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Prevalence and Risk Factors for Multidrug-Resistant Tuberculosis in Republic of Georgia: A Population Based Study

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SUMMARY

Setting: Multidrug-resistant TB (MDR-TB, defined as resistance to at least isoniazid and rifampin) has emerged as a serious global public health problem, especially in former Soviet republics. The extent of the problem in Georgia has been incompletely defined.

Objectives/Design: A population-based study was carried out between July 2005-May 2006 to determine the prevalence and risk factors for MDR-TB in Georgia.

Results: Among 1314 patients with AFB smear and culture positive pulmonary TB, 799 (60.8%) were newly diagnosed patients and 515 (39.2%) were previously treated. Overall, 733 (56%) patients had resistance to at least 1 anti-TB drug and 195(15%) had MDR-TB. Patients who had previously been treated for TB were significantly more likely to have MDR-TB than patients who were newly diagnosed and never previously treated (141/515 [27.4%] vs 54/794 [6.8%], OR=5.27, 95%CI 3.75-7.41]. In multivariate analysis, previous TB treatment (AOR=5.47, 95%CI 3.87-7.74) and female gender (AOR=1.58, 95% CI 1.02-2.32) were independent risk factors for the presence of MDR-TB.

Conclusions: Drug resistant TB including MDR-TB has emerged as a major public health problem in Georgia. Further TB control efforts being implemented to prevent the development of new cases of MDR-TB and to treat existing patients with MDR-TB are urgently needed.

Keywords

multi-drug resistant tuberculosis (MDR-TB); Drug Resistance Survey; Georgia

INTRODUCTION

Tuberculosis (TB) is widespread in the republics of the former Soviet Union (1). TB has emerged as major public health problem in the country of Georgia following the break up of the Soviet Union due to the sudden decline in socioeconomic status, increased poverty, a large number of internally displaced persons resulting from a civil war which occurred in 1991-1992 following Georgia's independence from the Soviet Union, and failure of TB control and other health services (2-4). According to the National Tuberculosis Program the annual incidence of TB rose from 29.5/100 000 to 165/100 000 between 1989 and 1996.

In 1996 National Tuberculosis Program (NTP) established a surveillance system for TB in Georgia. The Georgian NTP embraced the WHO-recommended Directly Observed Therapy, Short Course (DOTS) strategy but only in recent years has begun to aggressively implement DOTS. Despite modest declines, TB case rates remain high in Georgia. In 2005, the annual

new TB case notification rate (incidence) in Georgia was reported by the Georgian National TB Program (NTP) to be 97 cases per 100 000 population and total TB case notification rate (new and re-treatment cases) was 147 cases per 100 000 population (5). Prevalence of TB/HIV co-infection is 1.1% (4).

The prevalence and risk factors for multidrug resistant TB (MDR-TB) have been incompletely defined in Georgia. Drug resistant TB is thought to be a problem given that the Georgian NTP has observed high rates of treatment failure (4% of new sputum smear positive TB cases and 10% of retreatment smear positive TB cases), large numbers of chronic cases, and a suboptimal treatment success rates (68% of new sputum smear positive TB cases and 49% of retreatment smear positive TB cases) for the cohort of 2004(6). MDR-TB has been reported from inmates at Georgian correctional facilities in the late 1990s; the prevalence of MDR-TB was found to be 13% of all strains tested(7). Data from the civilian population has been limited. In a pilot study carried out at 4 sentinel sites in Georgia, 10.5% of newly diagnosed patients with TB and about 50% of re-treatment cases had MDR-TB(8). Thus, there has been a great need in Georgia for data from a representative sample to assess the degree of drug resistance. The purpose of our study was to assess the prevalence of and risk factors for drug resistant TB including MDR-TB in Georgia using a population-based approach.

METHODS

Study Design

This cross-sectional population-based study in the country of Georgia was carried out by the Georgian NTP based on WHO recommendations for an Antituberculosis Drug Resistance Survey (DRS)(9). The study took place from July 2005 to May 2006; all 75 NTP diagnostic centers in Georgia participated.

