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Prevalence and Risk factors for Drug Resistance among Hospitalized TB Patients in Georgia

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SUMMARY

Background—Tuberculosis control in Georgia follows the WHO recommended DOTS strategy and has reached Global TB Control targets in treatment of sensitive TB, but the management of drug resistant forms of TB still represents a serious problem. A country-wide Drug Resistance Survey (DRS) found that the prevalence of MDR-TB was 6.8% in new and 27.4% in previously treated TB cases.

Objective—To determine prevalence and risk factors for drug resistance among TB patients in order to improve DR-TB case management and control.

Methods—Extensive social, clinical and bacteriological data were collected from hospitalized patients (National Centre for Tuberculosis and Lung Diseases, Georgia, 2005–2007).

Results—Out of 605 patients DR-TB was found in 491 (81.2%) cases, MDR-TB was observed in 261(43.1%) [51 (23%) out of 222 New cases and 210 (55%) out of 383 Previously treated cases], mono-DR-TB in 130 (21.5%), poly-DR-TB in 67 (11.1%) and XDR-TB in 33 (5.5%) cases.

Study showed that female gender, living in densely populated capital, family TB contact and previous TB treatment are associated with risk for having MDR-TB.

Conclusions—Findings confirm the necessity of improvement of infection control measures and availability of standardized treatment for DR-TB patients.

Keywords

Multi-Drug Resistant Tuberculosis (MDR-TB); Risk factors; Georgia

BACKGROUND

Tuberculosis (TB) is a major cause of illness and death worldwide. WHO estimates 14.4 million prevalent TB cases including 0.5 million multi-drug resistant TB (MDR-TB) for the year 2006. (1)

Incidence of TB has been rising since nineties and reached its peak in the year 2001 in Eastern European region. After the collapse of Soviet Union the health system in Georgia has experienced a substantial reduction in health financing, decline in the access to health services and huge increases in out-of-pocket payments. In addition, civil wars, ethnic conflicts and

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thousands of internally displaced persons (IDP) have led TB to become a major public health threat in Georgia. (2,3,4)

In 1995, the Ministry of Health of Georgia established the National Tuberculosis Program (NTP) based on World Heath Organization (WHO) recommended Directly Observed Therapy Short Course (DOTS) strategy. The Georgian NTP reached 100% DOTS coverage in 2004. TB diagnosis and treatment is provided in the specialized TB facilities, but at this time the management of drug resistant TB represented a serious problem. (5,6)

Due to rising incidence of drug resistant TB worldwide the Georgian NTP with support of WHO conducted a country-wide Drug Resistance Survey (DRS) between July 2005 and May 2006. Total of 1693 patients from all 75 TB facilities of Georgia were enrolled in this study. Sputum smear microscopy, culture and the first line anti-TB drugs DST was provided for these patients. The DRS reported that the prevalence of MDR-TB was 6.8% in never treated and 27.4% in previously treated TB cases. (7)

It was important to obtain baseline data about the prevalence and risk factors for MDR-TB among hospitalized patients in Georgia, since the NTP has been implementing MDR-TB treatment. The National Centre for Tuberculosis and Lung Diseases (NCTBLD) hospital is a main TB referral hospital in Georgia; the hospital serves about 1200 TB patients from whole country annually. Country wide DRS study evaluated risk factors of MDR-TB. It was important to assess risk factors for MDR-TB in hospitalized patients in comparison with the results obtained from the population based study.

Treatment for MDR-TB was not available in the public sector until 2008, with the exception of a *Médecin Sans Frontières (MSF*) MDR pilot project in one Georgian region (Samegrelo-Zemo Svaneti, since 2006).(5,6) Currently the MDR-TB treatment is conducted according to the National Guideline for DR-TB. (8)

OBJECTIVE

To determine prevalence and risk factors for drug resistance among TB patients who undergo inpatient treatment at the NCTBLD to improve DR-TB case management and control.

METHODS

Participants and Data Collection

With support of the United States Civilian Research Development Foundation (CRDF) grant (GEX1-002711-TB-06) we conducted a cross-sectional study from March 2006 through December 2007 at the NCTBLD hospital. All TB patients hospitalized at the NCTBLD during the study period were asked to participate in the study. Of 972 patients who were approached only 605 patients agreed to participate. All TB patients enrolled were required to provide written informed consent (in their native Georgian language of Kartuli). The study was approved by the Ethics Committee of the NCTBLD and the Syracuse VAMC Institutional Review Board (IRB).

