ORIGINAL ARTICLE

Melioidosis in Pahang, Malaysia

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Summary

We conducted a retrospective review of 135 patients with melioidosis in Pahang from January 2000 to June 2003. Patients were mostly male (78.5%) and Malay (83%) with a median age of 51 years. Seventy four percent of patients were diabetic. Common presentations were pneumonia (40.7%), septicaemic without obvious source of infection (19.3%) and multiple organ involvement (15.6%). Only 32.7% were given appropriate antibiotics empirically. The overall mortality was 54% with most deaths (65%) occurring within 48 hours of admission. Patients with pneumonia, multiple organ involvement and septicaemic of unknown source had higher mortality as compared to patients with subcutaneous, musculoskeletal or single internal organ involvement without pneumonia ($p<0.001$). The relapse rate was 19.2%.

Key Words: Adult, Melioidosis, *Burkhoderia pseudomallei*, Mortality

Introduction

Melioidosis is caused by gram-negative bacilli, *Burkholderia pseudomallei*, which is a common soil and fresh water saprophyte espécially in rice paddy fields in tropical and subtropical areas. Affected individuals usually have direct contact with soil as well as underlying predisposing factors. It frequently causes abscesses in almost all organs, but the commonest presentation is pneumonia[1]. It was associated with very high in-hospital mortality especially in the septicaemic form (65%) in the 1980s in Malaysia[2]. In the past 20 years, several studies have shown that the mortality rate has been reduced to 19-37% with high dose ceftazidime, imipenem or cefoperazone-sulbactam therapy for at least two weeks[1,3,4,5]. Parenteral amoxicillin- clavulinic acid has been shown to give similar results but with higher treatment failure rates compared to ceftazidime[6]. The use of cotrimoxazole in addition to a beta-lactam is still controversial[1,4].

Pahang is one of 14 states in Malaysia. It has an area of 35,965 km^2 and a population of 1.28 million (738,000 were 18 years old in 2002 Malaysia Statistic Department). The main economic activity is agriculture, mainly rubber and oil palm cultivation. The incidence of melioidosis in this region is unknown. We conducted a retrospective study to determine the incidence, predisposing factors, clinical features, bacteriology, mortality and outcome of adult melioidosis in Pahang, Malaysia during the period from January 2000 to June 2003.

Materials and Methods

This is a retrospective study to identify cases of adult melioidosis (age above 18 years old) in Pahang during the period of January 2000 to June 2003. In Pahang, only Hospital Tengku Ampuan Afzan (HTAA) in Kuantan and Hospital Mentakab (HM) have facilities to

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perform culture and sensitivity tests for *B. pseudomallei*. We therefore identified all cases with positive culture for *B. pseudomallei* from blood, pus, cerebro-spinal fluid or other body fluids from the database of the microbiology departments of these two hospitals within the study period. Case records of patients were reviewed and relevant data was extracted, including demography, predisposing factors, clinical presentation, physical findings, laboratory and radiological investigations, antibiotic therapy and clinical outcome.

When a patient was found to have positive cultures for *B. pseudomallei* on more than one admission during the study period, the second and subsequent infections were considered as relapses. Patients with a culture-proved infection before the study period were considered to have a positive history of past infection. Appropriate antibiotic therapy was defined as antibiotic treatment with ceftazidime, cefoperazone-sulbactam, amoxicillin-clavulanic acid, imipenem or the combination of any of these drugs. The in-hospital mortality was calculated based on patients with a known outcome (after excluding patients who were discharged against the advice of doctors, transferred to another hospital or had an uncertain outcome).

Both HTAA and HM used the same method for isolation of *B. pseudomallei*. Body fluids were cultured in blood agar and MacConkey agar at 37°C and confirmation was done by observing the colony morphology, staining reaction, motility and biochemical tests (indole, triple sugar iron, citrate, phenyl deaminase, oxidase fermentative 1% glucose, urease, oxidase and DNAse). Sensitivity testing was performed by agar dilution method using BBL ™ Sensi-Disc™ Antimicrobial Susceptibility Test Discs (Becton, Dickinson and Company, USA). Blood samples were incubated in tryptic soy broth bottle (Roche) in BACTEC system and cultured in blood agar and MacConkey agar if positive growth was detected.