Sample size and Inclusion Criteria

A 100% sampling of all diagnostic centers was adopted for this study. Every patient self reporting to 75 TB facilities throughout Georgia who was registered as an acid fast bacilli (AFB) sputum smear-positive pulmonary TB case during the study period was included. A sputum smear-positive status was assigned according to WHO/IUATLD definitions (10). Previously treated sputum smear-positive pulmonary TB patients diagnosed during the survey period were enrolled in the study. Patients who were registered as a sputum smear-negative (ss−) case were not eligible for inclusion in the study during the inclusion period.

Study Organization and Data Collection

The National Tuberculosis Program network in Georgia is comprised of 75 TB units, 30 microscopy laboratories, 37 sputum collection spots, and one National Reference Laboratory (NRL) at the National Center for Tuberculosis and Lung Diseases (NCTBLD). Three sputum samples were collected from every patient suspected of having pulmonary TB who was seen at all 75 NTP TB units during the study period. Patients were instructed on how to collect sputum and supervised by a lab technician (11). Sputum smear microscopy of the specimens collected at the 37 sputum collecting spots was performed at the corresponding microscopic TB laboratories. One specimen from every patient that was AFB smear positive was sent to the National Reference Laboratory (NRL) in Tbilisi for AFB culture and drug susceptibility testing. A messenger (courier) system was established for delivering the sputa from 30 TB laboratories to the NRL. Sputum was transported in standard containers (70ml volume, firm, wide-mouthed, water-proof, with screwed-on cap) twice a week by couriers (the same as drivers). Three cars were used for this purpose – first one was moving through western Georgia, second one in eastern Georgia and the third one in Tbilisi. From the mountainous part of the country sputum samples were transported to nearest regional centers by lab technician.

At the microscopic laboratory, 1% cetyl pyridinium chloride was added to the sputum specimen as a preservative prior to transportation to the NRL.

Patient related data were collected on a standardized form by the physician providing care to the patient with suspected TB (9). The patient's treatment status (new vs. previously treated) was based on vigorous patient interview and validated by review of the patient's medical record whenever possible. The current TB recording and reporting system in Georgia was established in 1998; patients diagnosed with TB before 1998 had to be assigned treatment status based on history because medical records did not exist for these patients. In this case the accuracy of treatment history was confirmed by re-interviewing.

Definitions

A newly diagnosed TB case was defined as a patient who had never had treatment for TB or who received antituberculosis drugs for less than one month; retreatment cases were defined as patients who had a prior history of treatment with antituberculosis drugs for more than 1 month (12). Retreatment cases included relapses, treatment after failure, treatment after default and chronic cases. Multidrug resistant TB (MDR-TB) was defined as resistance to at least both isoniazid and rifampin(13). Mono-resistance was defined as resistance to a single first-line antituberculosis drug (isoniazid, rifampin, pyrazinamide, ethambutol, or streptomycin)(13). Polyresistance was defined as resistance to two or more of the first-line antituberculosis drugs, but not both isoniazid and rifampin.

Laboratory Methods

AFB smear microscopy—Smear status was assessed by the Ziehl-Neelson staining method (14).

AFB Cultures—Sputum specimens received at the NRL were decontaminated and treated with 4% NaOH solution for 20 minutes and then neutralized with a HCl/Phenol red solution. Specimens were centrifuged and the sediment was inoculated on to Lowenstein-Jensen media which was incubated at 37°C using standard methodologies as previously described (9).

Identification of *Mycobacterium tuberculosis* was done using the p-nitrobenzoic acid (PBN) and thiophene carboxylic acid hydrazine (TCH) resistance test (9).

Drug Susceptibility Testing (DST) was performed by the Absolute Concentration method(9). The following concentrations of first-line antibiotics were used in media: Streptomycin (S) – 4 □g/ml, Isoniazid(H) – 0.2 □g/ml, Rifampin (R) - 40 □g/ml, Ethambutol (E) - 2 □g/ml. DST for pyrazinamide (PZA) was not performed. The Georgian National Reference Laboratory participates in annual panels testing and validation of results and quality assurance was provided by the Antwerp WHO Supranational Reference Laboratory which indicates 100% concurrence.

