

ISOLATION OF MYCOBACTERIA
FROM STOOLS AND INTESTINAL BIOPSIES
FROM HIV SEROPOSITIVE AND HIV SERONEGATIVE PATIENTS
WITH AND WITHOUT DIARRHEA IN KINSHASA, ZAIRE
Preliminary results*

by

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Summary — To determine the role of mycobacteria as etiologic agent in HIV related enteritis in Africa, the following study was performed in 1986, in the department of Internal Medicine in Mama Yemo Hospital in Kinshasa, Zaïre. Stool and intestinal biopsies were obtained from HIV seropositive and HIV seronegative patients with and without diarrhea. Patients with known *Mycobacterium tuberculosis* infection were not enrolled. Acid fast bacilli were found in fecal smears of 6 (10%) of 59 HIV seropositive patients and in none of 41 HIV seronegative patients ($p = 0.04$). Isolation rates of mycobacteria were slightly lower in HIV seropositive patients than in HIV seronegative patients (25% vs 44%, $p = 0.08$) and significantly lower in patients with diarrhea than in patients without diarrhea (15% vs 44%, $p = 0.02$). *Mycobacterium avium-intracellulare* was the species most frequently isolated from stools, in 12% of the HIV seropositive and in 22% of the HIV seronegative patients. Mycobacteria were not isolated from any of the intestinal biopsies obtained in 17 HIV seropositive patients with persistent diarrhea and any of these biopsies showed histological evidence of a mycobacterial infection. This study suggests that mycobacteria do not seem to play a major role in causing diarrhea in HIV seropositive patients.

KEYWORDS: Mycobacteria; *Mycobacterium avium-intracellulare*; *Mycobacterium tuberculosis*; Diarrhea; HIV Infection; Zaïre

Introduction

Mycobacterium tuberculosis infection is a frequent complication in HIV infected individuals in Africa (5, 11, 21). The role of atypical mycobacteria as opportunistic agents in African AIDS patients remains to be established. In the United States and Europe, infections with atypical mycobacteria such as *Mycobacterium avium-intracellulare* (MAI) are observed more frequently in HIV infected individuals than *M. tuberculosis* infections (6, 17, 18, 19, 24, 25). Infections with atypical mycobacteria have been observed in 50% of AIDS patients in autopsy series (25). The clinical consequences of an infection with atypical mycobacteria in a HIV seropositive individual remain incompletely known. It has been suggested that the gastrointestinal is a portal of entry for the MAI (23) and that this organism may cause persistent diarrhea (9).

* Part of this work has been presented by Dr. Maraka Nembunzu as thesis to obtain the degree in Master of Science during the International Course on Biomedical Tropical Science at the Institute of Tropical Medicine, Antwerp, Belgium.

Fourty to 90 % of AIDS patients in Africa develop persistent diarrhea (3, 20), but in more than 50 % of those patients the cause of such diarrhea cannot be found (20,4). To determine the role of mycobacteria as etiologic agents in HIV related enteritis in Africa, we performed, mycobacterial cultures on stools and intestinal biopsies of HIV seropositive and HIV seronegative patients with and without diarrhea.

Material and methods

The study was performed in 1986, in Mama Yemo Hospital in Kinshasa, Zaire.

Patients

HIV seropositive and HIV seronegative patients with diarrhea of at least one week duration, seen as in- and outpatients at the department of internal medicine were recruited in the study. Diarrhea was defined as at least two bowel movements a day of unusual loose consistency. Persistent diarrhea was defined as at least 30 diarrhea days during the last two months. Consecutive HIV seropositive and HIV seronegative patients without diarrhea visiting the gastro-enterologic outpatient department were also enrolled. Patients known to have active pulmonary tuberculosis and/or patients treated with antituberculous drugs were excluded from the study.

Laboratory methods

Serum was tested for HIV antibodies by ELISA (Organon Technica, Oklahoma city, OK, US or Wellcozyme, Wellcome Diagnostics, Dartford, UK). Positive sera were confirmed by Western blot (Dupont de Nemours, Wilmington, DA, US) or by indirect immunofluorescence.

