INDIRECT HEMAGGLUTINATION ANTIBODIES AGAINST *BURKHOLDERIA PSEUDOMALLEI* IN NORMAL BLOOD DONORS AND SUSPECTED CASES OF MELIOIDOSIS IN MALAYSIA

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Abstract. Interpretation of the indirect hemagglutination test (IHA) for melioidosis in endemic areas is difficult because of the presence of antibodies in apparently healthy individuals. Fifty-three out of 200 healthy blood donors in Malaysia showed positive antibody titers (≥ 1 : 40) against *Burkholderia pseudomallei*. Seven percent had an IHA titer of 1 : 40, 11% had an IHA titer of 1 : 80 while 8.5% had a titer ≥ 1 : 160. Out of 258 sera sent for melioidosis serology, 7% of the patients had an IHA titer of 1 : 40, 9% had an IHA titer of 1 : 80 while 20% had an IHA titer of ≥ 1 : 160. If a titer of ≥ 1 : 80 is taken as cut off point for positivity, 29% of the patients had positive melioidosis serology. Increasing the positivity threshold may jeopardize the sensitivity of the test. A more specific and sensitive test is needed.

INTRODUCTION

Melioidosis is caused by a gram-negative bacillus, *Burkholderia pseudomallei*. The disease is said to be endemic in areas between latitudes 20° north and south of the equator. The clinical spectrum of melioidosis is wide, ranging from asymptomatic infection to acute fulminant septicemia with a mortality rate of 80-90% (Ashdown, 1981). Death frequently occurs 24-48 hours after onset. In a review of 50 septicemic melioidosis cases admitted to University Hospital, Malaysia, 65% of cases ended fatally (Puthucheary *et al*, 1992). The high mortality rate is frequently associated with clinical unawareness of the disease and delay in bacteriological identification of the bacterium. Melioidosis is one of the diseases in which serological diagnosis plays an important role in providing early diagnosis so that treatment can be given as soon as possible. A variety of serological tests have been developed for the diagnosis of melioidosis, including complement fixation, indirect hemagglutination (IHA), indirect fluorescent antibody (IFA) and enzyme immunosorbent assays (ELISA). The indirect hemagglutination test is the most widely used method as it is inexpensive and simple to perform. This method measures antibodies against polysaccharide antigens of *Burkholderia pseudomallei*. In non-endemic areas, a positive IHA result supported by appropriate clinical findings gives a strong indication of melioidosis until proven otherwise. Interpretation of IHA results in endemic areas is difficult because of the presence of antibodies in apparently healthy individuals. Malaysia is an endemic country for melioidosis as the organism can be isolated from soil samples from almost all states in Malaysia (Strauss *et al*, 1967, 1969 a,b; Ellison *et al*, 1969). We carried out indirect hemagglutination tests on normal blood donors to determine the prevalence of antibodies against *Burkholderia pseudomallei* in a healthy population and to correlate it with the IHA results obtained from serum submitted for melioidosis serology sent to our laboratory.

MATERIALS AND METHODS

Serum samples

100 serum samples from blood donors residing in and around Kuala Lumpur, and 100 serum samples from donors residing in Kluang district were included in this study. Kluang district was chosen to represent an area situated in the south of Peninsular Malaysia.

Serum samples from suspected melioidosis cases, or where melioidosis needed to be ruled out, received from government hospitals throughout Malaysia from January 1994 until December 1995 were assessed by the indirect hemagglutination test for melioidosis. The sera were kept at -20°C before conducting the test.

Indirect hemagglutination test (IHA)

The IHA was conducted following a modification of the method of Ileri (1965). Three local strains of *Burkholderia pseudomallei* were grown separately in protein-free broth at 37°C for 2 weeks, autoclaved and centrifuged. The supernatant was used as antigen and stored at 4°C after addition of 0.5% (v/v) of phenol as preservative. The optimal dilution of each antigen was determined by block titration against a positive serum of known titer and then pooled together. Sheep red blood cells were sensitized with the antigen for 30 minutes at 37°C. Excess antigen was removed by washing the sheep red blood cells twice with phosphate-buffered saline, pH 7.2.

Conduct of test

The sera to be tested were inactivated at 56°C for 30 minutes followed by absorption with unsensitized sheep red blood cells at room temperature for 15 minutes. The serum were then serially diluted with normal saline starting at 1 : 20 in a microtiter plate. Equal volumes of sensitized sheep red blood cells were added to the serum dilutions and then incubated at room temperature. Results were read after 2-4 hours.

