Short Report

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, nocardiosis was diagnosed in 4% ($n = 7$), HIV-positive cases were 17 (9.9%). 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 (1.17%) 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.[1,2]

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.[3–8] Primary pulmonary nocardiosis may be subclinical or pneumonic mimics pulmonary TB in clinical symptoms and radiological characteristics and it is often wrongly treated with anti-TB drugs.[9] The differential diagnosis usually includes malignancy, pulmonary TB, *Rhodococcus equi* (in HIV-infected patients) or fungal pneumonia, especially *Aspergillus* spp.[9]

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.[10]

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)[11] 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[12] 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 uL of serum or plasma added to the sample window in the test card and allowed to soak in;

(2) 20 uL of diluents added to the sample window;

(3) 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.[13] 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.[10,14] 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.[13] The extraction of mycolic acids and thin layer chromatography analysis of extracted mycolates were done as described by Hamid *et al.*[15]

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 methanolysates 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.*[16] assigned eight (2%) isolates from 400 patients to the genus *Nocardia* as *N. Africana*; Elhassan *et al.*[17] reported 10 (3%) samples with *Nocardia* infections; Lucas *et al.*[18] 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.[19] In areas where HIV-associated TB is common, some patients diagnosed as smear-negative for pulmonary TB will actually have nocardiosis.[7] 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.[20]

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%).[21] Contrary to the common belief that *Nocardia* infection affects only those who are immunocompromised, it affects both types.[2] 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.

References

1 Baily GG, Neill P, Robertson VJ. Nocardiosis: a neglected chronic lung disease in Africa? *Thorax* 1988;**43**:905–10

2 Minero MV, Marín M, Cercenado E, Rabadán PM, Bouza E, Muñoz P. Nocardiosis at the turn of the century. *Medicine (Baltimore)* 2009;**88**:250–61

3 Beaman BL, Beaman L. *Nocardia* species: host-parasite relationships. *Clin Microbiol Rev* 1994;**7**:213

4 Hwang JH, Koh WJ, Suh GY, *et al.* Pulmonary nocardiosis with multiple cavitary nodules in a HIV-negative immunocompromised patient. *Intern Med* 2004;**43**:852–4

5 Manfredi R, Sabbatani S, Marinacci G. Nocardiosis as the first opportunistic disease in a patient with missed HIV infection. Pathomorphism of presentation, clinical course, and evolution. *Recenti Prog Med* 2006;**97**:262–5

6 Muñoz J, Mirelis B, Aragón LM, *et al.* Clinical and microbiological features of nocardiosis 1997–2003. *J Med Microbiol* 2007;**56**:545–50

7 Rasheed MU, Belay G. Nocardiosis in HIV-seropositive clinically suspected pulmonary tuberculosis patients. *Trop Doct* 2008;**38**:34–5

8 Imai K, Koibuchi T, Kikuchi T, *et al.* Pulmonary nocardiosis caused by *Nocardia exalbida* complicating neumocystis pneumonia in an HIV-infected patient. *J Infect Chemother* 2011 (in press)

9 Olson ES, Simpson AJ, Norton AJ, Das SS. Not everything acid-fast is *Mycobacterium tuberculosis* – a case report. *J Clin Pathol* 1998;**51**:535–6

10 Saubolle MA, Sussland D. Nocardiosis: review of clinical and laboratory experience. *J Clin Microbiol* 2003;**41**:4497–501

11 The Sudan HIV/AIDS Working Group (SHAWG). Tackling the HIV Challenge in Sudan, The Way Forward, 2008. Available at: www.shawg.org (last accessed March 2011)

12 World Health Organization. Culture examination and identification. In: *Laboratory Services in Tuberculosis Control*. Geneva: WHO, 1998:196–200

13 Isenberg HD (ed). *Essentials Procedures for Clinical Microbiology*. Washington DC: ASM Press, 1998:171–359

14 Goodfellow M. The genus *Nocardia* Trevisan 1889, 9AL *In*: *Topley and Wilson's Microbiology and Microbial Infections.* Systematic Bacteriology. 9th edn, Vol 2. Balows A, Duerden BI (eds). London: Edward Arnold, 1998:464–89

15 Hamid ME, Minnikin DE, Goodfellow M, Ridell M. Thin layer chromatographic analysis of glycolipids and mycolic acids from *Mycobacterium farcinogenes*, *Mycobacterium senegalense* and related taxa. *J Med Microbiol Virol Parasitol Infect Dis* 1993;**279**:354–67

16 Hamid ME, Maldonado L, Sharaf Eldin G, Mohamed MF, Saeed NS, Goodfellow M. *Nocardia africana* sp. nov., a new pathogen isolated from patients with pulmonary infections. *J Clin Microbiol* 2001;**39**:625–30

17 Elhassan MM, Magzoob EF, El Rahman M, Saeed NS, Hamid ME. The occurrence of *Nocardia* species among patients with pulmonary infection. *Bahrain Med Bull* 2010;**32**:30–3

18 Lucas SB, Hounnou A, Peacok C, Beaumel A, Kadio A, Cock KM. Nocardiosis in HIV-positive patients: an autopsy study in West Africa. *Tuber Lung Dis* 1994;**75**:301–7

19 Subhash HS, Christopher DJ, Roy A, Cherian AM. Pulmonary nocardiosis in human immunodeficiency virus infection: a tuberculosis mimic. *J Postgrad Med* 2001;**47**:30–2

20 Biscione F, Cecchini D, Ambrosioni J, Bianchi M, Corti M, Benetucci J. Nocardiosis in patients with human immunodeficiency virus infection. *Enferm Infecc Microbiol Clin* 2005;**23**: 419–23

21 Martínez Tomás R, Menéndez Villanueva R, Reyes Calzada S, *et al.* Pulmonary nocardiosis: risk factors and outcomes. *Respirology* 2007;**12**:394–400

22 King AS, Castro JG, Dow GC. *Nocardia farcinica* lung abscess presenting in the context of advanced HIV infection: spontaneous resolution in response to highly active antiretroviral therapy alone. *Can J Infect Dis Med Microbiol* 2009;**20**:e103–6