Predictors of Severe Leptospirosis: A Multicentre Observational Study From Central Malaysia

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Abstract

Background: Leptospirosis is a re-emerging disease with vast clinical presentations ranges from subclinical or mild, to severe and fatal outcomes. Though leptospirosis can be managed well if diagnosed earlier, similar clinical presentations by several other febrile illnesses or co-infections often result in mis- or underdiagnosis, thereby lead to severe illness. Identification of clinical predictors for the severe form of the disease plays a crucial role in reducing disease complication and mortality. Therefore, we aimed to determine the clinical predictors associated with severe illness among leptospirosis patients from Central Malaysia through a prospective multicenter observational study.

Methods: Data were collected from case records of 83 confirmed leptospirosis patients comprising of 33 severe and 50 mild. Statistical analysis was performed using $\chi 2$ and multivariable logistic regression test with Epi info software.

Results: We identified mechanical ventilation, AKI, septic shock, creatinine level of >1.13mg/dL, ALT >50IU, AST >50IU, and platelet <150 x 109/L as factors associated with severe illness. AKI, ALT >50IU and platelet <150 x 109/L were defined as the independent factors for severity.

Conclusions: Lungs, liver and kidney involvement, and septic shock were found as the prognostic factors for severe leptospirosis. AKI, high level of ALT and low level of platelets were found to be independent predictors of severity.

Background

Leptospirosis is a globally distributed zoonotic disease caused by the pathogenic species of *Leptospira*, a genus of spirochete bacteria. Although this disease has a worldwide distribution, it is more common in the tropics due to its favorable transmission condition. In Malaysia, leptospirosis is an endemic disease. Occasional outbreaks have been reported and are mainly associated with forest and water activities, flood and crowded living environment [1–5].

The clinical presentation of leptospirosis varies from mild to severe, life-threatening and fatal outcomes. The symptoms are diverse, generally include fever and often shares presentations with other infectious diseases, such as malaria, dengue, and influenza. About 90% of leptospirosis is a subclinical or self-limited febrile illness, while the severe form is presented by sepsis and multiple organ failures [6]. The most severe form of leptospirosis is Weil's disease, seen in 5–15% of infected cases and typically involves multiple organs damages accompanied with jaundice, acute renal failure and hemorrhage and has a fatality rate of more than 10% [7, 8]. However, leptospirosis cases with lung involvement presenting with acute respiratory distress syndrome (ARDS) or severe pulmonary hemorrhage syndrome (SPHS) have the highest mortality rates of over 70% [7]. The presentation of leptospirosis appears to be distinct in different geographical areas worldwide [8]. This is particularly true as different geographical location may have different prevailing *Leptospira* species and serovars. The variations of intrinsic virulence among serovars and species have been asserted to partially explain the disease severity of mild and

severe forms of leptospirosis [9]. These differences may also lead to specific predictors of severe disease and mortality.

The two-extreme manifestation of leptospirosis which are mild/subclinical and severe illness with mortality cautions that the disease should not be taken lightly, and constant monitoring of patients is essential. For constant monitoring, it is important to determine the prognostic markers, but it is still not clear what factors that predict the transition from mild to severe/fatal illness. Several independent prognostic factors for fatal leptospirosis have been reported across the globe and these include older age, oliguria, hyperkalaemia, abnormal serum creatinine level, ARDS, pulmonary haemorrhage, elevated bilirubin level, hypotension, arrhythmia and altered mental status [8, 10, 11].

Earlier studies from Malaysia identified elevated levels of cytokines such as IL-6, IL17A and IL-22 as potential prognostic biomarkers with IL-17A as the independent predictor for leptospirosis associated fatalities [12]. Another study conducted in a suburban area in Malaysia identified age above 70, clinical presentations suggestive of organ dysfunction and intensive care requirement as predictors for fatalities [13]. Fish Low *et al*. (2020) reported hypocalcemia (calcium < 2.10 mmol/L), hypochloremia (chloride < 98 mmol/L), and eosinopenia (absolute eosinophil count < 0.040×10^9/L as clinical predictors for laboratory-confirmed leptospirosis when compared with clinically suspected leptospirosis cases [14]. Analysis of a set of data for clinically diagnosed leptospirosis cases in a tertiary care hospital in Malaysia showed gastrointestinal tract (GIT) presentation as one of the predictors for severe illness, the study also found that GIT symptoms are significantly associated with age (20–40 yrs), poor sanitation and crowded living environment of the patients [15].

