ORIGINAL ARTICLE

SURVIVAL TIME OF PATIENTS WITH MELIOIDOSIS; AN APPLICATION OF KAPLAN-MEIER SURVIVAL ANALYSIS

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ABSTRACT

Melioidosis is a contagious infection caused by the gram-negative bacteria - Burkholderia pseudomallei. The modes of acquisition are inhalation, ingestion, and inoculation. Misdiagnosis of melioidosis is frequently reported due to limited awareness of the disease and confusion with tuberculosis. Antibiotic resistance of causative agent, age group, and organ involved are the prognostic factors contributing to melioidosis's high fatality. Hence, the purpose of this current research was to compare the survival probability in melioidosis patients according to the prognostic factors. This study documented 453 cases from Kedah and Perlis from 2014 until 2018 using a retrospective method. Patient's medical information related to the study was extracted using a data collection sheet. All the data were analyzed using IBM SPSS Statistics. Kaplan-Meier Survival Analysis was used to determine the 5-year survival and median survival time. In contrast, the Log-rank test was used to compare the survival probability based on the demographics, underlying diseases, and prognostic factors. The overall 5-year survival was 85%, and the median survival time of melioidosis patients was 30 days. Prognostic factors that cause a significant difference in survival probability and median survival time of melioidosis patients were age group above 40 years old, diabetes mellitus and chronic lung disease as comorbidities, and bacteremia melioidosis. Melioidosis should be classified as an opportunistic infection that has high mortality in the immunocompromised host. Early diagnosis and appropriate treatment can increase the survival advantages of melioidosis patients.

Keywords: *Burkholderia pseudomallei; melioiodosis; median survival time; Kaplan-meier; prognostic factors*

INTRODUCTION

Melioidosis, also known as Whitmore's disease, has been endemic in Malaysia since 1913 due to an outbreak involving laboratory guinea pigs and rabbits at the research institute in Kuala Lumpur. Since then, cases have been reported in humans and local animals such as horses, monkeys, etc. A soil saprophyte causes *Burkholderia pseudomallei* (*B. pseudomallei*) and can be isolated from infected water and wet soils [1]. Several types of research have shown that melioidosis is an emerging infectious for several reasons. Although antimicrobial therapy is provided, the cases and fatality rate of septicemia in melioidosis in Malaysia remain significantly high [1].

Males usually have a higher potential to get infected due to involvement in agricultural occupations, especially for those without the benefit of protective clothing. Besides, employees who work in building sectors, members involved in rescue operations, and archaeologists are the clusters with a high potential for exposure to contaminated water or soil [2]. Three modes of transmission of melioidosis are inhalation, ingestion, and inoculation. Inhalation of dust particles from contaminated soil, drinking contaminated water, and direct contact with contaminated soil allow *B. pseudomallei* to penetrate wounds or skin abrasions, causing human infection [3]. The antibiotic is not the only therapeutic option to treat melioidosis. The more we rely on antibiotics, the more bacteria are resistant. As a result, treatment for infectious bacteria becomes much more challenging [4].

The mortality rate of melioidosis is affected by different predicting factors. Thus, survival analysis is essential to determine the duration between exposure and death for a patient with melioidosis. In the United States of America (USA), melioidosis, also known as "The Vietnam time bomb," reflects this disease's high tendency to recur [4]. According to Nathan et al. (2018), Malaysia is an endemic hot spot for melioidosis,

with an increased number of mortality cases reported per year, which is more than deaths resulting from tuberculosis and dengue. However, melioidosis research is still limited in Malaysia and is considered relatively unattainable. The burden of diagnosing melioidosis remains a challenge. The recent study still reported a low clinical suspicion of melioidosis among physicians, leading to misdiagnosis and mismanagement of melioidosis patients [3]. In Malaysia, there is a notable scarcity of published studies examining melioidosis, particularly those employing survival analysis in determining the associated factors of mortality from melioidosis. According to a preprint article authored by Mardhiah [25], recent studies, notably those conducted by Hasan *et al*. and Toh *et al*. [26], [27] predominantly utilize logistic regression as the primary statistical method to identify prognostic factors associated with mortality in melioidosis.