RESULTS

From the 200 healthy blood donors sera, a total of 53 blood donors (26.5%) showed positive antibody titers of > 1 : 40. Twenty-seven were from Kuala Lumpur and 26 were from Kluang district. Antibody titers of 1 : 40 were detected in 14 donors (7%). Twenty-two donors (11%) were shown to have antibody titers of 1 : 80, 11 donors (5.5%) had antibody titers of 1 : 160, 4 donors (2%) showed titers of 1 : 320 and 2 donors (1%) had titers of 1 : 640 (Table 1). Antibody titers ≥ 1 : 1,280 were not detected in any of the blood donors.

A total of 258 serum samples were received from goverment hospitals with the requests for IHA test. Out of these samples 94 patients (36%) were found to have IHA titers ≥ 1 : 40. Antibody titers of 1 : 40 were detected in 17 cases (7%). Twenty-four patients (9%) had an antibody titer of 1 : 80, 14 patients (5%) showed an antibody titer of 1 : 160, 13 patients (5%) had titers of 1 : 320, 5 patients (2%) had a titer of 1 : 640 and 21 (8%) patients had a titer of ≥ 1 : 1,280 (Table 2).

Table 1

IHA antibodies in 200 healthy blood donors.

Titer	Kuala Lumpur n = 100	Kluang District n = 100	Total n = 200
< 1 : 40	73	74	147 (73.5%)
1 : 40	10	4	14 (7%)
1 : 80	12	10	22 (11%)
1 : 160	3	8	11 (5.5%)
1 : 320	2	2	4 (2%)
1 : 640	0	2	2 (1%)
≥ 1 : 1,280	0	0	0 (0%)

Table 2

IHA antibodies in suspected melioidosis or where melioidosis needed to be ruled out.

Titer	No. of cases (Total = 258)
< 1 : 40	164 (64%)
1 : 40	17 (7%)
1 : 80	24 (9%)
1 : 160	14 (5%)
1 : 320	13 (5%)
1 : 640	5 (2%)
≥ 1 : 1,280	21 (8%)

DISCUSSION

The indirect hemagglutination test, when carried out in non-endemic areas, has been shown to be highly specific and sensitive. A patient with appropriate clinical presentations and reactive IHA test can be assumed to have melioidosis until proven otherwise. However, interpretation of the IHA result in an endemic area is difficult because people residing in endemic areas have antibodies without obvious clinical illness.

In determining the prevalence of human melioidosis using the IHA test, Ashdown and Guard in northern Queensland showed that out of 9,047 serum samples tested, 512 (5.7%) were reactive with titers ≥ 1 : 40 (Ashdown *et al*, 1984). A serologic surveillance carried out by Atthasampuna *et al* (1969) in Thailand using the IHA test showed that 15% of Thais had positive reactions. In a study of 1,592 army recruits and selected populations in Malaysia, 7.3% were shown to have hemagglutinating antibodies against *Burkholderia pseudomallei* (Strauss *et al*, 1969).

Antibodies against *Burkholderia pseudomallei* demonstrated in persons residing in endemic areas could be a result of subclinical infection. Based on the IHA test done on sera in non-endemic areas, a titer of ≥ 1 : 40 is normally considered positive, indicating that the patient had been exposed to the organism. In an endemic area, the cut-off titer of IHA antibody for melioidosis at ≥ 1 : 80 is reported to have specificity and sensitivity of 95% and 70%, respectively (Puapermpoonsiri *et al*, 1989). In our study, 53 (26.5%) apparently healthy blood donors were shown to have antibody titers ≥ 1 : 40. If a titer of ≥ 1 : 80 is taken as the cut-off point, 19.5% of the blood donors had reactive titers. Eleven percent of the positive blood donors had antibody titers of 1 : 80. If the positivity threshold is increased to ≥ 1 : 160, only 8.5% of the donors will show reactive titers. However, by increasing the positivity threshold, the sensitivity of the test will be reduced and only small improvements in specificity will be produced (Chaowagul *et al*, 1989). In this study similar distribution of IHA titer can be seen in blood donors irrespective of which area they came from. *Burkholderia pseudomallei* has been isolated from soils all over Malaysia. There is no question regarding the endemicity of the disease, however, limited studies have been conducted on melioidosis in Malaysia as compared to Thailand. Melioidosis is recognized as a common cause of community-acquired septicemia in the northeastern part of Thailand. No study has been conducted on the community-acquired septicemia in Malaysia, however, physicians should bear in mind the differential diagnosis of melioidosis in cases where patients present on admission with fever.

