Patterns of drug resistance in pulmonary tuberculosis cases in the Izmir district, Turkey

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──────── **SUMMARY** ────────

Antituberculosis drug resistance patterns were investigated among the new and previously treated pulmonary tuberculosis (TB) cases in Izmir district, retrospectively. Proportions of resistance patterns were determined using a number of resistant cases using as a denominator. Resistance to at least one drug was found in 304 (29.7%) patients in 1023 a total of tuberculosis cases. 182 new and 82 previously treated consecutive pulmonary tuberculosis cases were investigated. Patterns were examined as single and/or probable combinations of isoniazid (H), rifampicin (R), ethambutol (E) and streptomycin (S). Single drug resistance mode, mono S, and HS resistance patterns were the highest proportions in comparison with other modes and patterns in both new and previously treated cases. HRS pattern showed a significant proportion and proportions of quadruple mode were higher than triple mode in previously treated cases. Proportions of patterns associated with R were detected more than expected. Surveillance of proportions of anti-TB drug resistance is important as well as surveillance of resistance rates.

KEY WORDS: Drug resistance, Drug resistance pattern, New cases, Previously treated cases, Tuberculosis

INTRODUCTION

The emergence of drug resistant strains of TB is a global threat to tuberculosis prevention and control efforts (WHO, 2004). Poor or suboptimal tuberculosis control programmes in both industrialized and developing countries can lead to emergence of drug resistance, especially if the prevalence of tuberculosis is high (Karabay, *et al.*, 2004).

Resistance of *Mycobacterium tuberculosis* to drugs is a man-made amplification of spontaneous mutations in the genes of the tubercle bacilli (WHO, 2004). Treatment with a single drug - due to irregular drug supply, inappropriate pre-scription, or poor adherence to treatment- permits the multiplication of drug-resistant strains. Since drug resistance develops because of inadequate use of drugs, antituberculosis drug resistance surveillance is, together with the monitoring of treatment outcome, an essential tool for evaluating the quality of tuberculosis control programmes (Schwÿbel, *et al.*, 2000). Surveillance and analysis of local rates of TB drug resistance is helpful in the detection and monitoring of the extent of multi-drug resistance (MDR) strains, indicating the quality of TB control in the country. Knowledge of the prevalence of drug resistance in new cases guides the selection of drugs used in initial treatment of tuberculosis. Resistance of previously treated cases is believed to be closely related to the efficacy of the treatment programme, and early diagnosis of MDR-TB helps to prevent its transmission in the community (Zwolska, *et al.*, 2000).

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Turkey has a moderate prevalence of TB with 26 per 100000 notification rate and 18500 total notified cases in 2003 (EuroTB, 2005). It is estimated that real numbers are at least twice as high. In Izmir district, TB incidence between 1995 and1999 was reportedly 44.8 per 100.000 (Kocamıs, 2005). Official drug resistance data are not available as the national tuberculosis laboratory was set up very recently. Resistance to anti-TB drugs has many dimensions and measurements. The aim of the study is to evaluate the possible combinations and proportions of H, R, S and E and four resistant modes, i.e. mono, double, triple and quadruple resistance instead of notifying the incidence of plain resistance rates, in the new and the previously treated cases in the Izmir district.

METHODS

Setting
The study was carried out by collaboration between one of the seven chest clinics and the microbiology laboratory Izmir Chest Diseases and Chest Surgery Training Hospital the sole tertiary referral hospital of the Izmir district and Western region of Turkey for tuberculosis and chest diseases. This hospital was notified 60% of all new TB cases between 1999 and 2003 in Izmir district. According to official data approximately 20% new cases (4400 cases) were notified from the hospital out of the whole country in 1995-1999 (Kocamıs, 2005).

Patients
Data were retrospectively collected from records of hospitalized pulmonary tuberculosis cases seen in the clinic between June 1994 and December 1999. All consecutive microbiologically confirmed pulmonary tuberculosis cases were eligible for the study; those which lacked reliable data on prior treatment were excluded.

Definitions
Drug resistance in mycobacteria is defined as a decrease in sensitivity to a sufficient degree to be reasonably certain that the strain concerned is different from a sample of wild strains of human type that have never come into contact with the drugs (Mitchison, 1984). Monoresistance is defined as resistance to one of the first-line drugs. Any drug resistance is indicated as total resistance for a drug with and without accompanying other drug resistance.