Survival analysis can help the clinician determine the most effective treatment and provide higher survival advantages to the patient. Mardhiah *et al*. reported the prognostic factors of mortality from melioidosis based on the same population [5], [6], [7]. The current study compared the survival probability in patients with melioidosis according to demographics, underlying diseases, and prognostic factors.

METHODS

Study design, participants, sampling method, and sample size

A total of 453 melioidosis patients who were admitted to hospitals in Perlis and Kedah between 2014 and 2018 were retrospectively included in the study. Patients at least 15 years of age with microbiologically confirmed melioidosis were enrolled regardless of their bacteremic status. Patients having infected septicemia but caused by other bacteria, admitted less than 24 hours, and with HIV were excluded. A simple random sampling method was applied in the study. The total number of patients within the study period was 510 patients. The data was collected from the melioidosis registry, and data was retrieved using standard study proforma. The outcome of the study was measured as dead and censored. Since the data was collected between 2014 and 2018, patients who did not experience the event of interest during the study period, withdrew from the study, or lost to follow-up were considered censored. Patients' records were reviewed and analyzed for demographic characteristics, treatment history, underlying disease, and final clinical diagnosis. In addition, patients were categorized into two clusters based on their age: more than 40 years old or below. The age grouping was conducted based on previous studies, which stated that patients are more susceptible to risk factors associated with melioidosis, especially type 2 diabetes mellitus, after age 40 [8].

The sample size calculation based on survival analysis was determined using Power and Sample Size Calculation software, with a significance level (α) of 0.05 and pre-determined power (1-β) of 0.90. The sample size was determined from the available literature [9] on the associated factors of recurrent melioidosis using the Cox Model and Kaplan Meier. The parameters used are HR (The detectable hazard ratio of those with bacteremic melioidosis to those with localized melioidosis was decided by the researcher based on clinical expert) =1.5, m_1 (The median survival time of melioidosis patients to relapse was obtained from literature) = 2.17, m (Ratio of melioidosis patients with localized infection to those with bacteremic infection were obtained from the literature=392/134) = 2.93, A (the accrual time during which patients were recruited) = 60, and F (additional follow-up time) = 9. A final sample size of 338 patients was determined based on addition of patients with localized (252 patients) and bacteremic melioidosis infections (86 patients). The adjusted sample size was 423 patients, accounting for an estimated 20% missing data. As the study was part of comprehensive research covering multiple objectives, the final sample size of 453 patients was taken by selecting the largest calculated sample size for each objective within the entire study. This emphasizes its significance within the scope of this research. A total of 45 patients were excluded from the study, with 18 patients aged less than 15 years old, 23 patients admitted less than 24 hours, and four patients with HIV. To obtain 453 patients as calculated, the patient IDs were set from 1 to 465, and the list of patients was randomly selected based on a Simple Random Sampling Using Excel by NajibMY [17].

Outcomes

The survival time for the event observations measured from admission to hospital with culture-confirmed melioidosis until time to death, where events were defined as a patient who died from melioidosis during the study period. The date of admission with culture-confirmed melioidosis was set as the time of entry (T0), while the last date at the hospital (death or discharge or transfer to another hospital) was set as T1. The follow-up time was between the difference between T1 and T0. This analysis was censored for death due to causes other than culture-confirmed melioidosis, loss to follow-up, and lived until the study ended. The classification was based on the final clinical outcome, which was recorded as discharged well, at own risk (AOR) discharge, transferred to other hospitals, or died. The survival time for a patient who died during the study period was calculated from the date of admission to the hospital to the date of death and measured in

days. The 5-year overall survival in the study was defined as the percentage of patients in the study who were alive five years after their admission to the hospital due to diagnosis of melioidosis.

Statistical analyses

The statistical analysis tool used in this study was IBM SPSS Statistics 24. For descriptive statistics, the total number of melioidosis patients and their outcome (dead or censored) were calculated respectively for each variable. The categorical data was stated in frequency in percentage form. Kaplan-Meier survival analysis was used to determine melioidosis patients' survival probability and their median survival time. A Log-rank test was used to compare the survival probability according to the demographic, underlying diseases, and prognostic factors. A P-value of less than 0.05 was determined as a statistically significant difference.

