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SHORT COMMUNICATION

Drug susceptibility of *Mycobacterium tuberculosis* in HIV-infected and -uninfected Ethiopians and its impact on outcome after 24 months of follow-up

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SUMMARY

From a prospective cohort study on tuberculosis/human immunodeficiency virus (TB/HIV) interaction in Addis Ababa, Ethiopia, drug susceptibility results were available for 94 TB patients (46% HIV-infected). Resistance to one or more drug(s) was detected in 21 (22.3%) and multidrug resistance in five (5.3%) patients. Occurrence of resistance was not related to HIV status or outcome after 24 months of follow-up. However, among HIV-infected TB patients who died during follow-up, survival time in those with a resistant *Mycobacterium tuberculosis* strain was significantly shorter compared to those with a sensitive strain (6 vs. 13 months). Early detection of drug resistance and timely treatment change can therefore have a positive impact on survival in HIV-infected TB patients.

KEY WORDS: tuberculosis; drug resistance; HIV-1; long-term follow-up; Ethiopia

THE SPREAD of drug-resistant tuberculosis (TB) and high rates of human immunodeficiency virus (HIV) TB co-infection are a serious threat to national TB control programmes, particularly in countries where both diseases are endemic.[1] In Ethiopia, culture and drug susceptibility testing for mycobacteria are not performed routinely; as only limited data on drug-resistant TB are available,[2–5] we assessed the prevalence of drug-resistant *Mycobacterium tuberculosis*. The prospective setting of the study enabled us to study the influence of drug resistance and HIV infection status on outcome 24 months from the start of TB treatment.

STUDY POPULATION AND METHODS

The study took place in the southwestern outskirts of Addis Ababa, an area with an estimated population of 59 890 (census 1994). From February 1999 until March 2001, patients were recruited among those presenting at the two local health facilities and suspected of having pulmonary tuberculosis (PTB) (smear-positive or smear-negative) and/or tuberculous lymphadenitis (also referred to as extra-pulmonary tuberculosis [EPTB]). Inclusion criteria were: age $\geqslant$15 years, residence in the area, willingness to participate in the study (with informed consent for HIV testing), and start on TB treatment. Three sputum samples from each participant were examined microscopically for acid-fast bacilli (AFB) at the local facility, then pooled and cultured for mycobacteria at the Armhauer Hansen Research Institute (AHRI). For patients suspected of tuberculous lymphadenitis, needle aspiration was performed.

Participants were counselled about HIV, a chest X-ray was taken and blood was drawn for HIV testing and other HIV-related parameters using standard techniques. A study nurse collected data on socio-demographic characteristics and past medical history using a standardised questionnaire.

Mycobacterial isolates were identified and tested for susceptibility to isoniazid (INH), streptomycin (SM), rifampicin (RMP) and ethambutol (EMB)[6] at the Ethiopian Health and Nutrition Research Institute (EHNRI). For personnel training and external quality control, testing was repeated for a selection of the *M. tuberculosis* isolates available at the World Health Organization Mycobacteria Supranational Reference

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Laboratory, Institute of Tropical Medicine (ITM), Antwerp, Belgium. The same selection was tested using molecular detection of rifampicin resistance in the *rpoB* gene (rifoligo typing)[7] at the Mycobacterial Reference Laboratory, National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands.

Twenty-four months after the start of treatment, the study nurse visited all participants at home to register their outcome as healthy, sick, dead or lost to follow-up. Sickness was not necessarily related to tuberculosis. In case a participant had died, time but not cause of death was recorded.

Data were analysed using the STATA statistical package, version 6.0 (Stata Corp, College Station, TX, USA). Categorical variables were compared across groups using the χ^2 test, and continuous variables were compared using the Mann-Whitney U test; $P < 0.05$ was considered significant.

Ethical clearance

The National Ethical Clearance Committee approved the study protocol. Informed consent was obtained from all study participants.

RESULTS

During the 2-year study period, 256 of 490 patients diagnosed with PTB or tuberculous lymphadenitis fulfilled the inclusion criteria and were enrolled. There were no significant differences in age and sex composition of TB patients enrolled versus those not enrolled. Baseline characteristics of the 209 participants for whom a mycobacterial culture result was available are shown in Table 1. Positive cultures were more numerous among HIV-negative than HIV-positive participants, but the difference was not significant. However, it was significant when only participants with PTB were analysed (57 HIV-negative vs. 44 HIV-positive, $P = 0.04$). The culture-positive group differed significantly from the culture-negative group in terms of age and TB type, but not in terms of sex and history of previous TB treatment.