Sputum smear microscopy, culture, first and second line DST was provided to all patients involved in our study. TB patients enrolled in the study completed a questionnaire containing demographic information (address, age, sex, ethnicity, marital status, number of children and family size), education and social history (e.g., employment type, monthly revenue, and living conditions before TB diagnosis) Information was collected regarding smoking, alcohol abuse, drug abuse, imprisonment, homelessness and internally displaced peoples (IDP) as well as concomitant diseases such as hepatitis, diabetes, peptic ulcer and hormone therapy. If a study

subject was a previously treated case, information regarding the previous treatment outcome was collected. HIV testing is routinely performed in the hospital for TB patients; however the questionnaire did not contain information regarding HIV status.

Definitions

New case (Never treated case) was defined as a patient who had never had treatment for TB or who received anti-tuberculosis drugs for less than one month. Information was collected by history and review of medical records. (9)

Previously treated case was defined as patient who had a prior history of treatment with anti-tuberculosis drugs for more than 1 month. **Previously treated cases** included relapses, treatment after failure, treatment after default and chronic cases.(9)

Mono-drug resistant TB (Mono-DR-TB) was defined as resistance to a single first-line anti-tuberculosis drug (i.e., isoniazid, rifampicin, pyrazinamide, ethambutol, or streptomycin). (10)

Poly-drug resistant TB (PDR-TB) was defined as resistance to two or more of the first-line anti-tuberculosis drugs, but not both isoniazid and rifampicin.(10)

Multi-drug resistant TB (MDR-TB) was defined as resistance to at least both isoniazid and rifampicin.(10)

Extensively drug resistant TB (XDR-TB) is resistance to at least isoniazid and rifampicin (MDR-TB), plus resistance to any one of the fluoroquinolone drugs and to at least one of the three injectable second-line drugs (i.e., amikacin, capreomycin or kanamycin).(11)

Primary resistance (drug resistance among new cases) was defined as the presence of resistant isolates of *M. tuberculosis* in patients who, in response to direct questioning, denied 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.(12)

Secondary resistance (drug resistance among previously treated cases) was defined as the presence of resistant isolates of *M. tuberculosis* in patients who, in response to direct questioning, admitted having been treated for tuberculosis for 1 month or more or, in countries where adequate documentation is available, in a patient for whom there is evidence of such a history.(12)

Laboratory Methods

AFB smear microscopy—Smear status was assessed by the Ziehl-Neelson staining method.(13)

AFB Cultures—Sputum specimens received at the National Reference Laboratory 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 onto Lowenstein-Jensen media which was incubated at 37°C using standard methodologies as previously described.(13)

Identification of *Mycobacterium tuberculosis* was done using the p-nitrobenzoic acid (PBN) and thiophene carboxylic acid hydrazine (TCH) resistance test.(13,14)

Drug Susceptibility Testing of first line drugs was performed by Absolute Concentration method on solid Lowenstein-Jensen media with following concentrations of drugs in the media.

Streptomycin (S) – 4μg/ml, Isoniazid(H) – 0.2μg/ml, Rifampicin (R) – 40μg/ml, Ethambutol (E) – 2μg/ml. (13) National Reference Laboratory of Georgia participates in annual panels testing and external quality assurance for first line DST was provided by the Antwerp WHO Supranational Reference Laboratory with 98% concurrence.(14)

Drug Susceptibility Testing of second line drugs was performed by Proportion method on solid Lowenstein-Jensen media based on the methodologies recommended by Antwerp WHO SRL. Following second line drugs with subsequent concentrations in media were used Amikacin (Amk) - 30μg/ml, Capreomycin (Cm) - 40μg/ml, Ofloxacin (Ofx) - 2μg/ml, Ethionamide (Eto) - 40μg/ml, Cycloserine (CS) - 60μg/ml, PAS - 0,5μg/m. (13)

Statistical Analysis

Data were entered and statistical analyses were performed using Epi Info version 3.3.2. Univariate 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

