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ORIGINAL ARTICLE

PRIMARY RESISTANCE OF *MYCOBACTERIUM TUBERCULOSIS* TO ISONIAZID, STREPTOMYCIN, RIFAMPIN, AND ETHAMBUTOL IN PULMONARY TUBERCULOSIS

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Abstract

Background-Paucity of information about primary drug resistance in this region and the necessity of obtaining information due to worldwide emergence of multidrug resistance tuberculosis (MDR-TB) prompted this study.

Methods-A total of 165 specimens from cases with pulmonary tuberculosis were selected in 3 consecutive years by the non-probable convenience method. This sample included about 1/3 of all sputum/washing-positive pulmonary tuberculosis (TB) cases . Direct microscopy we used according to the "International Union against Tuberculosis and Lung Disease" (IUATLD) guideline; for culture, isolation and sensitivity test we applied the WHO guideline in order to compare our results with those of national and international studies on primary drug resistance. Relapse cases or patients with previous history of anti-TB treatment and mycobacteria other than Mycobacterium tuberculosis (including Mycobacterium bovis) were excluded from the study by careful enquiry of records and laboratory data.

Results-Seventeen cases were excluded from the study and the remaining 148 had the clinical and laboratory criteria of primary drug resistance. Mean age of the patients was 44.01±18.23 and 56% were males. All patients had clinicoradiological findings of pulmonary tuberculosis. Single primary drug resistance to isoniazid (INH) and streptomycin (SM) were 4.05 % and 8.78 % and total primary resistance were 7.43% and 12.83 % respectively. Combined resistance to INH+SM was seen in 3.38% of cases, and no resistance was detected to rifampin (RMP) and ethambutol (ETB) amongst new patients affected by *Mycobacterium tuberculosis*. Resistance to RMP and ETB was noticed only in patients with a previous history of anti-TB therapy (secondary resistance) or in patients with non-tuberculous mycobacteria.

Conclusion-In this study which was conducted on patients with pulmonary TB, the highest primary resistance was towards SM and INH alone or to both of these drugs. There was no primary resistance to RMP or ETB, and hence the possibility of MDR-TB is negligible in our region.

Keywords • Mycobacterium tuberculosis • isoniazid • ethambutol • rifampin • streptomycin

Introduction

Drug resistance has been known since the discovery of the first anti-TB drug, streptomycin , in 1954 and the presence of resistant mutants in wild populations of mycobacteria has been well documented. Primary resistance is the resistance pattern seen in new patients, who have not been exposed to anti-TB drugs previously. Secondary resistance is the resistance pattern in patients with previous history of anti-TB treatment and is due to ineffective chemotherapy. Surveillance of the primary and secondary resistance patterns are important in assessing the

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quality of chemotherapy programs over several years and detecting errors in past treatments respectively.[1-4] This has been performed for years in many countries. Unfortunately, due to selection bias and the variety of methods used in the isolation and sensitivity tests, old studies are not comparable.[5-13] Scattered studies on drug resistance in Iran, mostly by the Pasteur Institute was not free of the above-mentioned surveillance errors.[3] Because of the emergence of multidrug resistance tuberculosis (MDR-TB), especially after the HIV epidemic, surveillance of the drug resistance by using a single universal method with applicability even to countries with poor economy has been one of the WHO priorities in estimation of the situation all over the world.[14] Our study is based on the WHO guidelines. Local facilities are utilized directly for assessing primary resistance and indirectly the quality control of the mycobacteriologic laboratory by comparing results with the national survey.

Materials and Methods

Specimens were obtained from patients with pulmonary tuberculosis, which were all diagnosed according to clinical and radiological findings. The specimens included sputum (83%), bronchial washes (20%), gastric aspirate (one case) and sinus pus (one case) and included a little more than 1/3 of all sputum/bronchial wash-positive cases. All these specimens were selected by the non-probable convenience method. The mean age and standard deviation (SD) of the patients was (44.01±18.23), among whom 56% were males. For direct microscopy we used the IUATLD guideline[15] and for culture, strain isolation and sensitivity we applied the WHO/ IUATLD guideline[16] for drug resistance surveillance with exclusion of the reference laboratory. Instead, some of our specimens were sent blindly to the National Tuberculosis Center, Tehran, for double check with the results of national drug resistance sponsored by WHO. The culture medium was prepared with local facilities and its quality was determined by the National Tuberculosis Laboratory, in Tehran. Critical concentration for isoniazid (INH), rifampicin (RMP), ethambutol (ETB), and streptomycin were 0.2, 40, 2, 40 mic/ml respectively. For interpretation of resistance we applied the proportion method. For isolation of mycobacteria we used the above-mentioned guideline with attention to colony type, pigmentation, niacin test, nitrate reduction test and TCH resistance test. Exact determination of non-tubeculous mycobacteria was not possible.

Results

A total of 165 specimens were studied, 56% of whom were males. The mean and standard deviation of their age was 44.01±18.23. *Mycobacterium tuberculosis* was isolated in 155 cases (148 new patients and 7 known cases) while non-tuberculosis mycobacteria including *M. bovis* were isolated in just 10 cases (8 of which were new cases).

