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The bacteriology of pulmonary tuberculosis in a population with high human immunodeficiency virus seroprevalence

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SUMMARY

SETTING: A public sector urban university hospital in Soweto, South Africa.

OBJECTIVE: To describe the utility of sputum smear microscopy and the prevalence of *Mycobacterium tuberculosis* resistance to antituberculosis drugs according to human immunodeficiency virus (HIV) serostatus in adults.

DESIGN: A retrospective descriptive study of consecutive cases using a record review.

RESULTS: We studied 412 adults with culture-proven pulmonary tuberculosis, of whom 185 (44.9%) were HIV-seropositive and had a significantly lower sputum smear positivity than HIV seronegatives (68% versus 79%, $P < 0.05$). Smear positivity was significantly higher in HIV-infected patients with CD4 counts $\leqslant 50/mm^3$ compared to those with CD4 counts of $201–300/mm^3$ ($P < 0.05$). In patients with and those without a history of previous treatment for tuberculosis, resistance to one or more antituberculosis drugs was found in 32.2% and 13.6% of cases, respectively, while resistance to both isoniazid and rifampicin (multidrug-resistant tuberculosis [MDR]) was found in 15.3% and 4.5% of patients, respectively. There was no significant difference in resistance between HIV-positive and seronegative patients.

CONCLUSION: A strong tuberculosis control programme and good surveillance will be required to prevent the further spread of MDR tuberculosis. Surveys such as these are useful for monitoring control programmes.

KEY WORDS: tuberculosis; pulmonary; HIV; diagnosis; resistance

THERE IS A WELL DOCUMENTED association between tuberculosis and human immunodeficiency virus (HIV) infection in sub-Saharan Africa.[1,2] In South Africa, tuberculosis is endemic, with case notification rates of 225/100 000 in 1994,[3] while the HIV epidemic continues to expand with an estimated 1.2 million adults infected by the end of 1994.[4] In 1995, 9% of women attending antenatal clinics in Soweto, South Africa were HIV seropositive (unpublished data).

There have been conflicting reports as to whether the sputum smear positivity rate in HIV-infected patients with pulmonary tuberculosis is the same as or lower than that in HIV seronegatives.[5,6] It has furthermore been suggested that HIV-positive patients with more advanced immunodeficiency and positive sputum cultures for *Mycobacterium tuberculosis* are less likely to have positive sputum smears.[7] Since sputum smear positivity is used as the basis for tuberculosis services in developing countries, it is important for these issues to be resolved.

Drug-resistant tuberculosis declined in South Africa between 1965 and 1988.[8] In the 1980s, primary resistance to isoniazid (INH) was 9.5% and acquired resistance was 15%, while combined resistance to isoniazid and rifampicin was less than 2%.

Against this background, we report on the HIV seropositivity and the bacteriological results of adults with culture-proven pulmonary tuberculosis at Baragwanath Hospital, Soweto, South Africa. We aimed to describe the utility of sputum smear microscopy and the prevalence of *M. tuberculosis* resistance to antituberculosis drugs according to HIV-serostatus.

PATIENTS AND METHODS

Baragwanath Hospital is a 3300-bed public university hospital serving an estimated population of 3 million people. The patient population included all adults aged 18–59 years who were diagnosed with culture-proven pulmonary tuberculosis between June 1995 and February 1996 and who were either in-patients or were seen at least twice as out-patients. A retrospective review of records was performed. HIV serostatus and CD4 counts were noted in those patients who had been tested by their doctor.

Serum samples were tested for HIV antibody by

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Table 1 Relation between human immunodeficiency virus (HIV) status and sputum smear positivity (according to number of negative specimens in smear negative patients)

	HIV-positive	HIV-negative	*P* value
Sputum smear positive (%)	121/185 (64.4)	176/227 (77.5)	0.0088
Smear positive with ⩾2 specimens negative (%)	121/178 (67.9)	176/223 (78.9)	0.0178
Smear positive with ⩾3 specimens negative (%)	121/170 (71.2)	176/216 (81.5)	0.0235

third-generation enzyme immunoassay (EIA, Abbott Diagnostic Products, Weisbaden, Germany), with positive specimens being confirmed by a second EIA, Wellcozyme (Murex Biotech Ltd, Dartford, UK). CD4 enumeration was performed on a Coulter XL-MCL (Coulter, Hialeah, FL, USA) as a whole blood lysis assay using Q-prep and CD3-ECD/T4-RD1/T8-FITC (both from Coulter). CD4 cells were defined as those cells in the automatically set 'lymphoid' gate that co-expressed CD3 and CD4.

