivity of the GeneXpert assay found in studies originate from the geographical features of the sampling locations, differences in sampling method, and MDR-TB and mutations on the *rpoB* gene in populations.

According to the WHO, among Middle Eastern countries, Iran has a high prevalence of TB with MDR of 1.3% in 2015 [28]. Mono-resistance to rifampicin is rare. Resistance to rifampicin is often a marker for drug resistance, and almost 90% of rifampicin-resistant strains are also resistant to isoniazid [4,6]. Surprisingly, resistance to isoniazid was detected in 3.1% of samples in our study, whereas MDR in new TB cases was obtained in 0.6 (1/162). The frequency of MDR-TB determined the efficiency of GeneXpert. In this study, the low positive predictive value of GeneXpert for rifampicin resistance was in the low prevalence of MDR-TB and the result may be confirmed by the proportional method.

According to the information mentioned above, and the results of the current study, we concluded that GeneXpert can be a quick and helpful method for the diagnosis of rifampicin-resistant TB that needs minimal technique and can be operated by non-specialist laboratory staff. Furthermore, GeneXpert can provide results in a short period of time, as it is not necessary to wait for smear results like in the proportional method. As a result, treatment can be started more quickly.

It seems that the results of GeneXpert must be confirmed by the standard proportional method. GeneXpert and proportional method complete but do not replace each other. This study was one of the first studies of GeneXpert in Iran. We

suggest that more studies need to be conducted in different regions of this country to evaluate the efficacy of GeneXpert, and whether it can be useful and effective in Iran.

Transparency declaration

The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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