Data Entry and Statistical Analysis

Data were double entered into a database using SDRTB3 software provided by WHO(15). The two databases were compared to ensure accuracy. Data were exported into a SAS 9.1 (SAS Inc., Cary, NC, USA) to carry out univariable and multivariable analyses. Univariable analysis was performed to assess risk factors for MDR-TB and Mantel -Haenszel Odds Ratios (OR), 95% Confidence Intervals and corresponding p-values were reported. To adjust for multiple covariates, we used a logistic regression method. Variables included in the final multivariate model were chosen a priori on the basis of the biological plausibility of their association with the outcome of interest, as well as on the basis of statistical and epidemiologic criteria. A *P*-value ≤ 0.05 was defined as statistically significant.

RESULTS

A total of 1693 patients who were AFB sputum smear positive (ss+) were enrolled in the study; 1449 (86%) had a positive culture for *M. tuberculosis*, 157 (10%)were culture negative and 87 (4%) had contaminated cultures (**Figure 1**). Drug Susceptibility Test (DST) results were available on isolates recovered from 1422 patients (98%).Clinical data regarding patient treatment status (new vs. previously treated) were unknown for 108 (7%) patients who were excluded from further analysis. Complete clinical and drug susceptibility testing data were available for 1314 patients with culture confirmed pulmonary TB who were included in the final analysis. Demographic information for those excluded from analysis (i.e., because of missing data or lack of having a positive culture) did not differ from those included in the final analysis (data not shown).

Among the 1314 patients included in the final analysis, 240 (18%) were female and 1072 (82%) were male. Median age was 37 years, 100% were Caucasian, 113 (9%) were incarcerated, and 1201 (92%) were from the civilian sector. A total of 799 (60.8%) were newly diagnosed patients with TB and 515 (39.2%) were previously treated cases (Table 1). Drug resistance to 1 or more antituberculosis drugs was found in *M. tuberculosis* isolates recovered from 733 (55.9%) of 1314 patients (Table 2); 581 (44.1%) were fully susceptible to first line antituberculosis drugs. The prevalence of resistance to first line anti-TB drugs was as follows: 32.7% of TB isolates were resistant to isoniazid, 15.8% to rifampin, 6.7% to ethambutol and 47.9% was resistant to streptomycin (Table 2).

Overall, isolates recovered from 195 (14.8%) of 1314 patients had MDR-TB; those with a prior history of TB treatment (“retreatment cases”) were significantly more likely to have MDR-TB than newly diagnosed patients (141/515 [27.4%] vs 54/799 [6.8%]; (OR=5.27, 95% CI, 3.75-7.41) (Table 2, Table 3). Risk factors for MDR-TB among the entire study population are shown in Table 3; risk factors for MDR-TB among newly diagnosed cases is shown in Table 4A and for retreatment cases in Table 4B. In multivariate logistic regression analysis, previous history of anti-TB treatment (retreatment case) (AOR=5.47. 95% CI, 3.87-7.74) and female gender (AOR=1.58, 95% CI, 1.02-2.32) were independent risk factors for having MDR-TB (Table 5). Among those with newly diagnosed TB, MDR-TB was more likely to occur in female than male patients (11% [19/176] vs 5.5% [35/623] of males; OR=2.15, 95% CI, 1.19-3.87, $p<0.01$).

DISCUSSION

A high prevalence of drug resistant TB including MDR-TB was found among patients in Georgia in this population-based study. The prevalence of MDR-TB was high: 6.8% in newly diagnosed cases, 27.4% in re-treatment cases and 14.8% of all cases. Risk factors for MDR-TB in multivariate analysis include: previous TB treatment (OR=5.47) and female gender (OR=1.60). Among those patients with newly diagnosed TB (i.e., no prior TB treatment), risk factors for MDR-TB included female gender (OR=2.15).