Polydrug resistance is resistance of *M. tuberculosis* strain to two or more of the first-line drugs. MDR is a special subgroup of polyresistance, in which there is resistance to at least rifampicin and isoniazid. Initial resistance is described as the resistant cases in which it is not known whether the patient has received prior treatment (Loddenkemper *et al.*, 2002).

Resistance among new cases is defined as the presence of resistant isolates of *M. tuberculosis* in patients who, in response to direct questioning, deny having had any prior anti-TB treatment (for as much as 1 month) and, in countries where adequate documentation is available, for whom there is no evidence of such a history. Resistance among previously treated cases is defined as the presence of resistant isolates of *M. tuberculosis* in patients who, in response to direct questioning, admit having been treated for tuberculosis for one month or more or, in countries where adequate documentation is available, in a patient for whom there is evidence of such a history (Loddenkemper *et al,.* 2002).

Microbiology
Lowenstein-Jensen (LJ) medium was used for cultures at least duplicate samples for every patient. Drug susceptibility testing (DST) of all isolates was done on LJ medium using the conventional proportion method described by Canetti *et al.* (Canetti *et al.*, 1969) Resistance was expressed as the percentage of colonies that grew on critical concentrations of the drugs. The ratio between the number of colonies growing on drug-containing and drug free medium should be greater 1% for resistance (10% for streptomycin). Drug critical concentrations were 0.2 µg/ml for H, 40 µg/ml for R, 2.0 µg/ml for E and 4.0 µg/ml for S. H37Rv strain is used for internal control strain for culture and DST (Canetti *et al.*, 1969; Laszlo *et al.*, 1997).

If more than one susceptibility test was performed per patient per year, initial test results were accepted. If both pulmonary and extra pulmonary isolates were analyzed for the same patient, pulmonary isolate was evaluated.

Resistance modes and patterns were determined

in proportional quantities using the numbers of resistant cases as denominator in this analysis.

Statistical analysis

Student's t-test was used for analyzing the significance of difference numerical and proportional quantities. P value of <0.05 was accepted as indicating statistical significance.

Proportions of resistance modes and patterns dealing with the references were calculated via the data, which is given the resistance rates and case numbers in the literature by authors.

The hospital ethics committee approved the study.

RESULTS

In all 1023 culture-positive cases were encountered from June 1994 to December 1999. Drug resistance was detected in 304 (29.7%) out of 1023 cases. Forty cases were excluded. Thus, 264 cases were eligible for the study. Drug resistance was detected in 182 new and 82 previously treated cases. Because the clinic was caring for male patients, all of the subjects of the study were male.

Most cases (approximately 85%; according to patients' records dealing with job, living standards, numbers of children, etc.) were living in low social-economic conditions. Eighty-two (31.06%) of 264 resistant cases were born at East and South-East of Turkey (the regions, which obtained the lowest living standards of country) and had moved to the West. Sixty-three (23.8%) cases had left the hospital of their own accord without completing the initial therapy.

The mean age of previously treated cases was 43.5 years (16-78) while the mean age of new cases was 39.2 years (14-83). Distribution of the cases according to age groups was seen in Table 1. Differences among age groups were not significant except for the 30-39 years interval. The proportion of drug resistant cases was significantly higher in the 30-39 year age group, and lowest in the 10-19 year age group (p<0.05).

From analysis of the data using the number of drug resistant-cases as denominator, we can make the following statements about proportions of resistance patterns:

Among new patients, single drug resistance was more common in new (69.2%) than previously treated cases. However, resistance to two, three, and four drugs was more common in previously

