ASIAN PACIFIC JOURNAL OF ALLERGY AND IMMUNOLOGY (2006) 24: 239-243

CASE REPORT

Mycobacterium avium and *Burkholderia pseudomallei* (Melioidosis) Coinfection in an HIV-positive Patient

Wadchara Pumpradit[1], Koya Ariyoshi[1], Wimol Petkanchanapong[2], Nuanjun Wichukchinda[2], Angkana Chaiprasert[4], Archawin Rojanawat[2], Pathom Sawanpanyalert[2] and Panita Pathipvanich[3]

SUMMARY A 29 year old HIV positive Thai female with CD4 count of 10 cells/mm^3 presented with chronic diffuse abdominal pain, fever, weight loss, anemia and leucopenia. Ultrasonography demonstrated diffuse upper abdominal lymphadenopathy with ascites. Microbiological and molecular work up of the specimen obtained by ultrasound-guided lymph node aspiration revealed co-infection with *Burkholderia pseudomallei* and *Mycobacterium avium*. Indirect hemagglutination, IgM-indirect fluorescent antibody, and IgG-indirect fluorescent antibody to *Burkholderia pseudomallei* were < 1:20, < 1:50 and < 1:50, respectively, at nine months, four months before the culture diagnosis and two months, eight months after the culture diagnosis of *Burkholderia pseudomallei* infection. The patient was treated initially with two weeks of intravenous ceftazidime, followed by oral cotrimoxazole, doxycycline and chloramphenicol. Clarithromycin and ofloxacin were added after the identification of *Mycobacterium avium* and its susceptibility test. The patients demonstrated clinical improvement with decreasing abdominal pain and resolution of fever.

Melioidosis is an infection caused by the gram negative bacteria, *Burkholderia pseudomallei,* a facultative intracellular pathogen[1-3] found in water and wet soil from rice paddy fields in endemic areas.[4] Its clinical presentation ranges from chronic localized infection characterized by abscess formation[5, 6] to fulminant septic shock. Northern Australia and Southeast Asia, particularly Thailand, are endemic areas. During the past 20 years, melioidosis has accounted for 20% of community-acquired septicemia in the northeastern part of Thailand.[7] Melioidosis affects those who have occupational exposure and the fatality rate is greater in people with specific comorbidities, such as diabetes mellitus, renal disease, cirrhosis, thalassemia, alcoholism and in people who are immunosuppressed due to immunosuppressive diseases or drugs.[7-9] However HIV-1 infection has not been found to be a predisposing condition for melioidosis.

We report a case of an HIV-1 infected Thai female who has developed abdominal lymphadenitis

From the [1]JICA-NIH Project, Ministry of Public Health, Thailand, [2]NIH, Ministry of Public Health, Thailand, [3]Lampang Hospital, Lampang, Thailand, [4]Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.
Correspondence: Wadchara Pumpradit
E-mail: wadchara.p@hivnat.org

caused by *Mycobacterium avium* complex and *B. pseudomallei.*

CASE REPORT

A 29 year old Thai female presented to our HIV daycare center, Lampang hospital, Thailand, with chronic diffuse abdominal pain, intermittent fever for approximately one month, which had increased in intensity over the past few days and weight loss of about five kilograms over the same period of time. The patient had a history of HIV infection since 1996 acquired through heterosexual contact with her husband. Absolute CD4 lymphocyte count at the initial visit was 10 cells/ mm^3. Viral load was not performed. The patient was not receiving antiretroviral treatment due to financial reasons. Examination revealed cachexia and diffuse abdominal tenderness without evidence of mass lesion. Complete blood count showed normocytic normochromic anemia with hemoglobin/hematocrit of 7.5 gm/dl/ 23.5%, white blood cell count 2,600, PMN 66%, lymphocytes 31%, monocytes 2%, eosinophils 1% and platelets 574,000. Ultrasonography of abdomen showed generalized upper abdominal lymphadenopathy with mild ascites. The patient was treated empirically for tuberculous lymphadenitis with antituberculous medications (Isoniazid, rifampicin, pyrazinamide and ethambutol for two months followed by isoniazid and rifampicin for 4 months). After six months of antituberculous therapy, her abdominal pain continued to worsen. Ultrasound-guided abdominal lymph node aspiration was performed. Bloody fluid with cell debris was obtained. Gram stain of the specimen was unrevealing. AFB stain showed 3 + AFB positive.