Resistance to antituberculosis drugs was described in patients with and those without a history of previous treatment for tuberculosis.

Sputum bacteriology

Acid-fast bacilli (AFB) were detected on direct smear of expectorated samples of sputum by fluorescent microscopy using a phenol-auramine stain (Auramine-O fluorochrome stain, South African Institute for Medical Research [SAIMR], Johannesburg), when at least 1–9 AFB/100 fields at 500 × magnification were seen (reported as scanty). Doubtful smears (1–2 AFB/300 fields) were checked by the Ziehl-Neelsen staining method.

Specimens were processed for culture using a modified Petroff's method.[9] Culture was carried out using both a Lowenstein-Jensen (LJ) agar slope and a BACTEC 12B vial (7H12 Middlebrook, Becton-Dickinson, Sparks, MD, USA).

The growth index in the BACTEC vial was measured on a weekly basis; an index greater than or equal to 20 was considered positive and the index was then read daily. Slide smears and agar plates of positive BACTEC vials were made to exclude the presence of contaminating non-mycobacterial bacteria. If such bacteria were present, decontamination was carried out and the vial re-cultured. LJ slopes were read once at 6 weeks for the presence of mycobacterial colonies.

Positive growth index BACTEC vials and colonies from LJ slopes were then inoculated into BACTEC vials for mycobacterial identification and sensitivity testing. Mycobacterial identification was carried out using the p-nitro-α-acetylamino β hydroxypropiophenone (NAP) system.[10]

Susceptibility testing was carried out in the BACTEC system.[11] Cultures were inoculated into vials containing isoniazid (0.1 μg/ml), streptomycin (0.1 μg/ml), rifampicin (2.0 μg/ml) and ethambutol (2.5 μg/ml), and one tenth of the inoculum was inoculated into a control BACTEC vial containing no antibiotics. Organisms found to be resistant were sent to the reference laboratory (SAIMR, Johannesburg) where the identification of the organism was confirmed as *M. tuberculosis* using a polymerase chain reaction (PCR) method developed by SAIMR. Multidrug resistance (MDR) refers to tubercle bacilli which were resistant to at least isoniazid and rifampicin.

RESULTS

There were 483 adults with positive sputum cultures for *M. tuberculosis*, of whom 412 (85.3%) were tested for HIV antibodies. Of those tested, 185 (44.9%) were HIV-seropositive. There was a statistically significant difference in the proportion of women compared to men who were HIV-infected: 89/172 (51.7%) women compared to 96/245 (39.2%) males ($P = 0.0236$).

Not all patients with negative sputum smears provided two or more sputum samples. At least two sputum specimens were tested for 178 of the 185 HIV-infected (96%) and 223 of 227 HIV-negative patients (98%). There was a significant decrease in sputum smear positivity in the HIV-positive group ($P < 0.05$) (Table 1).

CD4 counts were available for 97 HIV-seropositive patients (52.4%), of whom 62.9% had positive smears compared to 71.2% of the total HIV-positive group. Those patients with CD4 counts ⩽50/mm^3 had the highest smear positivity and those with CD4 counts of 201–300/mm^3 the lowest; this reached statistical significance ($P = 0.0218$) (Table 2).

Drug susceptibility testing was performed on cultures from 175 HIV-seropositive patients (95%) and 214 HIV-seronegative patients (94%). Resistance of tuber-

Table 2 Relation between CD4 count and sputum smear result in human immunodeficiency virus (HIV)-positive patients*

CD4/mm^3	Smear positive	Smear negative	% positive
0–50	23	7	76.6
51–200	17	12	58.6
201–300	5	9	35.7
>300	16	8	66.7

* Sputum smear negative patients had at least three negative specimens.