The sensitivity pattern of all mycobacteria (primary and secondary resistance to *Mycobacterium tuberculosis* and non-tuberculosis) and primary resistance in new patients are shown in Tables 1 and 2 respectively. Regarding Mycobacterium tuberculosis, 5 cases were resistant to both isoniazid and streptomycin and one case was resistant to 3 drugs. In non-Mycobacterium tuberculosis, secondary resistance to all drugs was seen in one patient while the other had resistance to 3 drugs.

Table 1. Primary and secondary resistance to INH, RMP, ETB and SM in Mycobacterium tuberculosis and non-tuberculosis patients.

Type of resistance	Resistance to isoniazid (%)	Resistance to streptomycin (%)	Resistance to ethambutol (%)	Resistance to rifampin (%)
Primary resistance to M. tuberculosis (n=148)	11 (7.43)	19 (12.83)	0	0
Secondary resistance to M. tuberculosis (n=7)	1 (14.28)	1 (14.28)	0	1 (14.28)
Primary resistance to M. non-tuberculosis (n=8)	5 (62.5)	3 (37.5)	3 (37.5)	2 (25)
Secondary resistance to M. non-tuberculosis (n=2)	2 (100)	2 (100)	2 (100)	1 (50)

Table 2. Primary resistance of *M. tuberculosis* to INH, RMP, SM and ETB (n=148).

Type of resistance	Number of resistant cases (%) [$CI_{95\%}$]
Total resistance to isoniazid	11 (7.43%) [6.9-8.2]
Total resistance to streptomycin	19 (12.83%) [7.4-17.4]
Single resistance to isoniazid	6 (4.05%) [3.2-4.7]
Single resistance to streptomycin	13 (8.78%) [4.5-14.2]
Multiple resistance to isoniazid and streptomycin	5 (3.37%) [0.4-6.3]
Overall resistance	25 (16.89%) [11-23]

Discussion

Our study was performed with the WHO/IUATLD laboratory guidelines and shows major primary resistance to INH and SM. This is not surprising because these two drugs were introduced earlier than other drugs. Amount or resistance to INH and SM is something between the observed percentages in well-developed and under-developed countries. We can draw few explanations for absence of resistance to ETB and RMP. First of all, the number of the specimens is not enough to reach a firm conclusion about the resistance to ETB and RMP. Between the published series, resistance to these two drugs has been low or very low in comparision to INH or SM and surveillance of more specimens (300-600) is needed for detecting a low resistance rate.[16] In collected data of 35 countries published by Pablos-Mendez[14] the mean primary resistance to RMP and ETB was 1.8% and 1% respectively and this is in favor of our results. In the report of Pablos-Mendez the highest resistance to ETB and RMP were from Latvia, the Republic of Dominican, Thailand and Estonia and this is explainable both with HIV infection and disrupted TB control system.[14,17] Second, in our study region, anti-TB drugs were not available for subscription by private physicians and this may contribute to good control of patients and prevention of treatment failure. Third, we can not absolutely rule out laboratory errors. Although, we possess no means for quality control of our laboratory by the WHO approved reference laboratories, but we had the opportunity for cross-checking of 22 specimens in the national drug resistance surveillance sponsored by the WHO. Results of this control have been depicted in Table 3.

As it is evident from Table 3, in 17 of 22 cases, there is complete concordance between our study and the national study. If we consider the clinical condition and laboratory results in patient1, 4 and 5 of Table 3, the correlation is better in the Tabriz study as compared to the national survey. Fourth, emergence of MDR-TB has a strong relation to HIV infection. Although all our patients were not screened for HIV, but random studies both in our patients and in TB patients originating from the general population of Tehran has revealed no evidence for HIV infection. But due to HIV epidemy in South-West Asia and problems of immigrants we should not ignore this major health-threat and reliance on directly observed therapy (DOT) is a key point for combating against TB in such situations.[17] Finally, delicate attention to the medical history of the patients is mandatory for surveillance of primary drug resistance. We have excluded 17 cases from the study due to previous anti-TB medication use or history of TB treatment. Resistance to all drugs including RMP and ETB is quite common in these patients and reluctance in history taking may

Table 3. Comparison of Tabriz and national study on drug resistance (n=22).

Tabriz Study	National Study	Comment
Fully susceptible MT strains (n=17)	100% concordant	Three patients were not new cases in Tabriz study
Resistant to INH+SM+ETB (n=1)	Resistant to INH+SM	Strain was not MT in Tabriz study
Fully sensitive to four drugs (n=1)	Resistant to INH+SM	MT strain
Resistant to INH (n=1)	Sensitive to four drugs	MT strain
Resistant to INH+SM+ETB (n=1)	Resistant to INH	Non-MT, pigmented in Tabriz study
Resistant to INH+RMP (n=1)	Resistant to INH	Known TB patient with history of treatment failure

contribute to a false positive increase in the prevalence of primary resistance.

In conclusion, our study shows a high primary resistance to SM and INH and absence or very low resistance to RMP and ETB. This correlates with a history of anti-TB medication use in our region. The first two drugs were introduced earlier than others. The prevalence of resistance to SM was highest in our study, hence we suggest replacement of this drug by ETB to obtain better results whenever possible. Reliance on DOT is also a major point in the prevention of resistance and for attaining successful results.

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