TABLE 1 - *Distribution of cases according to age groups.*

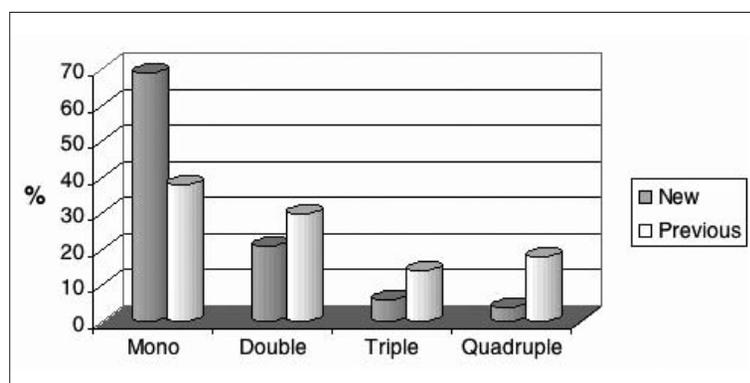| Age	10-19 N (%)	20-29 N (%)	30-39 N (%)	40-49 N (%)	50-59 N (%)	60 N (%)	Total
New	25 (13.7)	28 (15.3)	51 (28.0)	38 (20.8)	18 (9.8)	22 (12.0)	182 (100)
Previously treated	3 (3.6)	12 (14.6)	24 (29.2)	19 (23.1)	12 (14.6)	12 (14.6)	82 (100)
Total	28 (10.6)	40 (15.1)	75 (28.4)	57 (21.5)	30 (11.3)	24 (9.0)	264 (100)

FIGURE 1 - *Comparative distribution of the resistance modes between new and previously treated cases.*

TABLE 2 - *Numbers and proportions of new and previously treated resistant cases.*

Resistance patern	New resistant cases (n = 182)		Prev. treated resistant cases (n = 82)	
	n	%	n	%
Monoresistance				
H	23	12.6	5	6
R	18	9.8	6	7.3
S	59	32.4	12	14.6
E	26	14.2	8	9.7
Total	126	69.2	31	37.8
Double resistance				
HR	-	-	5	6
HE	4	2.1	3	3.6
HS	15	8.2	6	7.3
ES	13	7.1	5	6
RE	2	1.09	1	1.2
RS	4	2.1	4	4.8
Total	38	20.8	24	29.2
Triple resistance				
HRE	4	2.1	5	0.6
HRS	4	2.1	7	8.5
RES	-	-	-	-
HES	3	1.6	-	-
Total	11	6.0	12	14.6
Quadruple resistance (HRES)	7	3.8	15	18.2

treated than in new resistant cases (29.2% vs 20.8%; 14.6% vs 6.0%; and 18.2% vs 3.8%, respectively).

Figure 1 shows the comparison of the resistance modes in new and previously treated cases.

Among new cases, the four most frequent drug-resistance types, mono S (32.4%), mono E (14.2%), mono H (12.6%) and double HS (8.2%), accounted for 67.4% of all resistant cases.

Among previously treated cases the most frequent drug resistance pattern was HRSE (18.2%).

Proportions of any H, R and MDR patterns appeared higher in previously treated cases than new cases (p<0.05). Table 2 gives the details of the proportions of resistance patterns.

DISCUSSION

In Turkey, a country of 70 million inhabitants, tuberculosis is a major public problem. From 1980 to 2003 case notification rates of tuberculosis decreased from 80 to 26 per 100000. The rate of deaths due to TB was 262/100000 in 1945 in Turkey. Mortality rate declined from 7 to 4/100000 between 1990 and 2003 (Ozkara *et al*. WHO, 2005).

In Turkey, numbers of studies have been published about resistance rates in anti-tuberculosis drugs since 1953 (Ucan, 1994). Various resistance rates have been declared between 15%-45% as combined (new + previously treated) resistance (Ucan 1994; Yolsal *et al*., Dogan *et al.*, 2004; Bengisun *et al*., 2000; Ozsahin *et al*., 2000; Talay *et al*., 2003; Caglar *et al*., 2003; Ogul *et al*, 1999; Sevim *et al.,* 1999; Güneri *et al.,* 2004). In new and previously treated cases, 14%-37%, and 28%-63% rates had been reported, respectively (Uca, 1994; Yolsal, *et al.,* Dogan *et al.,* 2004; Bengisun *et al.,* 2000; Ozsahin *et al*., 2000; Talay *et al*., 2003; Caglar *et al*., 2003; Ogul *et al*, 1999; Sevim *et al*., 1999; Güneri *et al*., 2004; Kartaloglu *et al*., 2002). However, no study has been published mentioning the proportions of resistance modes and patterns. Proportions of the patterns shed light on the relationship between new and previously treated cases, amplification and major pathways of drug resistance creation.