IHA is the most widely used serological test for melioidosis in Malaysia. In the two year period of January 1994 to December 1995, there were 258 requests for the IHA test compared to only 50 requests in the two year period of 1992 until 1993. This rise could be the result of increasing awareness among physicians regarding the disease. Out of the 258 cases, 94 cases (36%) had antibody titers ≥ 1 : 40. If a titer of ≥ 1 : 80 is taken as the cut-off for positivity, 77 cases (30%) will be considered positive. However, a titer of 1 : 80 can be detected in 11% of blood donors. If the cut-off point is raised to > 1 : 160, only 20% of the suspected cases will be positive. This may raise the specificity of the test but its sensitivity may be affected. The IHA test can be negative in culture-proven melioidosis (Khupulsup *et al*, 1986) and also may show high titer (≥ 1 : 1,280)in septicemia due to other bacterial infections (Chaowagul *et al*, 1989). We could not follow-up the outcome of the suspected cases, whether they were confirmed melioidosis by culture or not because most of the requests came from hospitals situated outside Kuala Lumpur.

The IHA test is most useful as a screening test. It cannot be used to measure disease activity as the antibody may persist for several years after infection. Ashdown and other workers (Ashdown, 1981; Khupulsup *et al*, 1986) have shown that the IFA-IgM test using crude antigens can be used to measure disease activity but this test needs a fluorescent microscope and related expertise, thus limiting its use in smaller hospitals. The use of crude antigen may give rise to some cross reactions. Several tests have been developed using more purified antigens such as exotoxin and endotoxin (Ismail *et al*, 1987; Petkanjanapong *et al*, 1992). Detection of antigen direct from clinical sample was also developed but it still needs optimization (Lew *et al*, 1994).

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REFERENCES

Ashdown LR. Demonstration of human antibodies to *Pseudomonas pseudomallei* by indirect fluorescent antibody staining. *Pathology* 1981; 13 : 597-601.

Ashdown LR, Guard RW. The prevalence of human melioidosis in northern Queensland. *Am J Trop Med Hyg* 1984; 33 : 467-73.

Ashdown LR. Melioidosis and safety in clinical laboratory. *J Hosp Infect.* 1992; 21 : 301-6.

Atthasampuna P, Noyes HE, Winter PE, *et al.* Annual progress report of SEATO medical research study on melioidosis. Bangkok: SEATO, 1969; 257-64.

Chaowagul W, White NJ, Dance DAB, *et al.* Melioidosis: A major cause of community-acquired septicemia in northeastern Thailand. *J Infect Dis* 1989; 159 : 890-9.

Ellison DW, Baker HJ, Mariappan M. Melioidosis in Malaysia I. Method for isolation of *Pseudomonas pseudomallei* from soil and surface water. *Am J Trop Med Hyg* 1969; 18 : 694-7.

Ileri SZ. The indirect hemagglutination test in the diagnosis of melioidosis in goats. *Br Vet J* 1965; 121 : 164-70.

Ismail G, Embi MN, Omar O, Allen JC, Smith CJ. A competitive immunosorbent assay for detection of *Pseudomonas pseudomallei* exotoxin. *J Med Microbiol* 1987; 23 : 353-7.

Khupulsup K, Petchlai B. Application of indirect hemagglutination test and indirect fluorescent antibody test for IgM antibody for diagnosis of melioidosis in Thailand. *Am J Trop Med Hyg* 1986; 35 : 366-9.

Lew AE, Desmarchelier PM. Detection of *Pseudomonas pseudomallei* by PCR and hybridization. *J Clin Microbiol* 1994; 32 : 1362-32.

Petkanjanapong V, Naigowit P, Kondo E, Kanai K. Use of endotoxin antigens in enzyme-linked immunosorbent assay for the diagnosis of *P. pseudomallei* infections (melioidosis). *Asian J Allergy Immunol* 1992; 10 : 145-50.

Puapermpoonsiri S, Puapermpoonsiri P, Bhuripanyo K, Vilachi C, Auncharoen A. Indirect hemagglutination antibody titer to *Pseudomonas pseudomallei* in patients with melioidosis. In: Punyagupta S, Sirisanthana T, Stapatayayong B, eds. Melioidosis. Proceedings of National Workshop on Melioidosis, Bangkok: Bangkok Medical Publisher 1989; 193-6.

Puthucheary SD, Parasakhti N, Lee MK. Septicemic melioidosis: a review of 50 cases from Malaysia. *Trans R Soc Trop Med Hyg* 1992; 86 : 683-5.

Strauss JM, Jason S, Mariappan M. *Pseudomonas pseudomallei* in soil and surface water of Sabah, Malaysia. *Med J Mal* 1967; 1 : 31-2.

Strauss JM, Groves MG,Mariappan M, Ellison DW. Melioidosis in Malaysia. II. Distribution of *Pseudomonas pseudomallei* in soil and surface water. *Am J Trop Med Hyg* 1969a; 18 : 698-702.

Strauss JM, Alexander AD, Rapmund G, Gan E, Dorsey AE. Melioidosis in Malaysia. III. Antibodies to *Pseudomonas pseudomallei* in the human population. *Am J Trop Med Hyg* 1969b; 18 : 703-7.