Statistical Analysis

The data was analyzed using Statistical Package for the Social Sciences (SPSS) version 10 software package. Incidence was calculated by average number of new cases per year divided by year 2002 adult population (≥18 years old) in Pahang. Univariate analysis was done for all variables. Independent sample t-Test or Mann-Whitney U Test was used for continuous parameters depending on their normality. Categorical variables were tested using chi-square test. Factors found to be significant on univariate analysis were then subjected to multivariate analysis using binary logistic regression. Significance was taken at 0.05.

Results

Cohort study

During the study period, a total of 157 patients had positive culture for *B. pseudomallei* from the two labs in HTAA and HM. Most of the cases presented at the two largest hospitals namely HTAA (44.4%) and HM (36.3%); the remaining cases were from other smaller district hospitals. The calculated annual incidence of adult melioidosis in Pahang state was 6.07 per 100, 000 population per year. Only 135 patients' case records could be traced and analyzed. One hundred and six patients (78.5%) were male. There were 112 Malays (83%), 13 Chinese (9.6%), four Indians (3%), four aborigines (3%) and two Indonesians. Most patients were between 40 to 60 years of age and the median was 51 years (range: 19 - 87 years) (Table I). Patients without predisposing factors were significantly younger (median age 41 years) than patients with predisposing factors (median age 52 years) (p=0.012). Only six patients (4.4%) had a previous history of confirmed melioidosis and another six patients (4.4%) had previous infection possibly due to melioidosis but with negative cultures.

Underlying illness

Common underlying illnesses that predisposing them to melioidosis were diabetes mellitus (74%), followed by chronic renal failure (6%), chronic lung disease (3%) and Human Immunodeficiency Virus (HIV) infection (2.2%). Other predisposing factors were urinary stone disease (3), recent motor vehicle accident (MVA) (2), benign prostatic hyperplasia (BPH) (2), multiple myeloma (1), thalassemia trait (1), alcoholism (1) and steroid therapy(1). Twenty patients (14.8%) had no predisposing factor whereas 11 patients (8.1%) had more than one predisposing factors. Most of the patients presented in the months of March to April and November to January (Figure 1). Occupations were known only in 61 patients, of whom 24.6% were farmers.

Clinical presentations

Fever was the commonest presentation and was present in 93% (119/128) of the patients. Only 9 patients were afebrile on admission. The median duration of fever was 7 days with a maximum of 90 days. Two patients who were admitted with abrasion wounds and soft tissue injury following a motor vehicle

accident developed fever 18 hours after admission with positive blood cultures for *B. pseudomallei.* Thirteen of 133 patients (9.8%) had a blood pressure of less than 90/60mmHg on admission.

On admission, 23 patients had jaundice, 23 patients had hepatomegaly and 4 patients had hepatosplenomegaly. Ultrasound was done on only 11 patients with either hepatomegaly or hepatosplenomegaly and isolated liver abscess was detected in 2 patients, isolated splenic abscess in 2 patients and both liver and splenic abscesses in 2 patients. Three patients with splenic abscesses had no splenomegaly on physical examination. In contrast, 4 out of 21 patients (19%) with normal abdominal examination finding showed intra-abdominal abscesses on ultrasound. White blood counts on admission were more than $11X10^6$/L in 75 patients (55.6%) and less than $4X10^6$/L in 5 patients (3.7%).

Organ involvement and mortality

Isolated pneumonia was the commonest presentation occurring in 55 patients (40.7%) followed by septicaemic melioidosis without obvious source of infection (19.3%) (Table II). There were 21 patients (15.6%) with multiple organ involvement including 15 (71%) with pulmonary involvement.

Only 113 patients of our patients had a known outcome (after excluding patients who were discharged against medical advice and transferred to other hospitals without obvious outcome). Mortality according to organ involvement is showed in Table II. Patients with pneumonia, multiple organ involvement and septicaemic of unknown source had higher mortality than to patients with subcutaneous, musculoskeletal or internal organ involvement without pneumonia ($p<0.001$). The overall mortality was 54% with most deaths (65%) occurring within 48 hours of admission (Table III). Among patients with a known outcome, 37 (32.7%) were given an appropriate antibiotic empirically and the mortality was not different from patients who received no antibiotic, other antibiotics or a combination of ceftazidime with another antibiotic to which B. pseudomallei was susceptible ($p=0.551$).