In the present investigation, we performed a multicentered observational study to identify factors predictive of severe leptospirosis. Determining the predictors at early stage of the disease could greatly reduce the severe illness development and thereby mortality.

Methods

Study design

The study was conducted from January 2016 to December 2017 in three tertiary hospitals from two states in Central Malaysia; Hospital Serdang and Hospital Tengku Ampuan Rahimah (HTAR) in the state of Selangor and Hospital Teluk Intan in the state of Perak. A prospective multicenter observational study was performed on patients with confirmed leptospirosis (laboratory confirmation). The clinical data were collected upon patient admission. The patients were followed for up to four weeks after discharge. Laboratory confirmation is defined as positive by PCR or microscopic agglutination test (MAT) (first sample > 1:400; seroconversion or 4-fold increase in the paired samples) [16]. The genes used to detect the presence of leptospires in blood and serum were *lipL32* and 16S rDNA [17, 18]. The samples positive by 16S rDNA were subjected to sequencing (MyTACG Bioscience Enterprise, Malaysia) to identify the infecting *Leptospira* species [1]. The serovars used for MAT assay comprised Australis, Autumnalis,

Batavia, Canicola, Celledoni, Grippotyphosa, Hardjoprajitno, Icterohaemorrhagiae, Javanica, Pyrogenes, Tarrasovi, Djasiman, Patoc, Pomona (international serovars were obtained from World Health Organization, WHO) and IMR LEP 1; saprophyte, IMR LEP 115; saprophyte, IMR LEP 175; saprophyte, IMR LEP 803/11-Copenhageni, IMR LEP 27-Hardjobovis, IMR LEP 22-Lai (local serovars were obtained from the Institute for Medical Research (IMR), Federal Territory of Kuala Lumpur, Malaysia [19, 20].

Case definitions

Any patient who is laboratory confirmed for leptospirosis is included in the study. The cases were classified into two categories such as mild and severe (severe included dead patients). Mild illness is characterized by fever, headache, myalgia, conjunctival congestion, mild cough, lymphadenopathy, rash, anorexia, nausea and vomiting [21], while severe illness is defined as hospitalization plus jaundice, acute kidney injury, and or pulmonary involvement [1, 22].

Statistical analysis

All data such as the socio-demography of the patients, clinical presentation and selected blood profile were entered and recorded in Excel sheet. Epi info version 7 was used for analysis. Qualitative variables were compared through $\chi 2$ test. The relationship between mild and severe cases was evaluated using logistic regression, using severe as the dependent or outcome variable. Variables which had significant finding in univariate analysis for the severe cases were further analysed by multivariable logistic regression test to predict the independent variable for severity. Only p-value less than 0.05 with corresponding 95% confidence interval (CI) of more than one was taken as significant result.

Results

Descriptive analysis

A total of 83 patients with confirmed leptospirosis were recruited. Among the 83 positive cases, 57 were confirmed by 16S rDNA and the infecting species were identified as *L. interrogans* (n = 40; 48.2%), *L. kirschneri* (n = 16; 19.3%) and *L. wolffii* (n = 1; 1.2%). The socio-demography, clinical presentations and laboratory findings of all confirmed patients are shown in Table 1. The 83 patients comprised of 54 (65.06%) males and 29 (34.9%) females with a mean age of 41.92 years. Twenty patients had acute kidney injury (AKI). From the 83 confirmed leptospirosis patients, 11 (13.3%) died and 72 (86.7%) recovered. Among the 72 patients who recovered, 22 developed severe illness based on the definition given above (case definition in methods section).

