Drug-resistant pulmonary tuberculosis in western Turkey: prevalence, clinical characteristics and treatment outcome

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BACKGROUND: Although high antituberculosis (anti-TB) drug resistance rates have been reported in Turkey, the clinical characteristics and implications for the outcome of anti-TB treatment have not been fully investigated. We determined the prevalence of anti-TB drug resistance and examined demographic data, clinical characteristics and treatment outcome in relation to patterns of resistance.

METHODS: From the TB case registry of a university hospital and the two largest dispensaries in Manisa city, we identified all pulmonary TB cases with a culture-proven definitive diagnosis and antimicrobial susceptibility results for a 7-year period. We collected and analyzed demographic and clinical data and information on treatment outcome for those cases in relationship to anti-TB drug resistance.

RESULTS: Of 355 *M. tuberculosis* strains, 71.5% were susceptible to streptomycin, isoniazid, rifampicin and ethambutol. Any drug resistance and multi-drug resistance (MDR) rates were 21.1% and 7.3% and were higher in males (53% and 9%, respectively) than in females (22% and 1%, respectively). Drug resistance was significantly higher in old cases (acquired drug resistance) vs new cases (primary drug resistance), and was associated with treatment failure ($P<0.001$). The prevalence of MDR was significantly higher in the old cases (22.4%) than in the new cases (4.4%) ($P<0.001$). Symptoms, radiographic findings, associated diseases, and sputum smear positivity were unrelated to the development of resistance. The prevalence of any drug resistance and MDR was significantly higher in those with treatment failure than in patients with treatment success.

CONCLUSION: High resistance rates, particularly for acquired MDR, indicate a need for improvement in the TB control programme in our region.

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Tuberculosis (TB) is a serious threat to human health. Despite remarkable achievements, the estimated number of new cases of TB in the world during each of the past several years has steadily increased from 8.0 million in 1997 to 8.3 million in 2000, and is expected to reach 10.2 million in 2005.[1] Antituberculosis (anti-TB) drug resistance is a major factor threatening the success of TB control programmes.[2] Patients infected with bacilli resistant to multiple drugs are difficult to cure, and treatment is much more toxic and expensive. Epidemiological studies for the assessment of local resistance rates and the detection of multidrug-resistant (MDR) TB are therefore important to optimize drug therapy and prevent the dissemination of resistant strains in the community. Although high anti-TB resistance rates have been reported in various hospital-based studies in Turkey,[3-6] relationships

to demographic and clinical characteristics and effect on the outcome of anti-TB treatment have not been fully investigated in this country. The objective of the study was first to determine the prevalence of drug resistance among strains of *Mycobacterium tuberculosis* isolated from pulmonary TB cases in western Turkey, including the city of Manisa, which has 1.2 million inhabitants and is the most crowded city in the region. We then examined the relationship of anti-TB drug resistance to demographic data, clinical characteristics and treatment outcomes.

Methods

Using a computerized database, we reviewed the TB case registry and found all consecutive pulmonary TB cases with a culture-proven definitive diagnosis and results of antimicrobial susceptibility tests from cases diagnosed between January 1997 through December 2003 in the Celal Bayar University Hospital and two local dispensaries. The hospital is a 400-bed tertiary care center that serves Manisa district and is the only center that performs susceptibility testing for *M. tuberculosis*. Specimens collected in the dispensaries are sent to the regional tuberculosis laboratory for culture and susceptibility testing. We collected demographic and clinical information, including age, sex, history of pulmonary TB, clinical symptoms and signs, the extent of pulmonary involvement based on chest radiograms, associated diseases, treatment outcomes, and drug resistance results. Repeated isolates with the same antibiogram from the same patient were regarded as one strain. Clinical and outcome variables were analyzed with respect to drug resistance patterns.

In the regional tuberculosis laboratory, susceptibility testing of TB isolates to four first-line antimicrobial agents, isoniazid, rifampicin, ethambutol and streptomycin, was performed by the indirect proportion method with Lowenstein-Jensen medium, as described by Canetti et al.[7] The following drug concentrations were used to distinguish resistant isolates from susceptible isolates: streptomycin (S), 5 μg/mL; isoniazid (H), 0.2 μg/mL; rifampicin (R), 40 μg/mL; ethambutol (E), 2 μg/mL. In the university hospital, all *M. tuberculosis* isolates were tested using BACTEC 460 TB (BD Diagnostic Systems, Sparks, MD, USA) against the same drugs.[8] The following concentrations were used; streptomycin, 2.0 μg/mL; isoniazid, 0.1 μg/mL; rifampicin, 2.0 μg/mL; and ethambutol, 2.5 μg/mL. The *M. tuberculosis* H37Rv strain was used as a control strain.