Previous TB treatment has been noted to be a major risk factor for development of drug resistance (16-18). The WHO/IUATLD Global Project on Anti-tuberculosis Drug Resistance Surveillance reported that the retreatment case status was significantly associated with both MDR-TB and any drug resistance (17). Previous treatment was also demonstrated as the strongest determinant of MDR-TB in Europe in a systematic review based on studies from twelve European countries (19). The likelihood of having MDR-TB increases linearly with increasing total time of prior TB treatment(20). The current WHO recommendations for treatment of patients who have relapse or failed initial treatment (category II) includes administration of 5 first line drugs (adding streptomycin to the first line regimen) and may lead

to further amplification of resistance (16). New guidelines and the Global Plan to Stop TB emphasize the need to be able to do drug susceptibility testing and provide treatment for MDR-TB(21). In this regard, in December 2007 Georgia is starting second line treatment for the whole country. In addition, it is essential to prevent the development of new drug resistant cases through the implementation and expansion of the DOTS strategy(21).

In most reports, men have been noted to be at increased risk for having susceptible TB then females, but for MDR-TB females seem to be at higher risk(22). Three studies from former Soviet Union countries reported that men were at lower risk than women for having MDR-TB (19). The finding that newly diagnosed women with TB had an increased risk of MDR-TB in Georgia appears to be in line with findings from other studies carried out in the region and confirms similar findings from a pilot study undertaken in selected Georgian cities in Georgia (8). Reasons for the increased risk of MDR-TB among women in Georgia that we found in our population-based study are not entirely clear and require further investigation. One hypothesis is that this may be due to cultural reasons. Since MDR-TB treatment has not previously been available in Georgia and there have been no inpatient services in Georgia for patients with MDR-TB, these patients are likely to stay in their houses and become progressively more ill. Since the prevalence of HIV co-infection among TB patients in Georgia is low(4), it is possible that such patients may live for months or years after the diagnosis. It is likely that the care provided to these patients with MDR-TB was provided by women (e.g., their wives, mothers or sisters) thus placing women at increased risk for exposure to and the subsequent development of MDR-TB. Further investigation of household contacts of MDR-TB cases is needed including a case-control study to test our hypothesis and evaluate the reasons for these findings.

Our study had some limitations. A small percentage (14%) of cultures from AFB smear positive sptutum cases were culture-negative or contaminated and did not yield *M. tuberculosis*. Negative cultures from smear positive patient may in part be due to delays in transportation of specimens from around the country of Georgia to the NRL in Tbilisi where cultures were performed. However, demographic data did not differ between those AFB sputum smear positive patients that were culture positive and those who did not have a positive culture. In addition, sputum smear negative patients were not enrolled in the survey. Another limitation was that treatment status (i.e., newly diagnosed case vs. previously treated case) for some patients was assessed by history when medical records indicating treatment status were not available or did not exist. Finally, HIV status was not examined in the study population which is an additional study limitation. However, prior studies carried out in Georgia have indicated a low prevalence of HIV co-infection among patients with tuberculosis (e.g., 1% in a recent publication) (4).

CONCLUSION

In summary, MDR-TB has emerged as a major public health problem in Georgia and the prevalence of MDR-TB was found to be high in a population-based study (approximately 15% overall, 7% among newly diagnosed patients and 27% among previously treated patients). Prior treatment and female gender were associated with an increased risk of MDR-TB. Aggressive TB control efforts should be urgently implemented in order to prevent the development of new cases of MDR-TB and to treat existing MDR-TB patients. Georgian National Tuberculosis Program succeeded in reaching WHO targets for TB case finding and in 2006 the new smear-positive case detection rate was 109%.

Finding of this study has had several favorable outcomes. These include: 1) approval from the WHO Green Light Committee for procurement of second line drugs; 2) two DOTS-Plus pilot programs (1 in civilian sector with MSF and 1 in penitentiary sector with ICRC) have been implemented to begin treatment of persons with MDR-TB in Georgia; and 3) a proposal from

Georgia to the Global Fund Against AIDS, TB and Malaria proposal focusing on MDR-TB treatment and control was recently funded. A case-control study to investigate the reasons for the increased risk of MDR-TB among newly diagnosed females was funded and has been initiated.