Table 1
Socio-demographic, clinical presentation and laboratory findings

Socio-demographic, clinical presentations and laboratory findings	Mean ± SD or n (%)
Socio-demographic	54 (65.06%)
Gender: Male	
Female	29 (34.94%)
Age	41.92 ± 17.99
> 60	20(24.10%)
< 60	63(75.90%)
Clinical Presentations	81 (97.59%)
Fever	
Headache	34 (40.96%)
GIT involvement	53 (63.36%)
Myalgia	27 (32.53%)
Mechanical ventilation	6 (7.23%)
AKI	20 (24.1%)
Septic shock	9 (10.84%)
Laboratory findings	
Creatinine > 1.13 mg/dL	35 (42.17%)
Bilirubin > 32.49 µmol/L	20 (24.39%)
ALT > 50 IU	35 (42.17%)
AST > 50 IU	45 (55.56%)
HCT < 35%	38 (48.10%)
Platelet < 150 × 10^9/L	30 (36.14%)
HB < 11.5 g/dL	35 (42.17%)
WBC < 11 × 10^9/L	34 (40.96%)
Infecting species	40 (48.2%)
Leptospira interrogans	
Leptospira kirschneri	16 (19.3%)

Socio-demographic, clinical presentations and laboratory findings	Mean ± SD or n (%)
Leptospira wolffii	1 (1.2%)

Univariate analysis

Univariate analysis identified requirement of mechanical ventilation, AKI, septic shock, creatinine level of > 1.13 mg/dL, ALT > 50 IU, AST > 50 IU, and platelet < 150×10^9/L as significant association with severe diseases (Table 2).

Table 2
Univariate analysis between severe and mild infections

Variables	Severe cases (n = 33, 39.8%)	Mild cases (n = 50, 60.2%)	Odds ratio	95% CI	χ2 test	Corrected X^2 tailed p P < 0.05
Gender M:F	(70%, M)	(62%, M)	1.4	0.6–3.6	0.2	0.6
Older age > 60 years	32%	26%	1.3	0.4–4.4	0.04	0.8
Fever	97%	98%	0.7	0.04–10.8		1*
Headache	30%	48%	0.5	0.2–1.2	1.9	0.2
GIT involvement	55%	70%	0.5	0.2–1.3	1.4	0.2
Myalgia	36%	30%	1.3	0.5–3.4	0.1	0.7
Mechanical ventilation	18%	0%	UN	UN		0.0029**
AKI	48%	8%	10.8	3.2–36.9	15.7	0.00007**
Septic shock	21%	4%	6.5	1.3–33.4	4.4	0.035**
Creatinine > 1.13 mg/dL	70%	24%	7.28	2.7–19.5		0.00009**
Bilirubin > 32.49 µmol	33%	18%	2.2	0.8–6.2		0.18*
ALT > 50 IU	58%	32%	2.9	1.2–7.2	4.3	0.037**
AST > 50 IU	79%	40%	5.7	2.1–15.6	10.6	0.001**
HCT < 35%	56%	43%	1.7	0.7–4.3	0.9	0.3
Platelet < 150 × 10^9/L	58%	22%	4.8	1.8–12.6	9.4	0.002**
HB < 11.5 g/dL	48%	38%	1.5	0.6–3.7	0.5	0.5

Variables	Severe cases (n = 33, 39.8%)	Mild cases (n = 50, 60.2%)	Odds ratio	95% CI	$\chi 2$ test	Corrected X^2 tailed p P < 0.05
WBC < 11 × 10^9/L	42%	40%	1.1	0.5–2.7	0.0	1
Leptospira interrogans	68%	71%	0.9	0.27–2.7		1*
Leptospira kirschneri	32%	26%	1.4	0.4–4.4	0.04	0.8

*P-value based on Fisher-exact test

**Significant value

UN: Undefined

Multivariable analysis

Multivariable analysis on variables that showed significant association with severe cases, performed through the multivariable logistic regression analysis found that AKI with OR 12.2 (1.3-119.1), ALT > 50 IU with OR 7.1 (1.1–43.4) and platelet < 150 × 10^9/L with OR 6.6(1.5–28.4) were the independent risk factors associated with severity (Table 3).

