

Seroprevalence of melioidosis in diabetic patients in Taiwan

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Diabetes mellitus is a major predisposing factor for *Burkholderia pseudomallei* infection. This study surveyed serum samples from 356 Taiwanese patients with diabetes mellitus for anti-flagellin antibodies against *B. pseudomallei*. Antibody titer to *B. pseudomallei* was positive in 3.0% (11/365) of diabetes mellitus patients. All seropositive individuals were aged ≥ 60 , indicating that elderly and diabetic adults are at high risk of *B. pseudomallei* infection. In this study, diabetic females, who were usually housewives, had a seropositive rate of 81.1%. However, the incidence of melioidosis in males (usually working outdoors) was 93.7% based on clinical cases. We suggest that exposure of males and females to *B. pseudomallei* in this study was via different routes of infection.

Key words: Bacterial antibodies, *Burkholderia pseudomallei*, melioidosis, seroepidemiologic studies

Burkholderia pseudomallei is a causative agent of melioidosis, which is recognized as an emerging disease worldwide [1]. This disease was first described in Taiwan in 1984 [2], while a substantial increase in sporadic cases was reported after 1994 [3]. Because the clinical manifestations of melioidosis are quite protean, clinical diagnosis is often difficult, and the true incidence of melioidosis may actually be higher than suggested by current data [4]. Previous studies used impure or crude protein extracts from *B. pseudomallei* as antigenic targets for serologic methods. This resulted in cross-reaction with other bacteria in the sera, making the methods less specific [5-7]. Recently, we developed an indirect enzyme-linked immunosorbent assay (ELISA) using recombinant flagellin as molecular probes, with a sensitivity of 93.8% and specificity of 96.3% for distinguishing melioidosis from healthy Taiwanese donors [8]. We further reported a 2.8-5% seropositive rate for melioidosis in healthy donors in several parts of Taiwan using this indirect ELISA [9]. Whether this positive rate indicates that melioidosis in Taiwan will develop into an overt and widespread disease remains unclear. Underlying diabetes mellitus is a major

predisposing factor for melioidosis [10,11]. Over 50% of patients with melioidosis in Taiwan have concurrent diabetes mellitus [3]. This study surveyed the seroprevalence of melioidosis in patients with diabetes mellitus.

Materials and Methods

Serum samples

Serum samples were collected from 356 patients with diabetes mellitus who visited Kaohsiung-Veterans General Hospital or Kaohsiung Municipal Min-Sheng Hospital. All patients had a diagnosis of diabetes mellitus based on 1998 World Health Organization diagnostic criteria [12]. Patients with a previous history of melioidosis were excluded. The collected sera were maintained at -70°C . Serum samples were also collected from 16 patients with melioidosis treated at Kaohsiung-Veterans General Hospital. These cases were confirmed to be melioidosis by bacterial culture and seropositivity to specific flagella antibodies against *B. pseudomallei* [8].

Serologic tests

Seropositivity to *B. pseudomallei* was determined by indirect ELISA using a titer cut-off value of 1:256, in accordance with our previous study [8]. Briefly, the

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96-well polystyrene microtitre plates were coated with 1 µg/mL flagella antigens in a coating buffer (50 mM carbonate/bicarbonate buffer, pH 9.6) at 4°C, overnight. The plates were blocked for 2 h using 100 µL bovine serum albumin (1 mg/mL; GIBCO BRL, USA). After washing with saline-Tween solution [0.9% (w/v) sodium chloride, 0.05% (v/v) Tween-20 in phosphate-buffered saline, PBS] 3 times, the wells were incubated at 37°C for 1 h with 2-fold serial dilutions of various sera in PBS. Then, the wells were washed with saline-Tween solution and incubated with diluted (1:1000) anti-human immunoglobulin G (IgG) conjugated with peroxidase (Zymed, USA) at 37°C for 1 h. The wells were washed with PBS 3 times, and the 100 µL substrate solutions of 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) [ABTS] were added. The optical density at 450 nm of the finished reactions was read using a microplate reader (Anthos 2010, Austria). When the average of the optical density reading of the tested sample was ≥ 0.1 , the tested sample was considered positive for specific antibody in accordance with the procedures described in a previous report [8]. The highest dilution of the tested sample which still gave a positive result was considered the endpoint titer and listed on the data sheet.

Statistical analysis

Statistical analysis of the results was performed using chi-squared test. A value of $p < 0.05$ was considered significant.

Results

Antibody titer to *B. pseudomallei* was positive in 3.0% of patients (11/365) with diabetes mellitus (Table 1).

The age distribution revealed that the occurrence of seropositives in diabetes mellitus patients was dependent on age (Fig. 1A). These seropositivities in patients with both symptomatic (active melioidosis group) and asymptomatic infection (diabetes mellitus group without symptoms from melioidosis) were mainly distributed among patients aged >60 (Fig. 1A). The seropositive rate in diabetic females was higher than that in males. However, the number of patients with melioidosis was higher for males than females (Fig. 1B).

Discussion

B. pseudomallei is a saprophyte which was mainly found at a depth of 300-600 mm under crop-covered fields in an epidemiologic survey in Taiwan [13]. Adults exposed to soil contaminated with this bacterium were reported to have an increased risk of melioidosis in endemic areas [14]. Whether patients have acquired melioidosis in Taiwan though indigenous infection from a contaminated site is difficult to establish. However, a number of reports noted that patients with melioidosis in Taiwan had never traveled to endemic areas [3,15,16].