The specimen was also placed in Myco F/Lytic culture bottle and incubated in a Bactec 9240 blood culture system. Seventeen hours later, a positive signal was detected. Culture filtrate was subcultured in blood, MacConkey, Chocolate and 3% Ogawa agar. Gram stain of the filtrate showed gram negative bacilli with bipolar staining. Two days later gram negative non-lactose fermenter colonies were isolated. On triple sugar iron agar, it produced acid slant, neutral butt without hydrogen sulfide. The organism exhibited the biochemical characteristic of *Burkholderia pseudomallei*; oxidase positive, growth at 42°C, positive gas from nitrates, positive arginine dihydrolase, oxidizes glucose, lactose, mannitol, and maltose. The organism was sensitive to ciprofloxacin, ceftazidime, piperacillin, trimethoprim/sulfamethoxazole (cotrimoxazole), gentamicin and amikacin. It was found resistant to ampicillin, cefuroxime, imipenem, tetracycline, and cefoperazone/ sulbactam. Intravenous ceftazidime was administered for two weeks followed by cotrimoxazole, doxycycline, and chloramphenicol. Indirect hemagglutination (IHA), indirect fluorescent antibody technique (IFA) to detect IgM and IgG were performed on patient's archived samples at nine months and four months before the culture diagnosis and two months and eight months after the culture diagnosis. The results of IHA, IgM-IFA and IgG-IFA on all four time points were < 1:20, < 1:50 and < 1:50, respectively (Cutoff for positive IHA and IgM-IFA are > 1:160 and > 1; 200. Four fold rising titer of IgG-IFA in paired sera is considered positive for melioidosis).

The Myco F/Lytic culture bottle with the positive signal from the Bactec 9240 was sent for further testing. Twenty milliliters of culture filtrate were drawn from the MycoF/Lytic bottle with a sterile needle and syringe and centrifuged at 4,234 x *g* and 4°C for 10 minutes (Hereus Megafuge 1.0 R). The pellet was washed with TE buffer (10 mM Tris, 1 mM EDTA pH 8.0) and centrifuged again, resuspended in 1 ml of TE buffer. Half a milliliter of cell suspension was used for DNA extraction using QIAgen kit. Five microliters of DNA were used in one-tube, employing a nested-PCR-based assay for detection of *Mycobacterium tuberculosis* complex. [10] The result revealed no specific DNA band of *M .tuberculosis* complex. For the isolation of mycobacteria, 0.2 ml each of cell suspension were inoculated on two Loewenstein Jensen (LJ) egg based slants, incubated at 37°C and examined weekly. After three weeks of incubation, there were moderate numbers of cream-smooth colonies on the slant. They were acid-fast positive and further identified as *Mycobacterium avium* using the polymerase chain reaction and restriction analysis (PCR-REA) based on *hsp* 65 and *rpoB* genes of mycobacteria. Susceptibility to isoniazid (INH), rifampicin (R), streptomycin(S) and ethambutol (E) were performed on LJ according to recommendation of WHO and IUATLD.[11] The isolated strain revealed resistance to all four drugs. Sensitivity testing to amikacin (18 µg/

ml), ciprofloxacin (1µg/ml), clarithromycin (3µg/ml) doxycycline (6 µg/ml), cefoxitin (18µg/ml), imipenem (4µg/ml) and cotrimoxazole (30µg/ml) using drug-impregnated disc method on Middlebrook 7H10 agar supplemented with 10% OADC[12] were also performed. The *Mycobacterium avium* strain isolated from this patient showed sensitivity only to amikacin and clarithromycin. Clarithromycin and ofloxacin were then added to the patient treatment with clinical improvement. She became afebrile and her abdominal pain decreased.