Resistance was defined using guidelines of the World Health Organisation (WHO) and the International Union Against Tuberculosis and Lung Disease (IUATLD).[9] Any drug resistance was defined as resistance to one or more first-line drugs, whereas multidrug resistance was defined as resistance to both isoniazid and rifampicin with or without resistance to other agents. A patient who had not received prior anti-TB treatment for more than one month was classified as a new case. A patient who had received prior treatment for more than one month or who had been recorded as a treatment failure, relapse, return-after-default or a chronic case was classified as an old case or previously treated patient. Treatment failure was defined as failing anti-TB treatment, i.e., having began treatment for smear positive pulmonary TB and remaining smear positive at five months or later during the course of treatment. Relapse was defined as becoming smear-positive again after having been treated for TB and declared cured. Return-after-default was defined as interrupted treatment for more than two months after having received a total of at least one month of anti-TB treatment and then returning with bacteriologically confirmed TB. Chronic cases were patients who continued to be smear positive after the completion of a re-treatment regimen.[9] Patients diagnosed with TB who acquired resistance to one or more of the anti-TB drugs used during treatment were considered to have developed acquired drug resistance (old cases). Patients with TB who harboured organisms resistant to one or more drugs, but had never been previously treated for TB or had been treated for less than one month were considered to have primary resistance (new cases).

Response to treatment was categorized as a success or failure based on the clinical assessment, radiographic improvement or deterioration, and the results of follow-up microbiological examination of sputum. Treatment success was defined as resolution of radiologic changes, clinical improvement and negative sputum culture for *M. tuberculosis* at the end of the course of the treatment.

Data analysis was carried out using the SPSS version 10.0 sofware package (SPSS Inc, Chicago, IL, USA) for Windows. Pearson's chi-square or Fisher's exact test was used for proportions. P value of <0.05 was considered significant.

Results

From January 1997 to December 2003, antimicrobial susceptibility testing was performed for 355 strains isolated from patients with pulmonary TB in the study area. A total of 254 (71.5%) *M. tuberculosis* strains

were susceptible to all drugs tested. The frequency of any drug resistance and MDR was 21.1% (75 strains) and 7.3% (26 strains), respectively (Table 1). Any type of resistance was most common to isoniazid, with 60 strains (16.9%), followed by streptomycin (53 strains, 14.9%), ethambutol, (35 strains, 9.8%) and rifampicin, (32 strains, 9.0%). There were no significant differences in the prevalence of drug resistance across the 7-year period (P=0.8).

Of 355 pulmonary TB cases, 273 (77%) were males and 82 (23%) were females. The median age of the patients was 45±15.24 years for males and 44±18.79 years for females. There were no significant differences in drug resistance between age groups (Table 2). The prevalence of any drug resistance was 15.0% in males and 14.6% in females (P=0.9) for streptomycin, 17.9% in males and 13.4% females for isoniazid (P=0.3), 11.0% in males and 2.4% in females for rifampicin (P=0.01), 11.4% in males and 7.3% in females for ethambutol (P=0.2), respectively. Both rifampicin resistance and MDR were higher in males. There were no significant differences in drug resistance pattern with respect to educational level, socioeconomic status or living area (rural-urban).

The most common presenting signs and symptoms included chronic cough with sputum production, fever and weight loss (259 cases, 73%). Hemoptysis was associated with these signs in 82 cases (23%). Other symptoms and signs were present in 9 cases (3%) while there were no symptoms in 5 cases (1%). Drug resistance patterns were similar with respect to clinical symptoms and the extent of pulmonary involvement in chest X-ray findings. The sputum smear was positive in 311 (88%) cases, and 30% of these cases were resistant to at least one drug (68 any resistance and 24 MDR), but there was no difference in the drug resistance pattern between patients with positive and negative smears. Of the 355 cases, 79 (22.5%) had underlying diseases associated with pulmonary TB (diabetes mellitus, obstructive lung disease, malignancies, and others), but there was no relationship between resistance results and the presence of underlying diseases. The most common underlying disease was diabetes mellitus (34%).

The only risk factor for the development of drug resistant TB, other than sex, was history of treatment. Overall rates of resistance in new and old cases to one or more of the drugs tested were 25.3% and 44.8%, respectively (Table 3). The prevalence of MDR was significantly higher in the old cases (22.4%) than in the new cases (4.4%) (P<0.001).