When the national literature was examined for proportions of drug resistance patterns (Table 3), monodrug resistance mode was observed in higher proportions than poly-drug resistance modes in new cases. In previously treated cases, although monodrug resistance was the most frequent mode, double resistance mode was fluctuating in a wide range. Proportions of the triple and quadruple resistance mode were generally higher in previous cases than new resistant cases in the national studies. In our study, the proportion of quadruple resistance (18.2%) in previous cases was found elevated compared to other local studies.

Interestingly, the monoH proportion (12.6% in new and 6% in previous) was lower in both new and previous cases. This is bad news because R resistance is the major aspect for development the TB multidrug resistance, and monoR pattern (9.8%) was was relatively more frequent in new cases than in other national studies (1.1%-5.6%).

However, opposite to what was expected, the proportion of IR resistance was zero in new cases. This might be result of the low level of monoH proportion. For the same reason the IR proportion (6%) is lower in the previous cases in our district. Proportions of total MDR patterns show no significant differences among different studies (12%-25% in new and 18%-43% in previous).

When our findings were compared with the data from the WHO/IUATLD project on anti-tuberculosis drug resistance surveillance, proportions of resistance modes were found very close to results of global resistance.

However, proportions of monoH, HS, HRS and anyH patterns obtained were lower in both new and previous cases than the global proportions. As against, proportions of monoR, monoE, and anyE were found more in both groups. Proportions of MDR patterns in our study were seen in less than the average global proportions, possibly due to lower proportions of H combina-

TABLE 3 - *Comparison of our results with the global and national data.*

Patterns of resistance	New resistant cases			Prev. treated resistant cases		
	Our study %	*National studies (range) %*	*Global data %*	*Our study %*	*National studies (range) %*	*Global data %*
Monoresistance	69.2	55-79	60.9	37.8	24-71	35
H	12.6	41-43	26	6	18-25	17.9
R	9.8	1-5.6	3.2	7.3	0-8	4.6
S	32.4	5-25	29.4	14.6	4-30	10.6
E	14.2	0-30	2.3	9.7	0	2
Double resistance	20.8	13-24	24.9	29.2	25-40	25
HR	-	2.9- 4.2	3.8	6	12.2-21.4	10
HE	2.1	0-7	1.1	3.6	1.2-3.5	0.8
HS	8.2	2- 14	18.4	7.3	0-6	11.7
ES	7.1	0-1.9	0.6	6	0-1.8	0.4
RE	1.09	0-0.6	0.3	1.2	0-2.8	0.4
RS	2.1	0-12.6	0.7	4.8	0-12	1.7
Triple resistance	6.0	7-14	8.8	14.6	9-26	20
HRE	2.1	0-1.3	1	0.6	3.5-8.4	2.4
HRS	2.1	1- 12	5.8	8.5	9.7-17.8	15.3
RES	-	0-3.2	0.1	-	0-1.5	0.5
HES	1.6	0-4	2	-	0-2	2.2
Quadruple resistance (HRES)	3.8	1-7	5.3	18.2	3-19	19
ANY H	33	26-67	63	56	27-82	80
ANY R	21.5	11-66	20	52	19-74	54
ANY S	57.5	11-65	62	60	11-70	62
ANY E	32.5	2-43	12.6	45	2-43	29

tions. It is seen that proportions of monoH, monoS, HS and HRS resistance patterns were more prevalent than any other drug in both new and previous cases and HRS and HRSE proportions were much more frequent in the previous cases than in new cases in global research as well as in our study.

This relationship suggests amplification of resistance in our district as indicated by the WHO/IUATLD report for global trend.

In conclusion, with growing worldwide concern regarding TB drug resistance, a surveillance system is vital in providing the necessary data to monitor trends in TB drug resistance in Turkey. We believe that the proportions of the resistance patterns give us the resistance development pathways. Our results indicate that the proportions of TB drug resistance patterns in this country are similar to those in the overall global situation.

Although proportions of MDR patterns are not at a threatening level, proportions of monoR and anyR patterns are very high, necessitating closer monitoring of the treatment outcomes of individual patients as well as long-term follow-up for drug resistance on a nationwide scale. Drug resistance data are erratic in the country. Notification of the DST results along with clinical data is a key element for obtaining valid and representative information on drug resistance. Surveillance of anti-TB drug resistance should be adopted as an integral part of TB control programmes in Turkey.

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