Ethics

Approval of the study was obtained by Universiti Zainal Abidin Human Research Ethics Committee (UHREC) (protocol code: UniSZA/UHREC/2019/119) and Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia (MOH) [NMRR19-3090-46158 (IIR)].

RESULTS

Final clinical diagnosis of patients with melioidosis

Approximately 44.2% of study participants were diagnosed with pneumonia at the final clinical stage, while splenic abscess was the second most frequent final clinical diagnosis in the study participants (Table 1).

Median survival time of melioidosis patients

A total of 453 patients met the criteria and were selected as study participants. At the end of the study, 227 (50.1%) deaths were detected, and 226 (49.9%) patients were still alive and thus were considered censored. The median survival time of melioidosis patients in this study was 30 days, with a 5-year survival of 85.0% (Figure 1).

Comparison of median survival time according to demographic characteristics, underlying diseases, and prognostic factors

Most of the study participants (80.4%) were equal to or older than 40 years old, and males were the predominant (78.8%). Twenty-one percent of the patients were involved in high-risk occupations for melioidosis. Malays were the major population (88.0%) in this study's participant. Malays also had the longest median survival time (35 days) with a 5-year survival of 49.6% (95% CI: 0.0, 73.6) compared to other races. There was a significant difference between patients with an age group less than 40 years old and equal to or above 40 years old (***P***=0.005).

More than half of the study participants had underlying diabetes mellitus (69.8%). Only 0.2% of patients were alcohol abusers. The result indicated that the survival probability has differed between patients with or without diabetes mellitus. Patients with underlying diabetes mellitus had a longer median survival time (45 days) with a higher 5-year survival of 49.9% (95% CI: 0.0, 115.3) compared to patients without underlying diabetes mellitus. Other than that, patients with chronic lung disease have a higher 5-year survival (87.5%) but a shorter median survival time of four days (95% CI = 1.6, 6.4) compared to those without chronic lung disease. Chronic renal failure and chronic lung disease were recorded in 60 (13.3%) and 16 (3.5%) patients.

Table 1: Final clinical diagnosis of melioidosis patients admitted to hospitals in Kedah and Perlis (n=453).

Variable	Total n=453 n(%)	Died n=227 n(%)	Censored n=226 n(%)
Pneumonia	200(44.2)	120(60.0)	80(40.0)
Soft Tissue Abscess	47(10.4)	15(31.9)	32(68.1)
Septic Arthritis	28(6.2)	10(35.7)	18(64.3)
Osteomyelitis	2(0.4)	0(0.0)	2(100.0)
Prostatic Abscess	3(0.7)	0(0.0)	3(100.0)
Liver Abscess	39(8.6)	11(28.2)	28(71.8)
Splenic Abscess	48(10.6)	9(18.8)	39(81.2)
Meningoencephalitis	9(2.0)	3(33.3)	6(66.7)
Brain Abscess	5(1.1)	3(60.0)	2(40.0)
Pyelonephritis / UTI / Perinephric Abscess	13(2.9)	5(38.5)	8(61.5)

In this research, 86.8% of melioidosis patients had bacteremia, as shown in Table 2. There was a significant difference in survival probability and median survival time between patients with and without bacteremia (***P***<0.001). Melioidosis patients with bacteremia had a lower 5-year survival (83.2%) with 19 days of median survival time compared to non-bacteremia melioidosis. However, the median survival time of melioidosis patients without bacteremia cannot be determined in this study. Less than half of the study participants (31.6%) receive antibiotic treatment during melioidosis infection. Despite antibiotics being given to treat melioidosis, patients who received antibiotic treatment had shorter median survival time (27 days) and lower 5-year survival (48.6%) than those who did not receive antibiotic treatment.