DISCUSSION

The first case of melioidosis in an HIV patient in Thailand was reported in 1985.[13] The patient was a homosexual man who presented with recrudescent melioidosis. To our knowledge, there have not been any reports of a rare coinfection of MAC and *B. pseudomallei*. *B. pseudomallei infection* in this case was diagnosed with culture of specimen from sterile site which is the gold standard laboratory test. It is, however, interesting that the serological test for *B. pseudomallei* revealed negative result despite a long duration of illness prior to the definitive diagnosis. The negative result could be explained by the fact that the sensitivity of IHA, IgM-IFA, and IgG-IFA on culture-confirmed cases of melioidosis ranges approximately between 50-70%, 60%, and 45%, respectively.[14-20] In addition, this HIV-positive patient presented with advanced immunosuppression with CD4 count of 10 cells/ mm^3 which could further reduce the ability to mount the appropriate antibody response when compared to immunocompetent individuals as seen in other infectious diseases and immunizations.[21-25]

Despite its rarity, melioidosis deserves attention. Patients with melioidosis can have clinical presentations similar to and sometimes indistinguishable from tuberculosis and some chronic fungal infections. In a financially constraint setting where certain laboratory and radiological investigation can be expensive and sometimes not available, HIV patients who have melioidosis may be treated empirically for tuberculosis as happened in this case report. This case demonstrates that melioidosis should be considered in the differential diagnosis in patients presenting with severe systemic illness, in areas where melioidosis is endemic, especially when response to the treatment of a more usual pathogen is not obtained.

Thailand is considered a hyperendemic area for melioidosis. However, there is inter-regional variability. The infection rate in patients attending government hospitals in the northeastern region (137.9 per 100,000 in-patients) was significantly higher than those in the northern (18 per 100,000 in-patients), central (13.4 per 100,000 in-patients), and southern (14.4 per 100,000 in-patients) regions, respectively.[26] The patient presented in this report was born and grew up in the northern region and never traveled to other parts of the country. She worked in a grocery store. Except for HIV infection, the patient had no other medical problems. Interestingly no other known predisposing factors for melioidosis were identified.

An early attempt failed to demonstrate any relationship between HIV infection and melioidosis.[27] Out of 121 cases of melioidosis in Ubon Ratchathani province, northeastern Thailand, none was found to have HIV infection. A case-control study seven years later from northeastern Thailand found diabetes mellitus, pre-existing renal diseases, thalassemia, and occupational exposure (e.g. rice farming) as risk factors but not HIV infection.[9] Similarly in a prospective study of 252 cases of melioidosis over 10 years in northern Australia, HIV infection was also not a risk factor.[8]

However, a locally published study conducted in Khon Kaen, a province in the northeastern part of the Thailand, showed a significantly higher seroprevalence of melioidosis in HIV-1 infected patients when compared to healthy blood donor.[28] The proportion of individuals with an indirect melioidosis hemagglutination titer of more than 1:160 in HIV-1 infected patients and healthy donors are 21 out of 57 (36.8%) and 18 out of 100 (18%), respectively. By indirect immunofluorescent assay, HIV-1 infected patients who had IgG > 1:80 were 21 out of 57 (38%) whereas healthy donors who had IgG > 1:80 were 19 out of 100 (19%).

In addition, lymphocytes from patients who had recovered from melioidosis compared to control subjects showed a higher lymphocyte proliferation and higher interferon-γ production in response to *B.*

pseudomallei. There was also an increase in the percentage of activated CD4 and activated CD8 T cells.[28] Such findings indicated that cell mediated immune response may have some role in melioidosis. Therefore, it is conceivable that impaired cellular immunity found in HIV infection could render patients more susceptible to melioidosis.