In this study, at least, 2 patients out of 16 melioidosis cases had no history of travel to the endemic site. The 3% of seropositive rate in diabetes mellitus patients was similar to the 5% seropositivity (11/220) in healthy adults from our previous study [9]. Based on the 5% prevalence of the general population with an indirect ELISA (having 96.3% specificity and 93.8% sensitivity), the positive predictive value was only 57%. Thus, the actual rate of melioidosis with an inapparent infection may be as low as 1.71% in diabetes mellitus patients. Since diabetes mellitus is an important risk for

Table 1. The distribution of anti-*Burkholderia pseudomallei* flagellin antibody in the diabetes mellitus patient group

Antibody titer ^a	Patients with diabetes mellitus (age in years)						Total
	Male			Female			
	<40	41-59	>60	<40	41-59	>60	
$\leq 1:16$	15	23	30	17	26	42	153
1:32	7	22	29	9	15	25	107
1:64	1	15	19	4	8	12	59
1:128	2	1	15	1	5	12	36
1:256		1	1		1	3	6
1:512						2	2
$\geq 1:1024$					1	2	3
Seropositivity (%) ^b	0.00	1.60	1.10	0.00	3.60	7.10	3.00

^aAntibody titers in serum samples were determined reciprocally by using 2-fold dilution.

^bSeropositivity was determined with a cut-off value of 1:256 dilution.

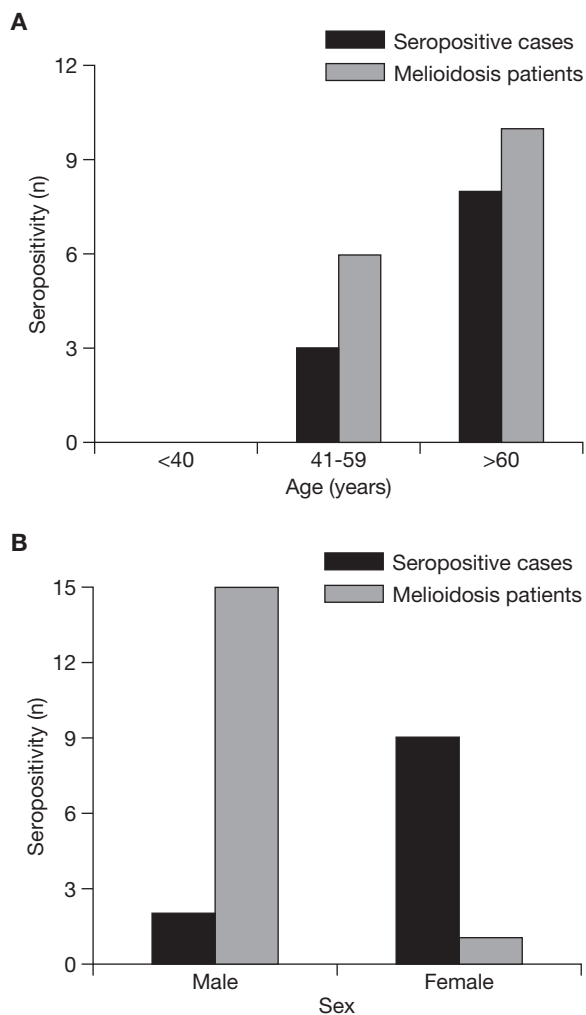


Fig. 1. Distribution of seropositive cases according to age (A) and gender (B). The black bars represent the numbers of seropositive cases in the diabetes mellitus patient group, and lighter bars the number of melioidosis patients (total, n = 16) from Kaohsiung Veterans General Hospital.

B. pseudomallei infection, the seropositive rates might be expected to be higher. However, diabetes mellitus patients did not have a greater prevalence of anti-*B. pseudomallei* antibodies in this study. This finding seems to agree with findings from a previous study that Taiwan has not developed into a hyper-prevalent area [9]. Determining the reasons for the lack of correlation between seroprevalence of melioidosis and diabetes mellitus will require further study using a well-matched cohort and controlling for confounding factors such as educational levels and occupational types. Methods for self-protection and decreasing the risk of occupational exposure to *B. pseudomallei* may be taught to patients with diabetes mellitus as part of a health-education program about their condition.

Males outnumbered females 3:1 in endemic areas, which is thought to represent occupational exposure to *B. pseudomallei* [11]. In this study from Taiwan, the seropositive rate in females with diabetes mellitus was higher than that in males. The females >60 years of age were usually housewives, suggesting a different mode of exposure to *B. pseudomallei* in these patients. Diabetic patients had a greater underlying susceptibility to melioidosis. Thus, no correlation was found in gender between symptomatic non-diabetic patients and diabetes patients with melioidosis.

In this study, seropositivity to *B. pseudomallei* antibody was mainly found among individuals >60 years of age in patients with or without diabetes mellitus. Those results confirm that immunocomprised patients or older adults are at increased risk from *B. pseudomallei* infection. The 3.0% seropositivity rate among diabetes mellitus patients highlights the need for physicians managing these patients in Taiwan to be aware of the possibility of community-acquired pneumonia and sepsis arising from *B. pseudomallei* infection, and to educate their patients about these increased risks.

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