Stool specimens (0.5 to 1 g) and intestinal biopsies were conserved in cetylpyridinium chloride (CPC) (22) and sent at room temperature to the Institute of Tropical Medicine, Antwerp. Conservation time before isolation procedures was started varied between 14 days and two months.

Fecal smears were made with saline on a slide and stained with Ziehl-Neelsen stain.

Mycobacterium cultures: three methods of stool decontamination were utilised: the method of Petroff (13), the method of Beerwerth and Schurmann (1) and the method of Wolinsky and Rynearson modified by Portaels *et al.* (15). Specimens were cultured on Löwenstein Jensen and Ogawa media (15). The media were checked every 15 days, during six months. Mycobacteria were identified using the identification schemes described by Jenkins *et al.* (10).

Intestinal biopsies (duodenal and rectosigmoidal biopsies) were obtained by endoscopy in HIV seropositive and HIV seronegative patients with persistent diarrhea and in HIV seropositive and HIV seronegative patients without diarrhea who underwent endoscopy for other reasons [abdominal

pain/discomfort (34 patients) gastrointestinal bleeding (2 patients), Kaposi's sarcoma (2 patients)]. Biopsies were taken from macroscopic normal appearing mucosa, except for five biopsies obtained from five HIV seropositive patients with slightly erythematous rectal mucosa. Intestinal biopsy specimens were also placed in 10% buffered formalin and processed using standard procedures. Sections were stained with haematoxylin eosin and periodic acid Schiff.

No systematic work up for enteric pathogens was performed.

Statistical analysis

Chi-square and Fishers' exact test.

Results

Patients' characteristics are shown in table 1. There were significantly more females among HIV seronegative patients and patients without diarrhea than among HIV seropositive patients and patients with diarrhea ($p < 0.03$). Mean ages of all patient groups were similar. Acid-fast bacilli (AFB) were found in fecal smears of 6 (10%) of 59 HIV seropositive patients, and in none of the 41 HIV seronegative patients ($p = 0.04$) (Table 1). Of the 6 patients with presence of fecal AFBs, mycobacteria (MAI) were cultured from the stools, in only one case. Isolation rates of mycobacteria were slightly lower in HIV seropositive compared to HIV seronegative patients (25 vs 44%, $p = 0.08$) (Table 1). Mycobacteria were isolated from significantly fewer patients with diarrhea than from patients without diarrhea (15% vs 45%, $p = 0.02$) (Table 1). This was so regardless the serological status of the patient. Types of mycobacterial species which were identified are shown in Table 2. MAI was the species most frequently isolated, in 12% of the HIV seropositive and 22% of the HIV seronegative patients. *M. tuberculosis* on the other hand was recovered from 7% of the HIV seropositive and in 2% of the HIV seronegative patients. In a few patients *M. gordonae*, *M. malmoense* and *M. simiae* were isolated.

TABLE 1
Results of mycobacterium cultures of stool and intestinal biopsies in HIV seropositive and HIV seronegative patients with and without diarrhea, Kinshasa, Zaire

	PATIENTS			
	WITH DIARRHEA HIV (+)*	WITHOUT DIARRHEA HIV (-)*	WITH DIARRHEA HIV (+)*	WITHOUT DIARRHEA HIV (-)*
MALES	19	9	12	3
FEMALES	7	5	21	24
MEAN AGE (years)	32	30	30	30
FECAL AFB	1/26 (4)	0/14	5/33 (15)	0/27
STOOL CULTURE	4/26 (15)	2/14 (14)	11/33 (32)	16/27 (59)
BIOPSY CULTURE	0/17	1/3 (33)	0/11	0/7

* N° (+) / N° tested (%)

TABLE 2
Types of mycobacteria isolated from stools of HIV seropositive and HIV seronegative patients in Kinshasa, Zaire