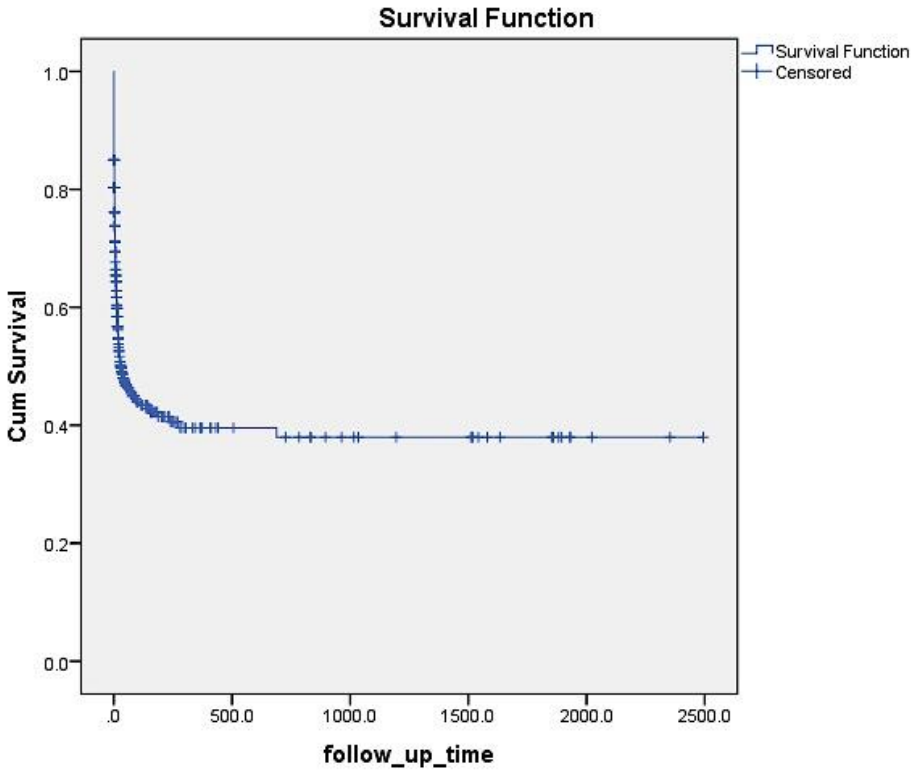

Figure 1. Kaplan-Meier Survival Analysis of Melioidosis Patients (n=453)

Table 2a: Comparison of Median Survival Time by Log-rank test among melioidosis patients admitted to Hospital in

Variable	Total n=453 n(%)	Died n=227 n(%)	Censored n=226 n(%)	Median Survival Time (95% CI)	***P***-value[b]
Demographic					
Age					
< 40 years [a]	89(19.6)	31(34.8)	58(65.2)	-(-, -)[e]	
≥ 40 years	364((80.4)	196(53.8)	168(46.2)	22.0(11.3, 32.7)	0.005
Races					
Malay [a]	399(88.0)	198(49.6)	201(50.4)	35.0(0.0, 73.6)	0.978[d]
Chinese	18(4.0)	9(50.0)	9(50.0)	30.0(4.2, 55.8)	0.641[d]
Indian	27(6.0)	17(63.0)	10(37.0)	27.0(16.2, 37.8)	0.526[d]
Other	9(2.0)	3(33.3)	6(66.7)	-(-, -)[e]	
Occupation					
High risk [a]	96(21.2)	46(47.9)	50(52.1)	45.0(0.0, 588.1)	
Low risk	177(39.0)	85(48.0)	92(52.0)	72.0(-,-)	0.797[c]
Unknown	180(39.7)	96(53.3)	84(46.7)	20.0(11.7, 28.3)	0.224[c]
Gender					
Male [a]	357(78.8)	180(50.4)	177(49.6)	30.0(1.2, 58.8)	0.744
Female	96((21.2)	47(49.0)	49(51.0)	24.0(0.0, 165.9)	

Table 2b: Comparison of Median Survival Time by Log-rank test among melioidosis patients admitted to Hospital in

