Mycobacterium tuberculosis and nontuberculous mycobacterial isolates among patients with recent HIV infection in Mozambique*,**

Donna pułaparzona przez Mycobacterium tuberculosis e micobakteirię nato-tuberculosas, wtednych pacjencjach recem-diagnostycznych, jako HIV pozywowych, Moçambique, Afryka

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Abstract

Objective: Mycobacteriosis is frequently diagnosed among HIV-infected patients. In Mozambique, where few patients are under antiretroviral therapy and the prevalence of tuberculosis is high, there is need for better characterization of mycobacteria at the species level, as well as for the identification of patterns of resistance to antituberculous drugs. Methods: We studied a sample of 503 HIV-infected individuals suspected of having pulmonary tuberculosis. Of those 503, 320 tested positive for mycobacteria through sputum smear microscopy or cultures of bronchoalveolar lavage fluid. Results: Acid-fast bacilli were observed in the sputum of 73% of the individuals presenting positive cultures. Of 277 isolates tested, only 3 were nontuberculous mycobacteria: 2 were identified as Mycobacterium avium and one was identified as M. simiae. Strains initially characterized as M. tuberculosis complex through polymerase chain reaction restriction analysis (PRA) of the hsp65 gene were later confirmed as such through PRA of the gyrB gene. Among the M. tuberculosis isolates, resistance patterns were as follows: to isoniazid, 14%; to rifampin, 6%; and multidrug resistance, 5%. Previously treated cases showed significantly higher rates of resistance to first-line antituberculous drugs. The most common radiological pattern was interstitial infiltrate (in 67%), followed by mediastinal lymph node enlargement (in 30%), bronchiectasis (in 28%), miliary nodules (in 18%) and cavitatory (in 12%). Patients infected with nontuberculous mycobacteria presented clinical profiles indistinguishable from those of other patients. The median CD4 lymphocyte count in this group was 134 cells/mm³. Conclusions: There is a strong association between tuberculosis and AIDS in Mozambique, as expected in a country with a high prevalence of tuberculosis. Although drug resistance rates are high, the isoniazid-rifampin regimen continues to be the appropriate choice for initial therapy.

Keywords: Tuberculosis; Mycobacteria, atypical; HIV; Acquired Immunodeficiency Syndrome; Drug Resistance, Multiple; Mozambique.

Resumo

Objetivo: A micobacteriose é frequentemente diagnosticada entre pacientes infectados pelo HIV. Em Moçambique, onde apenas um pequeno número de pacientes encontra-se sob tratamento anti-retroviral, e a tuberculose tem alta prevalência, existe a necessidade de melhor caracterização destes agentes bacterianos, em nível de espécie, bem como de se caracterizar os padrões de resistência às drogas antituberculosas. Métodos: Em uma coorte de 503 indivíduos HIV positivos suspeitos de tuberculose pulmonar, 320 apresentaram positividade para baciloscopia ou cultura no escarro e no lavado bronquico. Resultados: Bacilos álcool-ácido resistentes foram detectados no escarro em 73% dos casos com cultura positiva. De 277 isolados em cultura, apenas 3 mostraram-se tratar de micobactérias nato-tuberculosas: 2 Mycobacterium avium e uma M. simiae. Todos os isolados de M. tuberculosis inicialmente caracterizados através de reação em cadeia de polimerase (RCP) do gene hsp65 foram posteriormente caracterizados como tal através de RCP do gene gyrB. Resistência à isoniazida foi encontrada em 14% dos casos; à rifampicina em 6%; e multirresistência em 5%. Pacientes previamente tratados para tuberculose mostraram tendência à taxas maiores de resistência às drogas de primeira linha. O padrão radiológico mais frequente encontrado foi o infiltrativo intersticial (67%), seguido da presença de linfonodos mediastinais (30%), bronquiectasias (28%), padrão miliar (18%) e cavidades.

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oping countries than in developed ones. This has been attributed to prior exposure and acquired immunity. However, it might also be that some centers from developing countries are ill equipped to correctly identify such infections. In Mozambique, TB is the most frequently diagnosed mycobacterial disease, with a prevalence of 250/100,000 inhabitants and a mortality rate of 124/100,000. The prevalence of TB among HIV-infected patients is 47%.(6) Due to limited economic resources, NTM identification is not routinely performed in Mozambique. Therefore, one could question whether in some HIV patients with negative smear microscopy for acid-fast bacilli (AFB) and under TB treatment, as well as in those being treated for multidrug resistant TB (MDR-TB), as determined using the routinely performed sensitivity test, the disease is actually caused by NTM. This study provides insight into the problem in a selected population of HIV-infected patient in the city of Maputo.

Methods

The study was carried out in two large general hospitals located in Maputo: the Hospital Central de Maputo and the Hospital Geral de Machava. The first is the reference for TB in the southern region of the country, and the second is the national reference for TB cases. Most TB/HIV co-infected patients requiring hospitalization are admitted to one of those two facilities. The study was conducted from October of 2002 through August of 2004.