Treatment and relapse

Of 52 patients who were treated as melioidosis and discharged well, only 27 (51.9%) were given at least one appropriate antibiotic for at least two weeks, 12 patients (24.5%) were given one of the appropriate antibiotics but for less than two weeks and the remaining patients were given other antibiotics. Ten patients (19.2%) had culture proven relapses. Two (20%) of these patients died during a second relapse and 1 had multiple relapses. Patients who were given antibiotics other than the appropriate antibiotics had a 40% risk of relapse as compared to 11.1% for patients who received appropriate antibiotics for at least two weeks ($p=0.046$). Relapse occurred in 25% of patients who were given appropriate antibiotics but less than two weeks. Among patients who were discharged well without subsequent relapse, 16 patients (38.1%) defaulted follow up and 2 (4.7%) died at home. Twenty- four patients (57.1%) were well at the latest review (mean follow up duration 7.1±3.8 months).

Culture and sensitivity tests

Blood cultures were positive in 124 of 135 patients (including 3 patients with positive pus swab culture and 1 with positive sputum culture). In patients with negative blood culture, *B. pseudomallei* were isolated in pus (7), urine (2), joint fluid (1) and both urine and joint fluid cultures (1). In patients with more than one isolate, the organism showed the same sensitivity pattern. The organisms isolated were commonly sensitive to cefoperazone-sulbactam (100%), imipenem (99.1%), ceftazidime (99.2%), tetracycline (87.2%), ciprofloxacin (83.9%) and amoxycillin-clavulinic acid (81.9%). Resistance to trimethoprim-sulfamethoxazole was reported in 34.4% of the isolates.

Factors affecting mortality

Of the 113 patients with known outcome, 52 patients survived and 61 patients died. There was no significant difference in term of their socio-demographic characteristics. Univariate analysis was done to find out factors that contributed to mortality. Most patients who died had a shorter duration of fever before admission, positive blood culture in isolation, septicaemia of unknown source, pneumonia or multi-organ involvement; a lower platelet count, higher serum creatinine level, higher serum bilirubin level and lower serum albumin level (Table IV). However, when all these significant factors were analyzed using binary logistics regression, factors that were independently associated with the mortality of melioidosis patients were duration of fever (OR 0.91, 95%CI 0.85-0.98), platelet count (OR 0.99, 95%CI 0.98-0.99), blood urea (OR 1.1, 95%CI 1.0-1.2) and presence of pneumonia, multi-organ involvement and septicaemia of unknown source (OR 6.5, 95%CI 1.5-27.5).

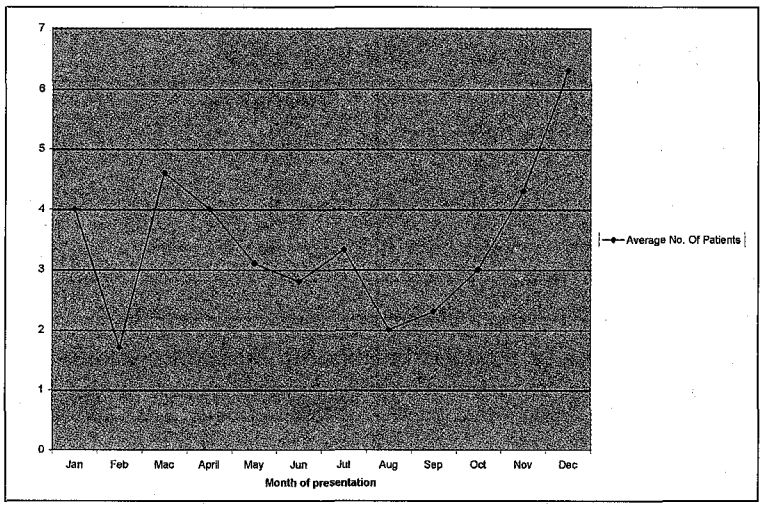

Fig. 1: Bar chart showing average number of melioidosis cases per year by month of presentation in Pahang, Malaysia (January 2000-June 2003)

Table I: Table showing number of melioidosis cases by age in Pahang, Malaysia (January 2000-June 2003)

Age(years)	Number (%)	
<30	10	(7.4)
30-40	18	(13.3)
40-50	35	(25.9)
50-60	41	(30.4)
60-70	25	(18.5)
>70	6	(4.4)
Total	100	(100)

Table II: Number of melioidosis cases and in-hospital mortality by foci of infection in Pahang, Malaysia (January 2000-June 2003).

Foci of infection	Number (%) (n=135)	In-hospital mortality (n=113)
Pneumonia	55 (40.7)	68.8
Septicemia with unknown source	26 (19.3)	47.4
Multiple organ involvement	21 (15.6)	73.7
Subcutaneous abscess or musculoskeletal involvement	20 (14.8)	18.8
Involvement of internal organs*	13 (9.6)	18.2
Total	135 (100)	54.0

*Organ involvement (e.g. liver or spleen or kidney or brain) other than lung, skin, subcutaneous tissue or musculoskeletal involvement.