Underlying diseases					
Diabetes Mellitus					
Yes [a]	316(69.8)	153(48.4)	163(51.6)	45.0(0.0, 115.3)	
No	137(30.2)	74(54.0)	63(46.0)	16.0(6.5, 25.5)	0.043
Chronic Renal Failure					
Yes [a]	60(13.3)	35(58.3)	25(41.7)	16.0(8.6, 23.4)	
No	393(86.8)	192(48.9)	201(51.1)	38.0(0.0,82.325)	0.276
Alcohol Abuse					
Yes [a]	1(0.2)	0(0.0)	1(100.0)	-	
No	452(99.8)	227(50.2)	225(49.8)		0.591
Chronic Lung Disease					
Yes [a]	16(3.5)	10(62.5)	6(37.5)	4.0(1.6, 6.4)	
No	437(96.5)	217(49.7)	220(50.3)	33.0(0.0, 67.9)	0.047
Other Immunocompromised State					
Yes [a]	5(1.1)	3(60.0)	2(40.0)	10.0(5.7,14.3)	
No	448(98.9)	224(50.0)	224(50.0)	30.0(0.0, 60.7)	0.714
Prognostic factors					
Previous Melioidosis Infection	14(3.1)	4(28.6)	10(71.4)	689.0(0.0,1621.2)	
Yes [a]	439(96.9)	223(50.8)	216(49.2)	27.0(0.9,53.1)	0.134
No					
Bacteraemia Status					
Yes [a]	393(86.8)	219(55.7)	174(44.3)	19.0(12.3,25.7)	
No	60(13.2)	8(13.3)	52(86.7)	-(-,-) [e]	<0.001
Antibiotic received					
No [a]	310(68.4)	161(51.9)	149(48.1)	30.0(0.0,68.9)	
Yes	143(31.6)	66(46.3)	77(53.8)	27.0(0.0,54.3)	0.580
Smoking Status					
Unknown	419(92.5)	212(50.6)	207(49.4)	30.0(0.0, 64.6)	0.727 [c]
Yes	18(4.0)	9(50.0)	9(50.0)	9.0(4.0, 14.0)	0.543 [c]
No [a]	16(3.5)	6(37.5)	10(62.5)	-(-,-) [e]	

[a]Reference group.
[b]Level of significance alpha was set at 0.05
[c]Bonferroni correction was applied for variables which has ≥ 3 level by correcting the level of significance alpha (a / number of pairs = 0.05/3 = 0.017)
[d]Bonferroni correction was applied for variables which has ≥ 3 level by correcting the level of significance alpha (a / number of pairs = 0.05/4 = 0.0125)
[e]Result could not be determined.

DISCUSSION

A high EI level indicates that the students were This study provides the 5-year overall survival and comparison of median survival times based on demographic characteristics, underlying diseases, and prognostic factors among melioidosis patients. It was a limited published article on application of Kaplan-Meier on melioidosis. Despite an extensive search, one study by Limmathurotsakul *et al*. provides important insights into the survival time of patients with relapse and reinfection of melioidosis using Kaplan-Meier survival analysis. The study found that the median time to relapse was 26 weeks, with interquartile range (IQR) of 10 – 72 weeks, which means that half of the patients who experienced a relapse did so within this time frame. In contrast, the study found that the median time to reinfection was 111 weeks (IQR = 59 – 164 weeks), significantly longer than the median time to relapse [9]. This suggests that patients who experience reinfection may have a longer period of disease-free survival before experiencing another infection.

The results of the current study suggest that patients with underlying diabetes mellitus had a longer median survival time of 45 days with a higher 5-year survival of 49.9% (95% CI: 0.0, 115.3) compared to those without diabetes mellitus. Interestingly, these findings are consistent with another study that reported a survival advantage for patients with diabetes after developing melioidosis [10]. This study included 1160 patients with melioidosis and found that patients with diabetes taking glyburide had a survival advantage compared to those not taking the medication. The log-rank test showed statistical significance for this survival advantage. The total duration of follow-up in this study was 11,845 patient days, with a median duration of follow-up of 6.5 days, according to the research in Northeast Thailand, which has shown that the expression of CX3CR1 in diabetic patients can increase survival during infectious disease, including melioidosis [11]. This may be due to alterations in the immune response, producing of high antibody titers and double-negative T cells against *B. pseudomallei*.

Melioidosis patients with an age group less than 40 years old had higher survival compared to those with an age group more than 40 years old. This outcome is consistent with a review done in Northeastern Malaysia [8]. The study found that found that patients aged over 40 years had a 6.47-fold increased chance of mortality (95% CI: 1.7, 23.8) [8]. The immune system protects the host from infections and malignancies, regulates wound healing, and self-identifies from other foreign organisms. T and B cells have reacted and reproduced actively to fight against infectious microorganisms and cancerous cells. They transform highly proliferative naïve cells into less proliferative effectors and memory cells. Rapid clonal replication imposes enormous proliferative stress on immune cells, leading to the progressive deterioration of protective immunity. These may explain why older patients are highly susceptible to infections such as melioidosis [12]. However, in another recent study in Malaysia, demographic factors such as age and gender were not independently predictive of mortality in that study population [26]. The risk of mortality in melioidosis patients appears to be influenced by age, as indicated by findings from various studies. In Thailand, a study by Hantrakun *et al*. (2019) reported a 1% risk of mortality, with a confidence interval (95% CI) ranging from 1 to 4.29 [13].