All patients over 11 years of age admitted with suspicion of pulmonary TB and for whom the HIV status was known were evaluated. The diagnosis of TB was made on the basis of complaints, clinical findings, and chest X-ray findings, as well as of the results of smear microscopy for AFB and culture. Patients were categorized clinically into four classes: asymptomatic (Class I); symptomatic less than 50% of the day (Class II); symptomatic more...
than 50% of the day (Class III); in bed most of the time (Class IV). Patients were questioned regarding previous treatment for TB, which was also checked in patient records. Strict patient confidentiality of test results was guaranteed, and all participating patients (or their parents/guardians) gave written informed consent. The ethics committees of the involved Institutions approved the study protocol.

A sample of peripheral blood was drawn from each enrolled patient in order to determine the levels of albumin, lactate dehydrogenase, and hemoglobin, as well as to perform a complete blood count and CD4 lymphocyte count.

Chest X-rays were taken at admission. Findings were categorized into the following patterns: interstitial, alveolar consolidation, nodules, cavitations, bronchiectasis, and mediastinal lymph node enlargement.

**Smear microscopy for AFB, culture, and drug sensitivity tests**

Sputum samples and bronchoalveolar lavage fluid were processed by Ziehl-Neelsen, fuchsin, or auramine staining. In all cases, Löwenstein-Jensen and Stonebrink culture media were used simultaneously for sputum or bronchoalveolar lavage fluid cultures. Tests of drug sensitivity to isoniazid, streptomycin, ethambutol, and rifampin were conducted according to the guidelines established by the World Health Organization and the International Union against Tuberculosis and Lung Disease. We defined MDR as resistance to rifampin and isoniazid. All tests were carried out at the National Reference Laboratory of Tuberculosis, located in Maputo. The Laboratory participates in an international quality assurance program sponsored by the *Istituto Superiore di Sanità* di Roma, in Rome, Italy.

Polymerase chain reaction restriction analysis of the hsp65 gene was used for species level identification of isolates. Strains of the *M. tuberculosis* complex were further identified with amplification and restriction of gyrB gene products. These tests were performed at the State University at Campinas Bacterial Pathogenesis Laboratory, located in Campinas, Brazil.

Patients presenting negative smear microscopy for AFB were submitted to flexible fiberoptic bronchoscopy. The bronchoalveolar lavage fluid was obtained after instillation of 5-mL aliquots of saline into the most affected segmental or lobar bronchi.

**HIV testing**

A blood sample was collected for HIV testing. Rapid immunochromatographic assays were used for the detection of HIV-1 (Unigold® test; Trinity Biochem, Dublin, Ireland) and HIV-2 (Determine Abbott®; Abbott Diagnostics, Park, IL, USA). Results

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**Figure 1 -** Patients involved in the study according to the test results. AFB: acid-fast bacilli; TB: tuberculosis; DST: drug sensitivity tests; and NTM: nontuberculous mycobacteria.
were further confirmed by rapid enzyme-linked immunosorbent assay.

**CD4 counts**

To determine CD4 lymphocyte counts, we used flow cytometry (Epics-XL-MCL; Beckman Coulter, Fullerton, CA, USA) in accordance with the manufacturer recommendations.

**Statistical analysis**

Data were analyzed using the Stata Intercooled program, version 8.2 (Stata Corp., College Station, TX, USA). Univariate analysis was used to describe demographic variables and variables related to drug resistance, using the chi-square test to determine statistical significance. For hematological parameters, the Mann-Whitney test was performed.

**Results**

A total of 503 HIV-infected patients were recruited (Figure 1). Among those 503, smear microscopy for AFB and culture had been performed in sputum or bronchoalveolar lavage fluid specimens for 447. Positive mycobacterial culture results were obtained in 320 patients. Of those 320, 235 (73.4%) also presented a positive smear microscopy for AFB. All data presented here are related to the group of patients in which mycobacterial infection was confirmed through culture (n = 320). Drug sensitivity tests were performed in 258 isolates. Identification of mycobacteria to the species level was achieved in 277 isolates. A substantial number of isolates could not be identified (n = 43) or were not submitted to drug sensitivity tests (n = 62), most due to preservation problems during transport. Only 3 isolates were characterized as NTM. Table 1 shows the demographic characteristics of patients presenting mycobacterial infections, as confirmed through smear microscopy or culture. Most patients, regardless of gender, were in the economically productive age bracket. The predominance of black patients reflects the demographic profile of the country.

Weight loss, fever, and cough were the most frequent clinical findings observed, and 50.6% of patients were categorized as Class I. The most common radiologic pattern was interstitial (in 66.8%), followed by mediastinal lymph node enlargement (in 30%), and bronchiectasis (in 27.5%). Miliary nodules were seen in 17.8% of cases, and cavitation was seen in 12.1%. These data can be seen in Table 2. All three individuals infected with NTM presented clinical manifestations rather similar to those of the cases of *M. tuberculosis* infection. The median CD4 lymphocyte count was 151 cells/mm³, and the median total lymphocyte count was 1140.5 cells/mm³. Cavities seen on chest X-rays were associated with CD4 lymphocyte counts > 200 cells/mm³ (Figure 2).