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Table 1

Characteristics of Patients with Culture Confirmed Pulmonary Tuberculosis in Georgia (n=1314)

Patient Characteristics	n(%)
Caucasian	1314(100%)
Sex	
Female	240(18%)
Male	1074(82%)
Age Groups	
0-14	1 (0.1%)
15-24	209 (15.9%)
25-34	372 (28.3%)
35-44	298 (22.7%)
45-54	262 (19.9%)
55-64	92 (7.0%)
65 +	80 (6.1%)
Incarceration Status	
Incarcerated	113(9%)
Civilian	1201(91%)
Region	
East Georgia	605(46.0%)
West Georgia	596(45.4%)
Prison	113(8.6%)
Treatment History	
Retreatment Case	515(39.2%)
Newly Diagnosed	799(60.8%)

Table 2

Prevalence of Drug Resistant Tuberculosis among Patients in Georgia with Pulmonary Disease, July 2005-May 2006 (n=1314)

	Number (%)				Number (%) Total
	New Patients*	Treated Patients**			
Drug Resistance Pattern	**n=799**	**n=515**	**OR, 95%CI**	**P-value**	**n=1314**
Susceptible to all 4 drugs	406 (50.76)	175 (33.8)			581 (44.1)
Resistance to any drug	393 (49.20)	340 (66.1)	2.01[1.59-2.53]	<0.0001	733 (55.9)
Any Resistance to					
Isoniazid (H)	187 (23.40)	243 (47.2)	2.9 [2.31-3.73]	<0.001	430 (32.74)
Rifampicin R	61 (7.56)	147 (28.5)	4.8 [3.49-6.71]	<0.001	208 (15.81)
Ethambutol (E)	33 (4.1)	56 (10.9)	2.8 [1.82-4.5]	<0.001	89 (6.71)
Streptomycin (S)	330 (41.31)	299 (58.1)	1.97 [1.57-2.47]	<0.001	629 (47.86)
Monoresistance to					
Isoniazid (H)	49 (6.17)	28 (5.48)			77 (5.90)
Rifampicin R	4 (0.5)	4 (0.81)			8 (0.61)
Ethambutol (E)	3 (0.41)	0 (0.00)			3 (0.23)
Streptomycin (S)	193 (24.2)	91 (17.7)			284 (21.61)
Total	249 (31.15)	123 (24.01)			372 (28.31)
Multi-drug Resistance to					
H+R	6 (0.76)	6 (1.17)			12 (0.92)
H+R+E	0 (0.00)	2 (0.39)			2 (0.15)
H+R+S	27 (3.40)	83 (16.11)			110 (8.43)
H+R+E+S	21 (2.62)	50 (9.71)			69 (5.28)
Total	**54 (6.75)**	**141 (27.4)**	**5.27 [3.75-7.41]**	**<0.001**	**195 (14.8)**
Polyresistance to					
H+E	1 (0.13)	1 (0.20)			2 (0.15)
H+S	78 (9.8)	70 (13.60)			148 (11.26)
H+E+S	5 (0.63)	3 (0.59)			8 (0.61)
R+E	0 (0.00)	0 (0.00)			0 (0.00)
R+S	3 (0.38)	2 (0.40)			5 (0.41)
R+E+S	0 (0.00)	0 (0.00)			0 (0.00)
E+S	3 (0.38)	0 (0.00)			3 (0.23)

*New Patients=Patients never diagnosed with TB or treated for < 1 month with anti TB drugs

** Treated Patients=patients with previous exposure to TB treatment for ≥ 1 month

Table 3

Univariable analysis of risk factors for Multidrug Resistant Tuberculosis (MDR-TB) in Georgia