Drug susceptibility patterns and relation to patient characteristics and outcome of disease

Of 117 culture-positive samples, 94 isolates were available for identification and sensitivity testing at EHNRI. All 94 isolates were identified as *M. tuberculosis*. Resistance to one or more drug was detected in 21 (22.3%) participants and multidrug resistance (MDR), defined as resistance to at least INH and RMP, in five (5.3%) participants (Table 2). The association between resistance and previous TB treatment was significant ($P = 0.03$). Among the five participants with MDR-TB, two had never been treated for tuberculosis, resulting in 2.7% MDR among the 73 new cases.

In total, 29 isolates were subjected to external quality control and rifoligo typing. Results at EHNRI and ITM were largely in agreement: INH 100%, RMP 100%, SM 96% and EMB 92%. In three isolates of one patient, rifoligo typing missed RMP resistance as detected in the standard drug susceptibility testing at EHNRI and ITM. The accuracy of the rifoligo assay, calculated as the number of correctly identified strains divided by the total number of strains tested, was 26/29 (90%).

The occurrence of drug resistance was not related to HIV status; nine resistant strains came from 43

Table 1 Baseline characteristics of participants for whom a culture result was available in a TB/HIV interaction study in Addis Ababa ($n = 209$)

Characteristic	Participants with positive culture for *M. tuberculosis* ($n = 117$) *n* (%)	Participants with negative culture for *M. tuberculosis* ($n = 92$) *n* (%)	*P* value*
Sex			
Male	65 (55.5)	52 (56.5)	0.89
Female	52 (44.4)	40 (43.4)	
HIV status†			
Positive	52 (44.8)	51 (55.4)	0.12
Negative	64 (55.2)	41 (44.5)	
Age			
Mean (years, IQR)	30 (21–37)	33.6 (25–41)	0.028
Type TB			
PTB	102 (87)	67 (73)	0.009
EPTB	15 (13)	25 (27)	
History of previous TB treatment			
Yes	21 (18)	12 (13)	0.33
No	96 (82)	80 (87)	

* Comparing participants with a positive culture vs. participants with a negative culture.
† HIV status was unknown for one participant.
TB = tuberculosis; HIV = human immunodeficiency virus; IQR = interquartile range; PTB = pulmonary tuberculosis; EPTB = extra-pulmonary tuberculosis.

Table 2 Drug resistance in *M. tuberculosis* strains obtained from Ethiopian patients with new and previously treated tuberculosis

Drug resistance	Total *n* (%)	First isolates from patients with: New TB *n* (%)	Previously treated TB *n* (%)	Not known *n*
Total	94	73	19	2
Resistance to one or more drugs	21 (22.3)	13 (17.8)	8 (42.1)	0
Resistance to any of the following				
H	16 (17)	7	8	1
S	18 (19)	10	8	1
R	5 (5.3)	2	3	0
E	4 (4.2)	2	2	0
Multidrug resistance				
HSR	4	2	2	0
HSRE	1	0	1	0

TB = tuberculosis; H = isoniazid; S = streptomycin; R = rifampicin; E = ethambutol.

HIV-positive patients (21%) and 11 from 50 HIV-negative patients (22%); in one participant with a resistant strain, the HIV status was not known. It was also not related to outcome after 24 months, as 65% of the patients with a resistant strain and 67% of those with a sensitive strain were healthy (Table 3). Mortality was associated with HIV status: it was 4% in HIV-negative and 46.5% in HIV-positive TB patients. In the latter group, mortality was associated with CD4+ lymphocyte depletion at enrolment (median 9%, range 3–26). The occurrence of TB drug resistance did play a role in survival time. Among the 20 HIV-positive participants who died within 24 months of follow-up, five (median CD4+ at enrolment 7%, range 5–22) were infected with a resistant TB strain; of these, three had an MDR strain. The median time to death among these five 6 months after the start of treatment was significantly shorter than among the 15 HIV-positive participants (median CD4+ at enrolment 10%, range 3–26) who were infected with a sensitive TB strain at 13 months after start of treatment ($P = 0.01$, Mann-Whitney U test).

Table 3 Outcome in 93 culture-positive TB patients, 24 months after start of treatment, in relation to drug susceptibility and HIV status

	Outcome: Healthy *n* (%)	Dead* *n* (%)	Sick† *n* (%)	Lost to follow-up *n* (%)	Total *n*
Resistant					
M. tuberculosis and HIV+	4 (45)	5 (55)‡	0	0	9
M. tuberculosis and HIV−	9 (82)§	0	1 (9)¶	1 (9)	11
Sensitive					
M. tuberculosis and HIV+	13 (38)	15 (44)	3 (9)	3 (9)	34
M. tuberculosis and HIV−	36 (92)	2 (5)	0	1 (3)	39

*HIV status was not known for one subject with a resistant strain who died.
† Sickness at the time of home visit was not necessarily related to tuberculosis.
‡ Includes three patients with MDR-TB.
§ Includes one patient with MDR-TB.
¶ Patient had MDR-TB and was on retreatment at the time of home visit.
TB = tuberculosis; HIV = human immunodeficiency virus; MDR-TB = multidrug-resistant tuberculosis (resistance to at least isoniazid and rifampin); + = positive; − = negative.

