Prevalence of Nocardia species among HIV-positive patients with suspected tuberculosis

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SUMMARY The objective of the study was to determine the frequency of nocardiosis in HIV-positive and HIV-negative individuals clinically suspected of having tuberculosis (TB). The study population (n = 171) were those who attended chest hospitals in Khartoum State, Sudan, between January and March 2010. The patients suffered from pulmonary infections with positive acid-fast bacilli. Blood (n = 171) and sputum (n = 171) samples were collected simultaneously. Blood samples were tested serologically for the presence of antibodies using HIV/Intensified Combination Prevention (ICP) test and sputum were cultured onto Lowenstein Jensen slants according to standard methods. Isolates showing rapid growth characteristic of Nocardiae were subcultured and subsequently identified using glucose yeast extract agar medium. All candidates in the study population (n = 171) suffered from pulmonary infections with positive acid-fast bacilli. Five Nocardia species were isolated from HIV-negative patients whereas two were from HIV-positive patients. Nocardia spp. cause pulmonary infections (4.09%) in both immunocompetent (2.92%) as well as immunocompromised (11.7%) patients who attend chest clinics in Sudan.

Introduction
Recent increases in the reported frequency of human nocardial infections can be attributed to the widespread use of immunosuppressive drugs, improved selective isolation procedures and increased clinical and microbiological awareness. Nevertheless, in some developing countries where other chronic lung diseases, particularly TB, are prevalent, Nocardiae are either missed or misidentified in laboratory specimens.

Immunosuppression is the most important predisposing factor for pulmonary nocardiosis. Nocardia is a ubiquitous soil saprophyte but there are currently more than 30 species causing human clinical infections, notably Nocardia asteroides, N. farcinica, N. brasiliensis, N. nova and N. abscessus. These species cause a wide variety of diseases and have variable drug susceptibilities. Primary pulmonary nocardiosis may be subclinical or pneumonia mimics pulmonary TB in clinical symptoms and radiological characteristics and it is often wrongly treated with anti-TB drugs. The differential diagnosis usually includes malignancy, pulmonary TB, Rhodococcus equi (in HIV-infected patients) or fungal pneumonia, especially Aspergillus spp.

Accurate identification of Nocardia species has become increasingly important as differences among the species have emerged in terms of epidemiology, virulence and antibiotic susceptibility. Optimal therapeutic strategies depend on rapid and accurate identification of Nocardia spp. Molecular methods for identification, such as polymerase chain reaction, restriction enzyme analysis and sequencing, offer a time-saving alternative to conventional methods that involve the assessment of growth characteristics and colony and microscopic morphology and biochemical and susceptibility testing.

Reports have suggested that there is usually a delay in the diagnosis of pulmonary nocardiosis of about six weeks (range: 2 weeks – 4 years) which is attributed to a lack of awareness. The usual reason for requesting culture studies for the detection of Nocardia spp. is that a patient has not responded to the usual anti-bacterial or anti-TB treatment. Recent advances in medical technology and the increased number of immunocompromised patients, such as those with organ transplants or HIV infection, led to an increase in opportunistic infections.

According to the Sudan HIV/AIDS Working Group (SHAWG) an estimated 500,000 people living with HIV/AIDS were recorded in the country. Despite the fact that the epidemiological data is so limited, it is believed that the country is in the early stages of a HIV/AIDS epidemic and nocardiosis has been recognized in recent times as an unusual opportunistic infection associated with HIV.

The present study aimed to determine the frequency of nocardiosis in HIV-positive and HIV-negative individuals clinically suspected of having TB.

Methods

Ethical clearance

The study was approved by the National Ethics Committee, Ministry of Health, Sudan. Written consent was obtained from every patient before they were enrolled in the study.
Study design

The study was a prospective, cross-sectional study conducted in Khartoum State. One hundred and seventy-one patients who attended the Abu-Anga Hospital for Chest Diseases and El-Shaab Teaching Hospital during the period from January to March 2010 were tested for HIV serologically and were examined for the presence of acid-fast bacilli. The patients were suspected of having TB. A patient was included in the study if found to be positive for acid-fast bacilli. Data were collected by using a questionnaire. Sputum specimens were collected according to the World Health Organisation criteria in sterile, plastic, wide-mouth and leak-proof containers, wrapped with sufficient absorbent material to soak up any leak. All specimens were placed into a container that is hard to break and within a robust outer plastic box. Sputum specimens were not saliva but coughed up from the lungs after three mouth washes. Specimens were processed on the same day.

HIV screening

Under sterile conditions, 5 mL whole venous blood specimens were withdrawn from patients included in the study. The blood specimens were collected in plain containers (without anticoagulant). Serum was separated by centrifugation at 2000 rpm for 5 min. Then a HIV screening test was done using the immunochromatography technique (ICT; Biomeraux Inc, Marcy l’Etoile, France), the assay procedure as follows:

1. 10 μL of serum or plasma added to the sample window
2. Interpretation of results between 5–20 min by colour line appeared in the result window.