Table 1. Antituberculosis drug resistance results in *M. tuberculosis* strains

Drug Resistance	No.	%
Sensitive to all 4 drugs	**254**	**71.5**
Monoresistance		
H	21	5.9
R	5	1.4
E	10	2.8
S	22	6.2
Total monoresistance	**58**	**16.3**
Multidrug resistance		
H+R	6	1.7
H+R+E	1	0.3
H+R+S	7	2.0
H+R+E+S	12	3.4
Total multidrug resistance	**26**	**7.3**
Other patterns		
H+E	5	1.4
H+S	5	1.4
H+E+S	3	0.8
R+E+S	1	0.3
E+S	3	0.8
Total other patterns	**17**	**4.7**
Total	**355**	**100**

H: isoniazid, R: rifampicin, E: ethambutol, S: streptomycin

Of the 355 cases, 285 (80%) were cured while 67 (20%) were regarded as treatment failures. Three cases moved out of the region. Of the 67 cases with treatment failures, 45 completed the treatment but had persistent positive cultures. Fourteen patients returned after default and 8 patients died. The prevalence of any drug resistance and MDR was significantly higher in those with treatment failure (22.4% for streptomycin, P=0.06; 28.9% for isoniazid, P=0.006; 22.4% for rifampicin, P<0.001; 19.4% for ethambutol, P=0.008 and 17.9% for MDR, P=0.001) than in patients with treatment success (13.3% for streptomycin, 14.4% for isoniazid, 6.0% for rifampicin, 8.4% for ethambutol, 4.9% for MDR, P=0.001) (Table 4).

Discussion

Precise epidemiological data on the rate of drug resistance to anti-TB agents are important for the success of treatment and the control of TB. According to the Department of Tuberculosis of the Ministry of Health, the population of Turkey in 2002 was

66.6 million, the number of new cases of TB was 18 038 and the incidence of TB was 27 per 100 000 population. These data were included in the WHO Report in 2002.[10] However, it is known that these numbers do not reflect the true rates due to insufficient notification of cases in Turkey.

We found that the rate of any drug resistance and MDR in *M. tuberculosis* strains in our region was 21.1% and 7.3%, respectively. Isoniazid resistance was the most common (16.9%). Rates in new and old cases, which are expected to be different, were 25.3% and 44.8% for any drug resistance and 4.4% and 22.4% for MDR, respectively. The most common form of acquired resistance was also against isoniazid (13.8% and 32.8%, for any and MDR, respectively).

Table 2. Drug resistance by sex.

Sex	Susceptible		Any Drug Resistance		MDR		Total	
	No.	%	No.	%	No.	%	No.	%
Male	195	71.4	53	19.4	25	9.2	273	100
Female	59	72.0	22	26.8	1	1.2	82	100
Total	**254**	**71.5**	**75**	**21.1**	**26**	**7.3**	**355**	**100**

*Chi square test, $P<0.02$

Table 3. Drug resistance rates (%) by history of treatment.

Drug resistance pattern	New cases (n=297)	Old cases (n=58)	*P* value (new vs. old)
Any drug resistance	25.3	44.8	0.001
S	12.1	29.7	0.001
H	13.8	32.8	0.001
R	5.7	25.9	0.001
E	8.4	20.7	0.005
Multidrug resistance	4.4	22.4	0.001
H+R	1.0	5.2	0.05
H+R+E	–	1.7	0.16
H+R+S	1.7	3.4	0.32
H+R+E+S	1.7	12.1	0.001

Table 4. Treatment outcomes of patients with drug-resistant tuberculosis.

	Treatment Success		Treatment Failure		Total	
	No.	%	No.	%	No.	%
Susceptible	211	84.1	40	15.9	251	100
Any drug resistance	60	80.0	15	20.0	75	100
MDR	14	53.8	12	46.2	26	100
Total	**285**	**81.0**	**67**	**19.0**	**352**	**100**

Chi square test, $P=0.001$

Resistance rates reported in previous studies carried out in different regions of the country were similar to our results.[3-6,11-14] The acquired MDR rate (22.4%) in our study is among the highest reported in Turkey (Table 5). Even though there was not a significant increase in the resistance rates in the past 7 years, the high level of acquired MDR rates in our region may be a serious problem for treatment and control of TB in coming years.