Chronic lung disease contains different conditions, such as airflow obstruction, emphysema, and chronic bronchitis. A consistent trend is observed regarding the impact of chronic lung disease on the mortality risk of melioidosis patients. In a study conducted by Hanson and Smith (2019), utilizing multivariable logistic analysis, it was found that individuals with chronic lung disease had four times the odds of dying compared to those without this condition (95% CI: 1.84, 8.93). Similarly, in research by Currie, Ward, and Cheng (2010), the odds of mortality for patients with chronic lung disease were reported to be 50% higher than for those without chronic lung disease (95% CI: 1.1, 2.4) [14], [15]. These consistent findings across studies emphasize the significant association between chronic lung disease and lower median survival time in melioidosis patients.

The current study showed that bacteremia melioidosis contributes to the lower survival of patients with melioidosis. A Singapore study reported that bacteremic patients reduced the risk of surviving melioidosis by 98.0% (OR: 0.02, 95% CI: 0.00, 0.25) [19]. Many other studies are also allign with the current study's findings [18], [21]. Bacteraemia melioidosis happens when *B. pseudomallei* begins to disseminate through the blood system from the origin of the infection to other organs. Spreading of the bacteria to other organs can cause colonization of the bacteria inside the deep abscess that interrupts the normal functions of the organ. Thus, secondary focal dissemination is common in melioidosis patients with bacteremia status [16]. A review conducted in northeastern Malaysia showed that 76.6% of melioidosis patients are bacteremic, while disseminated infection and focal infection were 28.5% and 78.5%, respectively [17]. A case study in China suggested that antibiotic resistance of *B.pseudomallei* against ceftazidime and inappropriate initial treatment might cause the development of septic shock in melioidosis patients [20]. Bacteraemia significantly increases the mortality rate of melioidosis patients due to the high risk of developing severe sepsis and fatal septic shock associated with multi-organ organs in those patients [22]. Bacteraemia causes a poor prognosis in melioidosis patients, and septic shock is a significant predictor of high fatality within 24 hours of admission [23]. Thus, melioidosis patients who show bacteremia status should receive intensive care.

The study did not control for potential confounding variables, such as socioeconomic status, lifestyle factors, or comorbidities, which could have impacted the results. In addition, the study's retrospective design did not allow for the measurement of patients' delayed diagnosis or treatment or the possibility that some patients may have died before the diagnosis results came out. These limitations could have introduced biases and confounding factors in the study and affected the validity and generalizability of the findings. Therefore, further studies with more robust designs and appropriate adjustments for potential confounders are needed to provide more insights into the prognostic factors of mortality from melioidosis and outcomes of melioidosis.

CONCLUSIONS

In summary, the findings highlighted that the median survival time of melioidosis patients in this study was 30 days, with a 5-year survival of 85.0%. Patients with underlying diabetes mellitus exhibited a longer median survival time, consistent with previous studies, which reported a survival advantage for diabetic patients with melioidosis. Age was a significant factor, with patients under 40 years old having a higher survival probability, while patients aged 50 and above displayed thymic changes that may contribute to increased susceptibility to infections. Other than that, bacteremia, a severe form of melioidosis, was highlighted as a critical factor leading to lower survival, emphasizing the importance of intensive care for patients with bacteremia status.

Overall, while this study provides valuable insights into potential prognostic factors associated with the survival time of melioidosis patient, the limitations of the study should be considered when interpreting the results. Further research with larger sample sizes, prospective designs, and better control for confounding variables is necessary to enhance our understanding of the prognostic factors of mortality from melioidosis and outcomes of melioidosis.

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Competing interests
The author reports that no conflicts of interest are involved in this research. The datasets used for the current study are available from the corresponding author on reasonable request.

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