Table III: Table showing time of death after admission in Pahang, Malaysia (January 2000-June 2003).

Time of death	Number (%)	
Within 24 hours	26	(38.8)
Between 24 to 48 hours	14	(20.9)
Between 48 hours to 7 days	15	(22.4)
Between 7 to 14 days	2	(3.0)
More than 14 days	10	(14.9)
Total	**67**	**(100)**

Table IV: Clinical Characteristics of Subjects in Pahang, Malaysia (January 2000-June 2003).

	Dead	Alive	p
Past infection			0.065
Yes	1 (16.7%)	5 (83.3%)	
No	55 (54.5%)	46 (45.5%)	
Possible	5 (83.3%)	1 16.7%)	
Predisposing factors			0.616
Yes	52 (53.1%)	46 (46.9%)	
No	9 (60.0%)	6 (40.0%)	
Duration of fever (N=111) median (range)	5 (30) days	9(90) days	0.004
Site of culture positive			0.002
Blood only	6 (59.2%)	42 (40.8%)	
Blood plus other sites	0 (0.0%)	8 (100.0%)	
Other sites only	0 (0.0%)	2 (100.0%)	
Blood pressure (N=112)			0.131
Hypotensive	9 (75.0%)	3 (25.0%)	
Normal	52 (52.0%)	48 (48.0%)	
Empirical antibiotic regime (N=112)			0.551
No antibiotic	13 (48.1%)	14 (51.9%)	
Appropriate antibiotic	23 (62.2%)	14 (37.8%)	
Other antibiotic	17 (56.7%)	13 (43.3%)	
Combination therapy	8 (44.4%)	10 (55.6%)	
Severity of infection			< 0.001
Severe[1]	56 (65.1%)	30 (34.9%)	
Not severe	5 (18.5%)	22 (81.5%)	
Hb (g/dL, mean ± standard deviation)	11.3 ± 2.9	11.1 ± 2.3	0.603
TWC[2] ($X10^9$ /L, median (range))	12.1 (37.2)	11.2 (55.0)	0.369
Platelet ($X10^9$ /L , median (range)) (N=112)	151.5 (426.0)	206.0 (352.0)	0.001
RBS[3] (mmol/L, median (range)) (N=104)	14.2 (33.2)	13.1 (32.5)	0.525
Blood Urea (mmol/L, median (range)) (N=112)	12.0 (60.0)	6.3 (50.7)	< 0.001
Creatinine (µmol/L, median (range)) (N=83)	175 (775)	89 (1570)	< 0.001
Bilirubin (µmol/L, median (range)) (N=75)	23.5 (296.5)	15.0 (60.2)	0.015
Albumin (g/L, mean ± standard deviation) (N=77)	25.3 ± 5.9	28.0 ± 6.1	0.048

[1] Severe infection is defined as the presence of pneumonia, septicemia of unknown source or multi-organ involment
[2] Total White Count
[3] Random Blood Sugar

Discussion

The incidence of melioidosis varies between countries and also between different parts of the same country. In Thailand, it is most commonly seen in the northern region and the incidence was 3.6-5.5 per 100,000 per year[7]. In northern Australia, the incidence was 16.5 per 100,000 per year[3]. We are not aware of any published data on the incidence of melioidosis in Malaysia. We believe the incidence may vary among different states as agricultural activities differ in the various states. The incidence of 6.07 per 100,000 per year recorded in our study is comparable with Thailand. This may represent the incidence of melioidosis in most states in Malaysia. However, the incidence may be lower in states with limited agricultural activities especially in Kuala Lumpur or Penang. The picture seen in these two states may be similar to the incidence reported in Singapore (1.7 per 100,000 per year)[8]. The true incidence may be higher than reported as some patients may have negative cultures despite extensive investigation. In our study, there were 6 patients who had possible past infection. Three of these patients had a history of subcutaneous abscesses, one had pneumonia which responded to ceftazidime, one had septic arthritis and another had multiple liver abscesses with positive culture only during the subsequent admission.

The majority of our patients were Malay (83%). This may be because more Malays in Pahang work in the agricultural sector. The male to female ratio of 3.6 to 1 reflects the importance of occupational exposure and is consistent with a study done in Northern Australia3. Occupations were recorded for 45% of our patients and a history of soil contact among non-farmers was not routinely sought by the doctor. This makes analysis of soil contact as a risk factor difficult. The median age of 51 years in our study was slightly higher than in other reports[3,7] due to the exclusion of paediatric cases.