Variables		No. MDR-TB cases/total cases (% MDR)	OR	95% CI
Retreatment case*				
	Yes	**141/515(27.4%)**	**5.27**	**3.75-7.41**
	No	**54/799 (6.8%)**		
Age				
	≥37	**96/644 (15%)**	**0.9**	**0.69-1.29**
	<37	**97/648 (15%)**		
Sex				
	Female	**38/240(16%)**	1.14	**0.77-1.48**
	Male	**157/1074(14%)**		
Prison				
	Yes	**13/113 (12%)**		
	No	**182/1201(15%)**	**0.79**	**0.43-1.48**
Region				
	Prison	**13/113(12%)**		
	West Georgia	**71/473 (15%)**	**1.27**	**0.65-2.52**
	East Georgia	**111/728(15%)**	**1.26**	**0.66-2,45**

* Retreatment Case=Previous exposure to TB treatment for ≥ 1 month

Table 4A

Univariable analysis of risk factors for Multidrug Resistant Tuberculosis (MDR-TB)among Newly Diagnosed Patients with Tuberculosis in Georgia (n=799)

Variables	No. MDR-TB cases/total cases (% MDR)	OR	95%CI
Age Groups			
0-14	**0/1 (0%)**		
15-24	**13/167 (7.8%)**		
25-34	**17/217 (7.8%)**	**1.01**	**0.45-2.33**
35-44	**6/156 (3.8%)**	**0.47**	**0.14-1.38**
45-54	**13/155 (8.4%)**	**1.08**	**0.45-2.62**
55-64	**3/53 (5.7%)**	**0.71**	**0.13-2.74**
65 +	**2/50 (4.0%)**	**0.49**	**0.05-2.31**
Sex			
Female	**19/176 (10.8%)**	**2.15**	**1.19-3.87**
Male	**35/623 (5.5%)**		
Prison			
Yes	**5/70 (7.1%)**	**1.07**	**0.32-2.80**
No	**49/729 (6.7%)**		
Region			
Prison	**5/70 (7.1%)**		
West Georgia	**10/364 (5.2%)**	**1.4**	**0.39-4.05**
East Georgia	**30.365 (8.2%)**	**0.86**	**0.25-2.35**

Table 4B

Univariable analysis of Risk Factors for Multidrug Resistant Tuberculosis (MDR-TB)among Patients Previously Treated for Tuberculosis in Georgia (n=515)

Variables	No. MDR-TB cases/total cases (% MDR)	OR	95%CI
Age Groups			
0-14	**0**		
15-24	**8/42 (19.0%)**		
25-34	**40/155 (25.8%)**	**1.48**	**0.60-4.00**
35-44	**46/142 (32.4%)**	**2.03**	**0.84-5.49**
45-54	**28/107 (26.2%)**	**1.50**	**0.59-4.21**
55-64	**10/39 (25.6%)**	**1.46**	**0.45-4.88**
65 +	**9/30 (30%)**	**1.81**	**0.53-6.33**
Sex			
Female	**19/64 (29.7%)**	**1.14**	**0.60-2.08**
Male	**122/451 (27.1%)**		
Prison			
Yes	**8/43 (18.6%)**	**0.58**	**0.23-1.32**
No	**133/472 (28.2%)**		
Region			
Prison	**8/43 (18.6%)**		
West Georgia	**70/232 (30.2%)**	**0.53**	**0.20-1.24**
East Georgia	**63/240 (26.3%)**	**0.64**	**0.24-1.51**

* Retreatment Case=Previous treatment with anti-tuberculosis medications for ≥ 1 month

Table 5

Multivariate Logistic Regression Analysis for Independent Risk Factors for Multidrug Resistant Tuberculosis (MDR-TB) in Georgia

Variables	Adjusted Odds Ratio (AOR)	95% Confidence Interval
Retreatment Case	5.47	3.87-7.74
Female	1.58	1.02-2.32
Age	1.03	0.74-1.42
Prison	0.98	0.51-1.92

Retreatment Case=Previous exposure to TB treatment for ≥ 1 month

Prison=Being incarcerated during the study Age ≥37 years old