Table 3
Multivariable logistic regression using severe as dependent variable for independent predictor of severity in leptospirosis

Variables	Odds Ratio	95% C.I.	Coefficient	S.E.	Z-statistic	P-value
AKI	12.2	1.3-119.1	2.5	1.2	2.2	0.03**
Mechanical ventilation	326908.2	0.0–1.0 × 10^{12}	12.7	265.5	0.05	0.96
Septic shock	0.3	0.01–5.1	-1.3	1.5	-0.9	0.4
ALT > 50 IU	7.1	1.1–43.4	1.9	0.9	2.1	0.04**
AST > 50 IU	1.9	0.3–10.3	0.6	0.9	0.7	0.5
Creatinine > 1.13 mg/dL	3.9	0.8–19.9	1.4	0.8	1.6	0.1
Platelet < 150 × 10^9/L	6.6	1.5–28.4	1.9	0.7	2.5	0.01**
CONSTANT	*	*	-15.9	265.5	-0.06	0.9

Discussion

The clinical manifestation and presentation of leptospirosis are broad and only specific when it becomes severe. This presents challenges to clinicians not only to make a correct diagnosis but also to give the best management and treatment to patients to prevent progressing to severe disease and mortality. Several studies have outlined the prognostic factors associated with severe and fatal leptospirosis, however, the predictors could be different between geographical location depending on the socio-demography, type of occupation, prevailing *Leptospira* species, serovars and strains [8, 23].

In this study, seven variables (mechanical ventilation, AKI, septic shock, creatinine level of > 1.13 mg/dL, ALT > 50 IU, AST > 50 IU, and platelet < 150×10^9/L) were found to be associated with severe illness. The independent predictor of severity included AKI, ALT > 50 IU and platelet < 150×10^9/L. A recent study from Malaysia also showed the association of ALT with severe leptospirosis [24]. In another study conducted in the suburban area in Malaysia, factors that were associated with mortality included patients above 70-years with symptoms of oliguria and breathlessness, requirement of vasopressor support, invasive ventilation and steroid use [13]. The present study and two other studies in Malaysia [13, 24] also showed lung involvement as one of the predictors of severity and mortality in leptospirosis. Leptospirosis patients with pulmonary hemorrhage were also reported in several case reports including travelers who visited Malaysia [25, 26, 27]. These factors were found to be the independent factors for mortality in studies conducted in several other countries [8, 28, 29]. These findings indicate that although the kidney is the main target for leptospiral infection, it can also infect the lung and significantly contribute to severity and mortality in leptospirosis. Similar to the present investigation, studies from other countries have also reported the association of exaggerated AST response and higher mean of aspartate/alanine aminotransferase ratio (AAR) with severity and mortality in leptospirosis [23].

In our recent study, we identified three species of pathogenic *Leptospira (L. interrogans, L. kirschneri and L. kmetyi)* causing leptospirosis in Central Malaysia with *L. interrogans* as the most common species [1]. The investigation on the association between infecting species and disease outcome (severe illness) did not show any significant association.

The data obtained from the present study provides baseline information on the factors associated with severe leptospirosis and this needs to be further evaluated on a larger sample size covering different geographical locations in Malaysia and across the globe.

Conclusions

We identified mechanical ventilation, AKI, septic shock, creatinine level of > 1.13 mg/dL, ALT > 50 IU, AST > 50 IU, and platelet < 150×10^9/L) are associated with severe illness. AKI, high level of ALT and low level of platelet were defined as the independent factors for severity.

Declarations

Acknowledgments

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Authors' contributions

NP, LTLT¸ AMS, MYY, ZS, and VKN designed the study. NP interpreted the data and wrote the first draft of the paper. LTLT and VKN took the responsibility for reviewing and editing the draft paper. LTLT¸ AMS, MYY, ZS, and VKN were responsible in the acquisition of funding for this study. All authors agreed and approved the final version of this manuscript..

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Availability of data and material

Not applicable.

Ethical approval and consent to participate

The ethical clearance for this study was obtained from the Medical Research and Ethical Committee (MREC), Ministry of Health Malaysia (NMRR-15-2148-27536). Written informed consent was obtained from all patients who participated in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interest.

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