From March 2006 through December 2007 605 patients were included in our study. 222 (36.7%) patients were new TB cases, and 383 (63.3%) were previously treated TB cases. Susceptible *M. tuberculosis* isolates were found in 114 (18.8%) [73 (32.88%) new and 41 (10.70%) previously treated] cases and drug resistant *M. tuberculosis* isolates were found in 491 (81.2%) [149 (67.12%) new and 342 (89.3%) previously treated] cases; Mono DR-TB was diagnosed in 130 (21.5%) [69 (31.08%) new and 61 (15.93%) previously treated] cases; PDR-TB was diagnosed in 67 (11.1%) [23 (10.36%) new and 44 (11.5%) previously treated] cases; MDR-TB was diagnosed in 261 (43.1%) [51 (22.97%) primary resistance, 210 (54.83%) secondary resistance] cases; XDR-TB was diagnosed in 33 (5.5%) [6 (2.7%) primary resistance, 27 (7.04%) secondary resistance] cases.

Detailed information about resistance for first and second line anti tuberculosis drugs is given in table 1. In the text bellow special attention is paid to the more interesting results.

Patients included in the study showed a much higher rate of mono-resistance to streptomycin (S) compared to the rates of mono-resistance to isoniazid (H), rifampicin (R) or ethambutol (E). Primary resistance to S was higher than the secondary resistance [out of 105 (17.3%) S-resistant strains 60 (27.03%) were primary, 45 (11.75%) – secondary] (see table 1).

Resistance for first line anti-TB drugs with combinations (H+S), (H+R+S) and (H+R+S+E) were most frequent and in combinations (H+R+S) and (H+R+S+E) secondary resistance rates exceeded the rates of primary resistance (see table 1).

In spite of the absence of approved or formal second-line drug treatment of drug-resistant tuberculosis resistance for Amk was founded in 54 (8.9%) [11 (4.95%) primary resistance, 43 (11.23%) secondary resistance] - isolates; for Cm in 51 (8.4%) [12 (5.4%) primary resistance, 39 (10.18%) secondary resistance] - isolates; for Ofx in 75 (12.4%) [14 (6.3%) primary resistance, 61 (15.93%) secondary resistance] - isolates; Special attention needs to be given to both primary and secondary resistance to Eto141 (23.3%) [37 (16.7%) primary resistance, 104 (27.15%) secondary resistance] (see table 1). From 360 isolates resistant to H, 68 (19%) were also resistant to Ofx and 131 (36.4%) to Eto.

Descriptive frequencies of characteristics belonging to the TB population under study is given in table 2. Frequently TB was observed in patients from 30–50 age group - 341 (56,3%); in males - 478 (79%); in patients with secondary education - 581 (96%); in unemployed patients - 478 (79%); in unknown TB contacts - 377 (62,3%) and in previously treated cases - 383 (63,3%) (see table 2).

Univariate analysis was performed on predictors: age, sex, residential area, education level, TB contact, treatment history. High rates of MDR-TB cases was documented in female patients 72/127 56,7%) [OR=2.0; 95% 1.32<CI<3.9]; in patients living in densely populated capital 138/290 (47,6%) [OR=1.42; 95% 1.01<CI<2.01]; in family TB contacts cases 59/120 (49.2%) [OR=1.36; 95% 0.89<CI<2.06] and in previously treated cases 210/383 (54,8%) [OR=4.07; 95% 2.76<CI<6.01] (see table 3).

Multivariate logistic regression analysis showed that a previous treatment represents a clear risk for development of drug-resistant tuberculosis [OR=5.1; 95% 3.4<CI<7.7]); risk of development of drug-resistant tuberculosis was associated with female gender [OR=2.4; 95% 1.4<CI<4.0)]), living in densely populated capital (Tbilisi) [OR=1.5; 95% 1.05<CI<2.2]), and family TB contact [OR=1.4; 95% 0.84<CI<2.2]) (see table 4).

Based on our data, in spite of high occurrence of drug resistance, the following factors were not found to be associated with development of drug resistant tuberculosis: patient's age, marital status, education level, employment, monthly income, living conditions, tobacco smoking, alcohol abuse, drug abuse, imprisonment or forced displacement as well as presence of accompanying diseases: HIV/AIDS, hepatitis, diabetes mellitus, duodenal and gastric ulcer.