MYCOBACTERIA	HIV (+) PATIENTS N = 59	HIV (-) PATIENTS N = 41
M. tuberculosis	4* (7)	1 (2)
MAI**	7 (12)	9 (22)
M. gordonae	0	1 (2)
M. malmoense	2 (3)	0
M. simiae	2 (3)	5 (12)
Unidentified***	1 (2)	2 (5)

* N° of patients with positive culture (%)

** *Mycobacterium avium-intracellulare*

*** No growth in subculture

Intestinal biopsies were obtained from 38 patients (29 duodenal and 21 rectosigmoidal biopsies); in 13 patients a duodenal and a rectosigmoidal biopsy was obtained. Mycobacteria were isolated from only one biopsy (*M. malmoense* was isolated from a duodenal biopsy of a HIV seronegative patient with persistent diarrhea) (Table 1). No mycobacteria were cultured from the stools of this patient. In none of the biopsies histologic evidence of mycobacterium infection was found.

Discussion

AFBs were more frequently observed in fecal smears of HIV seropositive than of HIV seronegative patients. The explanation of this finding is unclear. In a study performed in Zambia, AFBs were observed with a similar frequency in fecal smears of HIV seropositive and HIV seronegative patients (7), but in this study stools had not been kept in a transport medium. In our study as well as in the Zambian study there was no correlation between a positive microscopy and a positive culture. This suggests that either Ziehl-Neelsen stains of fecal smears are not a reliable indicator of the presence or absence of mycobacteria in stool specimens or that the culture methods used were not sensitive enough.

Mycobacteria were less often isolated in HIV seropositive patients and in patients with diarrhea than in HIV seronegative patients and in patients without diarrhea. One explanation could be that because of the long conservation time of the stool specimens in CPC, certain mycobacteria strains which were particularly prevalent in HIV seropositive patients were unable to grow. A decrease of isolation rates of *M. tuberculosis*, after prolonged conservation in CPC has previously been observed (Portaels, unpublished). Another explanation could be that patients were using antibiotics at the moment stool specimens were obtained. HIV seropositive patients and patients with diarrhea are more likely to use antibiotics than HIV seronegative patients and than patients without diarrhea. Certain antibiotics, such as for example trimethoprim-sulfamethoxazole, a drug frequently used in AIDS and/or diarrhea patients, are able to inhibit certain atypical mycobacteria such as MAI (8).

Despite the fact that patients with known active tuberculosis were excluded from the study, in seven % of the HIV seropositive and two % of the

HIV seronegative patients, *M. tuberculosis* was isolated from their stools. This suggests that a considerable amount of active *M. tuberculosis* infections in Kinshasa remain undetected. Whether this is related to the fact that HIV potentially can alter the classic clinical presentation of *M. tuberculosis* infection (5,2), needs further study.

In none of the HIV seropositive study patients with diarrhea, mycobacteria were isolated from intestinal biopsies. Also in the Zambian study no correlation was found between chronic diarrhea and the presence of mycobacteria in the stool (7). These findings suggest that mycobacteria do not play a major role in causing diarrhea in HIV seropositive patients.

So far the significance of the isolation of mycobacteria other than *M. tuberculosis* from the stool of a HIV seropositive patient remains unknown. Interesting is the observation that *M. malmoense* and *M. simiae* were isolated in 9% of the patients. So far such mycobacteria, unlike *MAI*, were never isolated from the environment (10).

The results of this study should be considered as preliminary and should be confirmed by studies on patients for which more clinical information is available (including previous use of antibiotics) and in which intestinal biopsies and stools are examined and cultured without previous conservation in transport medium. Moreover, in prospective studies we need to determine the positive predictive value of the isolation of certain strains of atypical mycobacteria from the patients' stools for the development of generalised mycobacterial infections. Disseminated atypical mycobacterial infections have been reported in HIV seropositive patients from Africa (14), however the prevalence of such infections may be low (12) and the clinical manifestations associated with such infections in HIV infected patients remains to be determined.