**Mycobacteria identification**

Out of the 277 cultures in which mycobacteria were identified to the species level, 274 (99%) tested positive for *M. tuberculosis*, 2 (0.7%) tested positive for *M. avium*, and 1 (0.4%) tested positive for *M. simiae*. In the *M. tuberculosis* complex group, the gyrB polymerase chain reaction restriction analysis patterns confirmed *M. tuberculosis* in all isolates. Table 3 summarizes the drug resistance data. Sensitivity tests to antituberculous drugs were performed in 258 *M. tuberculosis* isolates and in 3 NTM isolates. New TB cases accounted for 179 (69%) of the patients, and 74 (29%) of the patients reported having previously been treated for
Resistance to the isoniazid-rifampin combination was higher among the previously treated cases than among the new cases. Overall, resistance to any single drug or combination of drugs occurred most frequently among the previously treated cases. As expected, the NTM isolated showed resistance to most of the drugs tested. Resistance to all of the drugs tested was found for *M. simiae*. Of the 2 *M. avium* isolates, one was sensitive only to rifampin, and the other was sensitive only to streptomycin.

**Discussion**

In Mozambique, AIDS and TB are firmly established. Comorbidities such as malarial infection, sickle cell anemia, malnutrition, and intestinal parasites contribute to the high mortality observed among such patients. In Mozambique, where data regarding TB and AIDS is incomplete, TB is ranked among the 10 leading preventable causes of deaths reported in Maputo. Although a nationwide TB treatment program has been run by paramedical staff since 1985, the results have been disappointing. The present study shows that TB/HIV co-infected patients in the Maputo area have low levels of hemoglobin and albumin, probably reflecting the above mentioned conditions, making the treatment of such patients even more difficult. Most of the affected individuals are in the economically productive age bracket, which implies a heavy socioeconomic burden. The clinical presentation of TB in the studied population was the classic triad of fever, sweats, and cough. Shortness of breath in combination with interstitial alterations seen on chest X-rays was a common finding, making *P. jirovecii* pneumonia one of the major differential diagnoses, especially among those with elevated serum lactate dehydrogenase, which was found in most of the patients evaluated. In a large case series study and review of the literature, the chest X-rays of TB/HIV co-infected patients were analyzed. The predominant findings were infiltrates, followed by interstitial infiltrate and lymphadenopathy. Low CD4 lymphocyte counts were associated with hilar/mediastinal lymphadenopathy and inversely associated with pleural effusion and cavitary disease. Our results corroborate those findings; NTM were isolated from sputum samples in only 3 cases. According

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Class I: asymptomatic; Class II: symptomatic less than 50% of the day; Class III: symptomatic more than 50% of the day; and Class IV: in bed most of the time.
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...disease, such as cavitary TB, carcinoma and chronic obstructive pulmonary disease. Transmission is thought to be airborne and fecal-oral since it has been detected in healthy human feces. The isolate found in this study was resistant to all common antituberculous drugs, as were M. simiae described in other case reports.

As expected, resistance of M. tuberculosis to at least one antituberculous drug was significantly lower among new cases of TB than among the previously treated cases. This pattern of resistance to at least one anti tuberculosis drug in previously treated cases is similar to that observed in other African countries. This high resistance rate among new cases might have been the result of concurrent situations: TB/HIV co-infection, which makes patients more susceptible when exposed to an environment in which there is continuous transmission of resistant mycobacteria; the use of certain antituberculous drugs for the treatment of other diseases; and decreased absorption of the antituberculous drugs due to concurrent diarrhea caused by parasitic diseases and intestinal bacterial infections, resulting in lower serum concentrations of the drug. The high resistance rates observed among the previously treated cases is of great concern, since it implies that conditions are conducive to the acquisition of additional resistance, leading to MDR-TB. Resistance to isoniazid was more common than was to other reports from developing countries, where the prevalence of TB is quite high, NTM isolation is rare. In one case, the probable causative agent was M. simiae. There have been few reports of M. simiae infection in HIV-infected patients. Although its association with human disease is not frequent, pulmonary infection with M. simiae has been reported in monkey trainers and in other individuals having close contact to these animals, as well as in patients with pre-existing pulmonary disease, such as cavitary TB, carcinoma and chronic obstructive pulmonary disease. Transmission is thought to be airborne and fecal-oral since it has been detected in healthy human feces. The isolate found in this study was resistant to all common antituberculous drugs, as were M. simiae described in other case reports.

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resistance to any of the other drugs tested and, at 14.9%, was considerably higher than 5.9% worldwide rate reported by the World Health Organization. According to that report, a higher than 10% rate of resistance to isoniazid and rifampicin can predict the development of MDR-TB. This finding increases concerns regarding the prophylactic use of isoniazid in HIV-infected individuals in Mozambique. The results of the drug sensitivity testing carried out in the present study indicates the need for strict enforcement of the directly observed therapy, short-course strategy, better epidemiological surveillance of TB cases, and coordination between AIDS and TB control programs.

References