DISCUSSION

In comparison with nationwide DRS results, higher rates of drug resistance were documented by our study data. The reason for the observed results is that the hospitalized patients were mostly previously treated TB patients (63.3%) which is higher proportion compared to the proportion of previously treated patients in population based DRS study (39.2%).(7) In addition, the study was hospital based and the study participants undergoing TB treatment in the NCTBLD hospital usually are smear-positive and have advanced forms of TB, since the hospital serves as a referral clinic treating severe forms of TB from the entire country.

High rates of resistance to S, H, R, and Ofx and especially of primary resistance are regrettable realities in most countries of Former Soviet Union (FSU), including Georgia.(2,3,4) This is explained by frequent and unjustified usage of these medicines in the network of general health care, creating serious problems for utilizing treatment courses in line with both DOTS and DOTS-plus strategies. The second line drugs are available without prescription in Georgia. General practitioners and ID doctors widely use these drugs for the treatment of infections other than TB. This is likely an important cause of the observed high rates of resistance to the second-line drugs especially quinolones.

The study has not examined the outcomes of the initial TB treatment and their association to the developed resistance. In addition, due to lack of universal access to the first- and second-line DST till 2007, adequacy of the TB treatment regimens could not be assessed.

The fact that the high rates of TB were documented in the age group of 30–50, is not unusual and is a problem in many other countries.(15,16,17)

According to our data, evidence of high rates of TB in unemployed, secondary education, low monthly income, alcoholic and drug addict patients is related, on the one hand, with socio-

economic situation in our country and on the other hand, with the fact that our inpatient clinic hosts exactly this category of patient.

The reason for high TB rates among persons with unascertained contact with TB, most probably is due to TB-associated stigma which causes concealing of illness. This problem is still unsolved in our country.(18,19,20)

Georgia has a low HIV prevalence of 0.1% in the year 2007.(20,21) As was expected a higher prevalence of 2.5% (15 HIV positive patients out of 605 study participants tested) was observed among the study participants, that can be explained by the study population being hospitalized TB patients.

Association of female gender with the development of drug-resistant tuberculosis may have a specific explanation in Georgia. Females are main caregivers in the family, thus spending a long period of time with a family member affected by TB. Women, therefore, may have an increased risk factor for development of DR-TB in Georgia. In country-based study conducted between July 2005 and May 2006 in Georgia similar hypothesis was formulated.(7) Higher rates of primary resistance among females was expected, however this was not observed in our study. We can suggest that previously treated women diagnosed to have MDR-TB had acquired primary resistance earlier in their life, but because there was no the first line drug susceptibility testing available in Georgia routinely till 2007 we could not confirm the primary resistance. In the majority of female study subjects family contact was not ascertained (only 34.7% of females reported family TB contact). A higher percentage was expected but stigma associated with TB could be the factor responsible for the observed result. Observation of family TB contact, of living in densely populated cities and of female gender as risk-factors for development of DR-TB suggests that both patients and their family members are not sufficiently knowledgeable about the disease and its prevention as well as effective infection control measures outside TB facility.(22,23)

We compared our results to the results observed in recent country based study. The same risk factors for development of MDR-TB were observed in both studies. The DRS showed that previous history of anti-TB treatment (OR=5.47. 95% CI, 3.87–7.74) and female gender (OR=1.58, 95% CI, 1.02–2.32) were independent risk factors for having MDR-TB.(7) In addition to those risk factors observed in the country based study, our study showed that history of family TB contact and living in densely populated capital as an independent risk factor for having MDR-TB.

CONCLUSION

Based on our findings it is recommended: to ensure adherence to the TB treatment of all TB patients under direct observation in accordance to DOTS strategy, to regulate the first- and second- line anti-TB drugs sale without prescription and to improve infection control measures in health care facilities. We suggest that a Knowledge, Attitude and Practice (KAP) survey should be undertaken in Georgian population. The results of such a study could then be used to develop an effective campaign, which will increase community awareness on TB.