Diabetic mellitus is the most commonly described predisposing factor in the literature. To our knowledge, the incidence of diabetic patients (74%) in our study is the highest reported (37% to 60%)[1,2,3]. This could be due to the higher prevalence of diabetes in Malaysia (more than 8%) as compared to Caucasians in Australia (5% to 6%) 10 and poor diabetic control in most of the diabetic patients[11]. Poor diabetic control is an important risk factor for melioidosis[9]. Both alcoholism and chronic lung disease were relatively uncommon in our study compared to Northern Australia[3] as most of our patients were Muslims and alcohol is prohibited in Islam. The incidence of cystic fibrosis is also low in Malaysia. We had three patients who were HIV serology positive without other predisposing factors which has not been reported earlier. BPH is known to cause urinary stasis and urinary tract infection but there was no publish data on BPH as a predisposing factor for melioidosis. We found two patients with BPH presenting with fever and dysuria. Both of their urinalysis suggestive of urinary trait infection and blood cultures were positive for *B. pseudomallei*. One of these patients also had diabetes mellitus and chronic renal failure. Unfortunately, urine cultures were not sent for these patients. Further study need to be done to look for possible association between BPH and melioidosis.

In this study, patients without predisposing factors were significantly younger than patients with predisposing factors. This could be related to undetected underlying predisposing factors such as thalassemia trait. In contrast, diabetes occurs more commonly in older patients and may be more easily detected.

Trauma to the skin due to motor vehicle accidents may be an important predisposing factor as contact of the wound with soil may result in direct inoculation of the organisms into the blood stream as seen in two of our patients. However, wound swabs were not taken from either patient to confirm our hypothesis. We concluded that melioidosis should be considered in immuno-compromised patients who develop fever after MVA in endemic areas and ceftazidime should be considered if the fever is not responding to the usual antibiotic.

In our study, 19% of our patients with normal abdominal examination were found to have intra-abdominal abscesses on ultrasound. This reflects the low sensitivity of physical examination in localization of intra-abdominal abscesses in melioidosis. In our study and most studies in Thailand, [1,7] prostatic abscesses were not commonly encountered as compared to 18% in Northern Australia[3]. This is probably due to under diagnosis as most physicians here do not perform CT scan routinely to locate intra-abdominal abscesses in confirmed cases. Therefore, we strongly suggest routine radiological investigation and evaluation of the sensitivity of ultrasound as compared to CT scan.

The mortality of melioidosis is extremely high especially in the bacteraemic form. The reported incidence of the bacteraemic form ranges from 46% to

60%[3,7] as compared to 91.8% in our study. The higher incidence in our study may due to late presentation of our patients who usually seek traditional treatment before presenting to us. Higher frequency of diabetes mellitus in our study may be another contributing factor as diabetics are more at risk for bacteraemic melioidosis[9]. Furthermore, paediatric patients who commonly present with localized melioidosis were excluded in this analysis[12] (we identified [14] paediatric cases and this is reported separately).

The overall in-hospital mortality (53.5%) in this study is higher than in other recent studies (19% to 37%)[3,4,5,7] as only 51.9% of patients received an appropriate antibiotic regime despite positive culture. This is probably due to lack of awareness among doctors regarding the treatment of melioidosis as reported from South India where the mortality was 58%[13]. Furthermore, there were also more bacteraemic patients in our study as compared to other studies[3,7]. We also found a higher risk of relapse following inappropriate antibiotic therapy which may result in mortality during relapse as seen in Thailand[14]. In view of the high mortality in septicaemic melioidosis, further studies should be carried out to evaluate possible approaches in reducing the mortality rate. To date, there is no evidence that early treatment of suspected cases with empirical ceftazidime may reduce mortality. In our study, empirical ceftazidime or other appropriate antibiotic appeared to have no effect on mortality even though combination therapy was associated with a better outcome. Further randomized studies are necessary to resolve this matter. The use of G-CSF in septicaemic melioidosis needs further evaluation.[15] Whether early diagnosis using test kits16, serology, monoclonal antibody[17] and polymerase chain reaction (PCR) [18] will reduce the mortality rate requires further research.

In conclusion, melioidosis in Pahang is commonly associated with diabetes mellitus and mortality remains extremely high despite antibiotic therapy.

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