Acknowledgements — We are grateful to the Commissioner of Health, Prof. Dr. K. Ngandu for his support; the Belgian-Zairian Medical Corporation, the European Economic Community, and the US Public Health Service for their financial assistance; to Prof. P. Gigase for reading the biopsies; to A. Van der Meer for typing the manuscript.

Isolément de mycobactéries de selles et de biopsies intestinales de patients VIH séropositifs et VIH séronégatifs avec et sans diarrhée à Kinshasa, Zaïre. Résultats préliminaires.

Résumé — Pour déterminer le rôle des mycobactéries comme agent étiologique dans la diarrhée liée à l'infection VIH en Afrique, l'étude suivante a été effectuée en 1986, au département de médecine interne à l'hôpital Mama Yemo à Kinshasa, Zaïre. Des selles et biopsies intestinales ont été obtenues chez des patients VIH séropositifs et VIH séronégatifs avec et sans diarrhée. Les patients connus comme tuberculeux étaient exclus de l'étude. Des bacilles acido-résistants ont été trouvés dans les frottis fécaux chez 6 (10%) de 59 patients VIH séropositifs et chez aucun des 41 patients séronégatifs ($p = 0.04$). Les mycobactéries étaient un peu moins fréquemment isolées chez les patients VIH séropositifs que chez les patients VIH séronégatifs (55% vs 44%, $p = 0.08$) et étaient significativement moins fréquentes chez les patients avec diarrhée que chez les patients sans diarrhée (15% vs 45%, $p = 0.02$). Les *mycobactéries avium-intracellulare* étaient l'espèce la plus fréquemment isolée, chez 12% VIH séropositifs et chez 22% des patients VIH séronégatifs. Chez aucun des 17 patients VIH séropositifs avec une diarrhée persistante, chez qui une biopsie intestinale a été obtenue, une infection à mycobactérie a été constatée ni histologiquement, ni par culture. Les mycobactéries ne semblent pas jouer un rôle important comme cause de diarrhée chez les patients VIH séropositifs.

Isolatie van mycobacteriën uit de stoelgang en intestinale biopsies bij HIV seropositieve en HIV seronegatieve patiënten met en zonder diarree in Kinshasa in Zaïre. Voorlopige resultaten.

Samenvatting — Om de rol van mycobacteriën te bepalen als etiologisch agent bij HIV geassocieerde enteritis in Afrika werd de volgende studie in 1986 uitgevoerd in de dienst interne

geneeskunde van het Mama Yemo Ziekenhuis in Kinshasa, Zaïre. Stoelgang en intestinale biopsies werden bekomen bij HIV seroposatieve en HIV seronegatieve patiënten met en zonder diarree. Patiënten met een gekende tuberculieuze infectie werden niet opgenomen in de studie. Zuurvaste staafjes werden gevonden in stoelgang uitstrijkjes van 6 (10%) van de 59 HIV seroposatieve patiënten en in geen enkel van de 41 HIV seronegatieve patiënten ($p = 0.04$). De frequentie van isolatie van mycobacteriën was lichtjes lager bij HIV seroposatieve patiënten dan bij HIV seronegatieve patiënten (25% vs 44%, $p = 0.08$) en was significant lager in patiënten met diarree dan in patiënten zonder diarree (15% vs 44%, $p = 0.02$). *Mycobacterium avium intracellulare* was het species dat het meest frequent werd geïsoleerd uit de stoelgang, in 12% van de HIV seroposiatieven en in 22% van de HIV seronegatieve patiënten. Uit geen enkel van de intestinale biopsies, bekomen bij 17 HIV seroposatieve patiënten met chronische diarree, werden mycobacteriën geïsoleerd en in geen enkele van deze biopsies werden mycobacteria vastgesteld bij histologisch onderzoek. Deze studie suggereert dat mycobacteriën geen belangrijke rol spelen in het veroorzaken van diarree bij HIV seroposatieve patiënten.

Received for publication on August 27, 1990.

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