Whether HIV-1 infected patients are more susceptible to melioidosis than general population is not known. However, the implication of such information is obvious. Not only would it help us to better recognize the disease in the HIV infected population but also to decide who should be targeted for preventive measures such as behavioral modification and possibly future vaccination.

ACKNOWLEDGEMENT

The authors wish to thank Dr. Chris Duncombe for editing and comments of the manuscript.

REFERENCES

1. Vorachit M, Lam K, Jayanetra P, Costerton JW. Electron microscopy study of the mode of growth of *Pseudomonas pseudomallei in vitro* and *in vivo*. J Trop Med Hyg 1995; 98: 379-91.
2. Jones AL, Beveridge TJ, Woods DE. Intracellular survival of *Burkholderia pseudomallei.* Infect Immun 1996; 64: 782-90.
3. Hoppe I, Brenneke B, Rohde M, Kreft A, Haussler S, Reganzerowski A, *et al.* Characterization of a murine model of melioidosis: comparison of different strains of mice. Infect Immun 1999; 67: 2891-900.
4. Wuthiekanun V, Smith MD, Dance DA, White NJ. Isolation of *Pseudomonas pseudomallei* from soil in north-eastern Thailand. Trans R Soc Trop Med Hyg 1995; 89: 41-3.
5. Wong KT, Puthucheary SD, Vadivelu J. The histopathology of human melioidosis. Histopathology 1995; 26: 51-5.
6. Vatcharapreechasakul T, Suputtamongkol Y, Dance DA, Chaowagul W, White NJ. *Pseudomonas pseudomallei* liver abscesses: a clinical, laboratory, and ultrasonographic study. Clin Infect Dis 1992; 14: 412-7.
7. Chaowagul W, White NJ, Dance DA, Wattanagoon Y, Naigowit P, Davis TM, *et al.* Melioidosis: a major cause of community-acquired septicemia in northeastern Thailand. J Infect Dis 1989; 159: 890-9.
8. Currie BJ, Fisher DA, Howard DM, Burrow JN, Lo D, Selva-Nayagam S, *et al.* Endemic melioidosis in tropical northern Australia: a 10-year prospective study and review of the literature. Clin Infect Dis 2000; 31:981-6.
9. Suputtamongkol Y, Chaowagul W, Chetchotisakd P, Lertpatanasuwun N, Intaranongpai S, Ruchutrakool T, *et al.* Risk factors for melioidosis and bacteremic melioidosis. Clin Infect Dis 1999; 29: 408-13.
10. Gengvinij N, Pattanakitsakul SN, Chierakul N, Chaiprasert A. Detection of *Mycobacterium tuberculosis* from sputum specimens using one-tube nested PCR. Southeast Asian J Trop Med Public Health 2001; 32: 114-25.
11. Tuberculosis Programme World Health Organization/International Union Against Tuberculosis and Lung Disease. Guideline for Surveillance of Drug Resistance Tuberculosis. Geneva: World Health Organization 1994. Document WHO/TB/94.178
12. Kent PT KG. Public Health Mycobacteriology. A guide for the level III Laboratory. U.S. Department of Health and Human Services, Public Health Services, Center for Disease Control, Atlanta, Georgia, U.S.A., 1985.
13. Tanphaichitra D, Sahaphong S, Srimuang S, Wangroongsarb Y. A case comparison of acquired immune deficiency syndrome (AIDS) in homosexual males with spindle-endothelial cell abnormalities and with recrudescent melioidosis. Asian Pac J Allergy Immunol 1985; 3: 200-4.
14. Mathai E, Jesudason MV, Anbarasu A. Indirect immunofluorescent antibody test for the rapid diagnosis of melioidosis. Indian J Med Res 2003; 118: 68-70.
15. Wuthiekanun V, Amornchai P, Chierakul W, Cheng AC, White NJ, Peacock SJ, *et al.* Evaluation of immunoglobulin M (IgM) and IgG rapid cassette test kits for diagnosis of melioidosis in an area of endemicity. J Clin Microbiol 2004; 42: 3435-7.
16. Wongratanacheewin S, Sermswan RW, Anuntagool N, Sirisinha S. Retrospective study on the diagnostic value of IgG ELISA, dot immunoassay and indirect hemagglutination in septicemic melioidosis. Asian Pac J Allergy Immunol 2001; 19: 129-33.
17. Ashdown LR, Johnson RW, Koehler JM, Cooney CA. Enzyme-linked immunosorbent assay for the diagnosis of clinical and subclinical melioidosis. J Infect Dis 1989; 160: 253-60.
18. Cheng AC, O'Brien M, Freeman K, Lum G, Currie BJ. Indirect hemagglutination assay in patients with melioidosis in northern Australia. Am J Trop Med Hyg 2006; 74: 330-4.
19. Wuthiekanun V, Desakorn V, Wongsuvan G, Amornchai P, Cheng AC, Maharjan B, *et al.* Rapid immunofluorescence microscopy for diagnosis of melioidosis. Clin Diagn Lab Immunol 2005; 12: 555-6.
20. Sermswan RW, Wongratanacheewin S, Anuntagool N, Sirisinha S. Comparison of the polymerase chain reaction and serologic tests for diagnosis of septicemic melioidosis. Am J Trop Med Hyg 2000; 63: 146-9.
21. Lange CG, Lederman MM, Medvik K, Asaad R, Wild M, Kalayjian R, *et al.* Nadir CD4+ T-cell count and numbers of CD28+ CD4+ T-cells predict functional responses to immunizations in chronic HIV-1 infection. Aids 2003; 17: 2015-23.
22. Lord A, Bailey AS, Klapper PE, Snowden N, Khoo SH. Impaired humoral responses to subgenus D adenovirusenovirus infections in HIV-positive patients. J Med Virol 2000; 62: 405-9.
23. Kroon FP, van Dissel JT, de Jong JC, Zwinderman K, van Furth R. Antibody response after influenza vaccination in HIV-infected individuals: a consecutive 3-year study. Vaccine 2000; 18: 3040-9.
24. Tawill SA, Gallin M, Erttmann KD, Kipp W, Bamuhiiga J, Buttner DW. Impaired antibody responses and loss of reactivity to Onchocerca volvulus antigens by HIV-seropositive