Isolation procedure

One hundred and seventy-one (n = 171) sputum samples were subjected to digestion-decontamination according to standard methods. The sputum samples were concentrated by centrifugation and the resultant preparations were used to inoculate Lowenstein-Jensen (LJ) slopes, which were incubated at 37°C for 14 days and then used to make smears which were examined with a standard Ziehl-Neelsen acid-fast stain.

Identification of isolates

Growth of actinomycetes was suspected on a morphological basis and confirmed using selected phenotypic properties. The isolates were subcultured and maintained on glucose yeast extract agar (GYEA) slopes at room temperature and as suspensions of mycelial fragments in glycerol (20% [vol/vol]) at −20°C. All the isolates were studied phenotypically and chemotaxonomically.

Detailed phenotypic characterization methods have been described by Isenbeg. The extraction of mycolic acids and thin layer chromatography analysis of extracted mycolates were done as described by Hamid et al.

Results

One hundred and seventy-one patients who approved were enrolled. All candidates in the study population (n = 171) suffered from pulmonary infections, nocardiosis was found in 4% (n = 7) and there were 9.9% (n = 17) HIV-positive cases. Five Nocardia spp. were isolated from HIV-negative patients and two were from HIV-positive patients.

The majority of the patients were males (119/171, 69.6%), compared with 52/171 (30.4%) females. Patients were grouped into three age groups: Group I (<20 years; 11.7%); Group II (21–49 years; 67.3%); and Group III (>50, 21.1%). One hundred and nine (63%) out of 171 patients were new cases, 29 (17%) patients were old cases, 25 (15.2%) patients were relapses, and eight (4.8%) had suffered treatment failure. Among the eight treatment failures there were three (37.5%) from whom Nocardia species were isolated.

The HIV/ICT results indicated that 17 of the 171 (9.9%) patients were HIV-positive (9.2% male and 11.5% female). There were 5.8% HIV-positives in Group II (21–49 years) compared to 4% in Group III (>50 years).

Seven LJ slopes out of the cultured 171 (4%) sputum samples showed growth and colony appearances characteristic of Nocardia species. The growth occurred within 2–3 days. The frequency of Nocardia spp. among Group II was 1%, compared to 3% in Group III. From these seven isolates, two (12%) were obtained from HIV-positive patients.

The main phenotypic properties used in this study were morphological, cultural, biochemical and physiological properties on GYEA. All seven isolates tentatively identified as Nocardia spp. showed orange, creamy and yellow wrinkled rough colonies. Some colonies were embedded into agar but others were smooth and easily detached.

All seven isolates showed the standard patterns of mycolic acid components when whole cell acid methanolsates were analysed by the thin layer chromatographic technique. The plates revealed single spots co-chromatographed with reference to Nocardia strain.

Discussion

The present study confirmed the occurrence of Nocardia spp. (4%) among Sudanese patients suffering from pulmonary infections. Similar figures have been previously reported: Hamid et al. assigned eight (2%) isolates from 400 patients to the genus Nocardia as N. Afericana; Elhassan et al. reported 10 (3%) samples with Nocardia infections; Lucas et al. in West Africa reported 10 (4%) patients with nocardiosis, including patients with definitive AIDS of whom four had been initially misdiagnosed as having pulmonary TB.

Patients with HIV infection are prone to developing pulmonary infections such as nocardiosis. It is often misdiagnosed as pulmonary TB since the manifestations are similar. In areas where HIV-associated TB is common, some patients diagnosed as smear-negative for pulmonary TB will actually have nocardiosis. Clinicians should be aware of this in HIV/immunocompromised patients with respiratory infections who fail to respond to anti-TB treatment. HIV should be considered in patients with CD4+ T cell counts below 50/μL and lung or pericardial involvement.

The present study revealed pulmonary nocardiosis with 4% frequency among the studied population. The infection
was diagnosed in both immunocompetent as well as immunocompromised patients who attended chest clinics in Sudan. Specific risk factors were found in 94% of patients. The predisposing conditions were treatment with steroids (64.5%), chronic obstructive pulmonary disease (COPD; 23%), transplantation (29%), HIV infection (19%) and alcoholism (6.5%).

Contrary to the common belief that Nocardia infection affects only those who are immunocompromised, it affects both types. We reported five cases from immunocompetent and two from immunocompromised patients. King et al.22 argue that Nocardia is an uncommon opportunistic pathogen in patients with HIV infection and they considered Nocardia in association with advanced CD4 depletion.

Conclusions
All candidates in the study population (n = 171) suffered from pulmonary infections (such as TB and others): 4% (n = 7) had nocardiosis and there were 17 (9.9%) HIV-positive cases. Therefore, Nocardia spp. did cause pulmonary infections in both immunocompetent and immunocompromised patients who attended chest clinics in Sudan.

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