Anti-TB drug resistance occurred predominantly in males (77%). We believe that the reason for this difference is largely due to the lifestyle of the two groups. Most women in our region are housewives and the men go out to work. Therefore males are less compliant to treatment and are more prone to acquire resistant strains through community contacts. Other studies from different countries have also demonstrated that sex and social circumstances in the community can influence the epidemiology of TB.[15,16]

In our study the most commonly observed symptoms and signs were chronic cough with sputum, fever and weight loss (73%) and the most common underlying disease was diabetes mellitus. Since screening for human immunodeficiency virus (HIV) in TB patients is not performed routinely in our country (except in high risk groups) because of the low prevalence of HIV-infected cases, we did not investigate whether or not HIV infection was a risk factor for resistance. Resistance rates did not differ significantly with clinical presentation, radiographic findings or smear positivity of sputum. The only risk factor for the development of drug-resistant TB, other than sex, was a history of anti-TB treatment. In our study, overall resistance in old cases and new cases were 45% and 25%, respectively. Experience from a number of successful national control programmes assisted by WHO or IUATLD suggests that when a national tuberculosis control programme has been well implemented for several years, the rate of acquired resistance is around 20% among old cases and the rate of primary resistance is usually 5% or less.[17] Our results are higher than these rates and the tuberculosis control programme in our region should be evaluated in order to prevent the emergence of new drug resistant cases. Treatment success in our cases was significantly lower in MDR TB (54%). But 80% of any drug resistant cases and 84% of all drug susceptible cases were treated sucessfully (Table 4). In our region, directly observed therapy (DOT) has been carried out only in small groups of cases who live in urban areas for the past two years, and our experience is

Table 5. Resistance rates of *Mycobacterium tuberculosis* isolates in different regions of Turkey.

Years	Authors/location	No. of patients	Any Drug Resistance (%)		MDR (%)	
			Primary	Acquired	Primary	Acquired
1992	Tahaoglu K/ Istanbul[3]	785	26.6	53.4		
1976-1997*	Bengisun JS/Ankara[4]	3319	39.2		5.8	
1984-89	Goral G/Bursa[11]	203		32.5		12.3
1990-95	Goral G/Bursa[11]	328		37.5		24.4
1999	Kilicaslan/Istanbul[6]	1370	19.9	40.4	3.1	18.5
1995-1999*	Balci I/Gaziantep[12]	106	40.2		7.6	
1999-2000	Kartaloglu Z/Istanbul[13]	365	23.8		2.7	
1998-2001*	Kisa O/Ankara[14]	470	14.9		1.7	
2000	Durmaz R/Malatya[15]	88	32.9		2.2	
1997-2003	Present study/Manisa	355	20.9	22.4	4.4	22.4

*The results in the studies are shown as combined resistance rates.

insufficient to measure the efficiency of this newly implemented programme.

There are some limitations in our study. Because we could not obtain the registries of all cases, our results are not representative of the region as a whole. However, this study provides a guide to drug resistance rates in our region. The other limitation is the difference in drug susceptibility testing in the university hospital and the regional tuberculosis laboratory. It is recommended by WHO that laboratories should use the susceptibility method with which they are most familiar provided that it is one of the 4 internationally recommended methods, namely the proportion method, the absolute concentration method, the resistance ratio method, or the BACTEC method.[9] This is necessary to eliminate variability due to disruption of routine testing when changing to a new testing procedure. Most countries employ the simplified variant of the proportion method using Lowenstein-Jensen medium.[9] This method has also been used in the regional tuberculosis laboratories of the Ministry of Health in Turkey because of the limited resources in the health budget. But most of the laboratories in university hospitals employ the BACTEC method, one of the rapid susceptibility testing methods, used to assure the earliest possible detection of resistance. It is important that the standardization of drug susceptibility testing for *M. tuberculosis* in all laboratories be implemented for accuracy of results. Although susceptibility testing in this study differed among cases, the methods and drug concentrations comply with WHO recommendations, and we considered the results to reflect accurate resistance rates.

In conclusion, we found that resistance rates, especially for MDR in old cases is high in our region. The findings indicate a need for improvement in the tuberculosis control programme in our region. Clinical features of the cases investigated in this study were not found to be risk factors for the development of resistance in TB. Drug resistance in males was higher than in females. History of therapy and treatment failure was associated with higher drug resistance. Many clinical and sociodemographic characteristics of patients with TB are well known,[18-20] but there are not sufficient studies exploring these features in drug-resistant TB. We believe that determination of the prevalence of drug-resistant TB and examination of clinical features and treatment outcomes possibly related to resistance will contribute to the control and the management of these cases.

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