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Table 1

Resistance to First- and Second - Line Tuberculosis Drugs in New and Previously treated Cases

Resistance	N=605		
	New cases n=222 (36,7%)	Previously treated Cases n=383 (63,3%)	Total N=605 (100%)
Sensitive TB	**73 (32,88%)**	**41 (10,70%)**	**114 (18,8%)**
Total DR-TB	**149 (67,12%)**	**342 (89,30%)**	**491(81,2%)**
Resistance for I Line TB Drugs			
Total Mono DR-TB	**69 (31,08%)**	**61 (15,93%)**	**130 (21,5%)**
H	8 (3,6%)	12 (3,13%)	20 (3,3%)
R	1 (0,45%)	3 (0,8%)	4 (0,7%)
S	60 (27,03%)	45 (11,75%)	105 (17,3%)
E	0	1 (0,3%)	1 (0,2%)
Total PDR-TB	**23 (10,36%)**	**44 (11,5%)**	**67 (11,1%)**
H+S	19 (8,6%)	33 (8,62,%)	52 (8,6%)
H+E	0	0	0
R+S	2 (0,9%)	3 (0,8%)	5 (0,8%)
R+E	0	1 (0,3%)	1 (0,2%)
S+E	1 (0,45%)	2 (0,52%)	3 (0,5%)
H+S+E	0	4 (1,04%)	4 (0,7%)
R+S+E	1 (0,45%)	1 (0,3%)	2 (0,3%)
Total MDR-TB	**51 (22,97%)**	**210 (54,83%)**	**261 (43,1%)**
H+R	2 (0,9%)	13 (3,4%)	15 (2,5%)
H+R+S	26 (11,71%)	85 (22,2%)	111 (18,3%)
H+R+E	0	7 (1,83%)	7 (1,2%)
H+R+S+E	23 (10,36%)	105 (27,4%)	128 (21,1%)
Resistance for II Line TB Drugs			
Amk	11 (4,95%)	43 (11,23%)	54 (8,9%)
Cm	12 (5,4%)	39 (10,18%)	51 (8,4%)
Ofx	14 (6,3%)	61 (15,93%)	75 (12,4%)
Eto	37 (16,7%)	104 (27,15%)	141 (23,3%)
Cs	11 (4,95%)	37 (9,66%)	48 (7,9%)
PAS	10 (4,5%)	26 (6,8%)	36 (6,0%)
Total XDR-TB	**6 (2,70%)**	**27 (7,04%)**	**33 (5,5%)**

Table 2

Frequencies of Risk Factors (N=605)

Risk factors	n (%)
Age	
<30	125 (20,7%)
30–50	341 (56,3%)
>50	139 (23,0%)
Sex	
Female	127 (21,0%)
Male	478 (79,0%)
Residential area	
Tbilisi (Capital)	290 (47,9%)
other regions	315 (52,1%)
Education level	
Higher	24 (4,0%)
Secondary	581 (96,0%)
Employment type	
Employed	127 (21,0%)
Unemployed	478 (79%)
TB contacts	
Family	120 (19,9%)
Prison	108 (17,8%)
Unknown	377 (62,3%)
HIV/AIDS	15 (2,5%)
Imprisonment	113 (18,7%)
Treatment history	
Never treated cases	222 (36,7%)
Previously treated cases	383 (63,3%)

Table 3

Univariate analysis of risk factors for MDR-TB

Variables	MDR-TB cases/total cases (% MDR)	OR	95%CI
Age			
30–50	153/341 (44.9%)	1,18	0.84–1.65
Others	108/264 (40.9%)		
Sex			
Female	72/127 (56.7%)	2.0	1.32–3.09
Male	189/478 (39.5%)		
Residential area			
Capital	138/290 (47.6%)	1.42	1.01–2.01
Other regions	123/315 (39.05%)		
Education level			
Secondary	245/581 (42.2%)	0.36	0.14–0.92
Higher	16/24 (66.7%)		
Family TB contact			
Yes	59/120 (49.2%)	1.36	0.89–2.06
No	202/485 (41.6%)		
Previous TB treatment			
Yes	210/383 (54.8%)	4.07	2.76–6.01
No	51/222 (23.0%)		

Table 4

Multivariate Logistic Regression Analysis of Revealed Risk Factors for MDR-TB

Term	Odds Ratio	95%	C.I.
Middle age	1, 0	0,7	1,4
Female gender	2,4	1,4	4,0
Living in densely populated capital	1,5	1,1	2,2
Secondary education	0,9	0,4	1,9
Family TB contact	1,4	0,8	2,2
Previous TB treatment	5,1	3,4	7,7