Table 3 Resistance to antituberculosis drugs according to HIV status in patients without a history of previous treatment for tuberculosis

	HIV-positive	HIV-negative	Total	*P* value
n	155	175	330	
Resistant to				
Isoniazid* (%)	13 (8.4)	11 (6.3)	24 (7.3)	0.6021
Isoniazid and rifampicin† (%)	10 (6.5)	5 (2.9)	15 (4.5)	0.1937
Other (%)	1 (0.6)	5 (2.9)	6 (1.8)	
Total (%)	24 (15.5)	21 (12.0)	45 (13.6)	0.4474

* Isoniazid alone or with streptomycin.
† With or without other drug resistances.

cle bacilli to one or more antituberculosis drug was found in 45 patients (13.6%) without a history of previous treatment for tuberculosis and 19 patients (32.2%) with a history of prior treatment (Tables 3 and 4). Isoniazid resistance, either singly or in combination with other drugs, was found in 14.9% of HIV-positives without a history of prior antituberculosis treatment. There was no statistically significant difference in resistance to drugs between HIV-positive and HIV-negative patients. Only 50% of patients with multidrug-resistant tuberculosis were sputum smear positive.

DISCUSSION

The high HIV seroprevalence in this series, which falls within the range of 17–49% found in patients with pulmonary tuberculosis in other African countries,[12] underlines the major impact that HIV infection will have on the number of active tuberculosis cases in a country with extremely high baseline tuberculosis rates. This is already being seen in our hospital where, for example, tuberculous meningitis is the commonest form of meningitis seen in adults.[13] There seems to be a disproportionately increased burden of tuberculosis in HIV-infected women compared to men. This observation reflects the approximately equal male-to-female ratio of HIV infection in this population and implies that HIV-seronegative men have more risk factors for TB reactivation than seronegative women.

The efficacy of diagnostic procedures for tuberculosis is reduced by dual infection with HIV. The significant decrease in sputum smear positivity in the HIV infected has a number of implications in developing countries, where resources for widespread use of culture are lacking. The delay in diagnosis between a negative smear, requesting a culture and then waiting for the result, which may still be negative, will have deleterious effects on patients in whom the course of tuberculosis is often compressed. Lack of access to HIV testing may result in the suspicion of tuberculosis being allayed in unidentified HIV-seropositives with negative sputum smears. There will be an increasing use of empirical antituberculosis treatment in the HIV infected, which will inevitably include many people without active tuberculosis and others with undiagnosed treatable infections. Ancillary techniques such as induced sputum with hypertonic saline nebulisation,[14] and new techniques such as PCR, may alleviate some of these problems if they can achieve wide application at low cost.

The finding that the most immunocompromised HIV seropositives based on CD4 count did not have lower rates of smear positivity has two possible explanations. Firstly, there is a high bacillary burden of *M. tuberculosis* in these patients, which can be demonstrated by positive smears in tissue samples.[15] Secondly, computed tomographic scans of the chest demonstrate microcavitation in many patients without cavitation on plain chest radiographs. There may be high bacillary counts in these cavities (Dr C Feldman, unpublished data).

There appears to be a large reservoir of patients with MDR isolates, reflecting the past failure of the TB control programme. In 1978 only 28% of patients referred

Table 4 Resistance to antituberculosis drugs according to human immunodeficiency virus (HIV) status in patients with a history of prior treatment for tuberculosis

	HIV-positive	HIV-negative	Total	*P* value
n	20	39	59	
Resistant to				
Isoniazid* (%)	3 (15.0)	3 (7.7)	6 (10.2)	0.6715
Isoniazid and rifampicin† (%)	4 (20.0)	5 (12.8)	9 (15.3)	0.7312
Other (%)	0	4 (10.3)	4 (6.8)	
Total (%)	7 (35.0)	12 (30.8)	19 (32.2)	0.9721

* Isoniazid alone or with streptomycin.
† With or without other drug resistances.

for TB treatment in Soweto completed 80% or more of the course.[16] Of particular concern is the high number of patients with MDR and with no history of previous treatment for tuberculosis. Only 50% of the MDR patients were smear positive, suggesting a lower risk of transmission compared to drug susceptible patients, a scenario different from that in the United States, where 70% of these patients were smear positive.[17] The MDR problem was not significantly higher in the HIV infected, a finding similar to that in other African countries.[5,18] The high level of INH resistance in the HIV infected militates against its widespread use alone as prophylaxis against tuberculosis in this community.