DISCUSSION

In the present study, we assessed the prevalence of drug-resistant *M. tuberculosis* among patients treated for TB between 1999 and 2001 in the urban setting of Addis Ababa. Of the study participants, 80% had never been treated for TB, and the prevalence of drug resistance among these was slightly higher than in studies from Addis Ababa performed in 1994,[2] 1996[5] and 1998.[3] Resistance to one or more drugs was 22.3% in our study compared with 14% in 1996.[5] MDR-TB was also found more often in the present study: 5.3% vs. 0.8%[5] in combined new and previously treated cases, and 2.7% vs. 1.2 % (1994[2]) and 0.6% (1998[3]) in new TB cases only. The percentage of MDR-TB was calculated to be 3.2% of all new TB cases worldwide in 2000,[8] and 2.3% of new cases in Ethiopia. The latter is close to the 2.7% MDR-TB among the new cases of TB identified in our study. However, figures from our study cannot be considered representative for the whole country, as >80% of Ethiopians reside in rural areas.

The rifoligo assay, a low cost test for the rapid detection of RMP resistance, was found to be useful with the Ethiopian *M. tuberculosis* strains, with a positive predictive value of 100%. However, its sensitivity and accuracy were lower than with *M. tuberculosis* isolates from Argentina and the Netherlands.[7] More strains from Ethiopia should therefore be tested to confirm rifoligo applicability on Ethiopian isolates before such testing is implemented locally.

As in other studies, we found a clear association between previous treatment and the occurrence of TB drug resistance.[9,10] No association was found between HIV infection status and TB drug resistance. This lack of association has been reported in Western countries,[11] sub-Saharan African countries[5,9,10,12] and worldwide.[8] It indicates that in Ethiopia also, HIV-positive TB patients are as likely as HIV-negative TB patients to have had previously treated and cured TB or to be infected with a resistant strain by transmission.

Little information is available about long-term follow-up of TB patients treated under the DOTS stategy in sub-Saharan Africa in relation to the drug susceptibility of their *M. tuberculosis* strain. We evaluated drug susceptibility with respect to outcome at 24 months and show that being infected with a resistant *M. tuberculosis* strain significantly shortened the

time of survival in HIV-infected TB patients. Of course, other factors such as CD4 lymphocyte depletion are major predictors of survival in such patients,[13] but TB drug resistance does have a negative impact. Therefore, early detection of TB drug resistance and a timely change in treatment should lead to a better outcome in these patients.

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RÉSUMÉ

Au cours d'une étude prospective de cohorte sur l'interaction tuberculose/virus de l'immunodéficience humaine (TB/VIH) à Addis-Abeba, Ethiopie, les résultats des sensibilités aux médicaments ont été disponibles chez 94 patients tuberculeux (TB) (46% infectés par le VIH). On a détecté une résistance à un ou plusieurs médicaments chez 21 patients (22,3%) et une multirésistance chez cinq (5,3%). La présence d'une résistance est sans relation avec le statut VIH ou avec le résultat obtenu après un suivi de 24 mois. Toutefois, parmi les patients TB infectés par le VIH décédés en cours de suivi, la durée de survie est significativement plus courte chez ceux porteurs d'une souche résistante de *Mycobacterium tuberculosis* que chez ceux dont la souche est sensible (6 vs. 13 mois). Une détection précoce de la résistance aux médicaments et une modification en temps utile de la thérapeutique peuvent dès lors avoir un impact positif sur la survie des patients TB infectés par le VIH.

RESUMEN

A partir de un estudio prospectivo de cohorte sobre la interacción tuberculosis/virus de la inmunodeficiencia humana (TB/VIH) en Addis Ababa, Etiopía, se pudo disponer de resultados sobre la sensibilidad a los medicamentos para 94 pacientes TB (46% infectados con VIH). En 21 pacientes (22,3%) se detectó una resistencia a uno o más medicamentos y en cinco (5,3%) se detectó una multirresistencia. La presencia de resistencia no estaba en relación con el estatus VIH ni con el resultado obtenido después de 24 meses de seguimiento. Sin embargo, entre los pacientes TB infectados con VIH que fallecieron durante el seguimiento, el tiempo de sobrevida de aquéllos con cepas resistentes de *Mycobacterium tuberculosis* era significativamente más corto, comparado con el de aquéllos con cepas sensibles (6 contra 13 meses). La detección precoz de la resistencia a los medicamentos y el cambio oportuno de terrapia pueden tener un impacto positivo sobre la sobrevida de los pacientes TB infectados con VIH.