onchocerciasis patients. Trans R Soc Trop Med Hyg 1996; 90: 85-9.
25. Kroon FP, van Dissel JT, Labadie J, van Loon AM, van Furth R. Antibody response to diphtheria, tetanus, and poliomyelitis vaccines in relation to the number of CD4+ T lymphocytes in adults infected with human immunodeficiency virus. Clin Infect Dis 1995; 21: 1197-203.
26. Vuddhakul V, Tharavichitkul P, Na-Ngam N, Jitsurong S, Kunthawa B, Noimay P, et al. Epidemiology of Burkholderia pseudomallei in Thailand. Am J Trop Med Hyg 1999; 60: 458-61.
27. Kanai K, Kurata T, Akksilp S, Auwanit W, Chaowagul V, Naigowit P. A preliminary survey for human immunodeficient virus (HIV) infections in tuberculosis and melioidosis patients in Ubon Ratchathani, Thailand. Jpn J Med Sci Biol. 1992; 45: 247-53.
28. Vimolsarte S, Wongweerakan P, Vimolsarte C, Leuprasert L. Study on melioidosis antibody, the opportunistic infection disease in AIDS patients in Khon Kaen province. J Office CDC 6 1997; 4: 17-23.
29. Ketheesan N, Barnes JL, Ulett GC, VanGessel HJ, Norton RE, Hirst RG, *et al*. Demonstration of a cell-mediated immune response in melioidosis. J Infect Dis 2002; 186: 286-9.