This study has several limitations. Since most of the patients were at least moderately ill and required referral or hospitalisation at the time of diagnosis, they may not be representative of all patients with tuberculosis in Soweto. The age group 18–59 years was chosen, as older people are not usually tested for HIV. Patients with MDR may be more ill and thus require hospitalisation more often than those with susceptible tuberculosis.[19] Only half of the HIV-seropositives had CD4 counts, but the fact that smear positivity was similar to that of the whole group suggests that they were representative, although the groups were small.

In conclusion, this is an area with a serious tuberculosis problem which will require major resource allocation and support in order to reach the World Health Organisation targets of 85% cure and 70% case detection rates. A strong programme with good surveillance is needed to prevent the further spread of MDR tuberculosis.[20] Surveys such as these are useful for monitoring control programmes.

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RÉSUMÉ

CADRE : Hôpital universitaire du secteur public dans la ville de Soweto, Afrique du Sud.

OBJECTIF : Décrire l'utilité de l'examen microscopique direct des crachats et la prévalence de la résistance de *Mycobacterium tuberculosis* à l'égard des médicaments antituberculeux selon le statut du virus de l'immunodéficience humaine (VIH) chez les adultes.

SCHÉMA : Etude descriptive rétrospective de cas consécutifs par une révision des dossiers.

RÉSULTATS : Nous avons étudié 412 adultes atteints d'une tuberculose pulmonaire prouvée par la culture, parmi lesquels 185 (44,5%) étaient séropositifs pour le VIH ; ces derniers avaient une diminution significative de la positivité de l'expectoration par comparaison avec les séroné-

gatifs (68% versus 79%, $P < 0,05$). La positivité de l'examen direct était significativement plus élevée chez les sujets infectés par le VIH dont les décomptes de CD4 étaient $\leqslant 50/mm^3$ par comparaison avec ceux dont les décomptes étaient de $201–300/mm^3$ ($P < 0,05$). Chez les patients avec ou sans antécédents de traitement antérieur pour tuberculose, la résistance à l'égard d'un ou de plusieurs médicaments antituberculeux s'observe respectivement chez 32,2% et 13,6% des cas, alors que la tuberculose à germes multirésistants (résistante à la fois à l'isoniazide et à la rifampicine) s'observe respectivement chez 15,3% et 4,5% des patients. Il n'y a pas de différence significative de résistance entre les séropositifs et les séronégatifs pour le VIH.

CONCLUSION : Pour prévenir la poursuite de l'extension des tuberculoses à germes multirésistants, un programme sévère de lutte antituberculeuse et une bonne surveillance sont indispensables. Des enquêtes comme celle-ci sont utiles pour la surveillance des programmes de lutte.

RESUMEN

MARCO DE REFERENCIA : Un hospital universitario de un sector urbano en Soweto, Sudáfrica.

OBJETIVOS : Describir la utilidad de la baciloscopía del esputo y la prevalencia de la resistencia de *Mycobacterium tuberculosis* a las drogas antituberculosas de acuerdo con la reacción sérica del virus de la inmunodeficiencia humana (VIH) en adultos.

MÉTODO : Un estudio retrospectivo y descriptivo de casos consecutivos utilizando una revisión de fichas clínicas.

RESULTADOS : Etudiamos 412 adultos con tuberculosis pulmonar con cultivo positivo, de los cuales 185 (44,9%) eran VIH-seropositivos y tenían una baja positividad en la baciloscopía del esputo comparada con los VIH seronegativos (68% versus 79%, $P < 0,05$). La positividad de la baciloscopía fue más alta en los VIH positivos con recuentos de CD4 $\leqslant 50/mm^3$ comparado con aquéllos con recuentos de CD4 de $201–300/mm^3$ ($P < 0,05$). En pacientes con y sin historia de tratamiento previo de tuberculosis, se encontró resistencia a una o más drogas antituberculosas en un 32,2% y 13,6% de los casos respectivamente, mientras que la resistencia tanto a la isoniacida como a la rifampicina (tuberculosis multirresistente, TMR) se encontró en un 15,3% y 4,5% de los pacientes, respectivamente. No existió una diferencia significativa en la resistencia entre VIH seropositivos y seronegativos.

CONCLUSIÓN : A fin de prevenir la tuberculosis multirresistente se requerirá de un programa de control de la tuberculosis sólido y de una buena vigilancia. Este tipo de encuestas son útiles para supervisar los programas de control.