Clinical manifestation, serology marker & microscopic agglutination test (MAT) to mortality in human leptospirosis

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Abstract. Leptospirosis is a potentially fatal zoonosis that is endemic in many tropical regions and causes large epidemics after heavy rainfall and flooding. Severe disease is estimated 5–15% of all human infections. Its mortality rate is 5-40%. MAT, isolation of the organism, or leptospiral DNA in PCR are used to confirm Leptospirosis. This cross-sectional analytic study recruited 26 hospitalized leptospirosis patients admitted to Dr. Moewardi Hospital Surakarta. The diagnosis was based on clinical, laboratory and epidemiological findings. The onset of the disease was the date when the first symptom started, and the end of the analysis was the date when the patient died or discharged. Modified Faine's score ≥ 25 tend to die (45.5%) while modified Faine's score 20 – 24 tend to heal (60%) (OR 1.250; CI 0.259-6.029; p=1.0). Seropositive IgM predicts mortality 7.8 times higher than seronegative IgM (OR 7.800; CI 1.162-52.353; p=0.038). MAT positive predict mortality 10.667 times higher than MAT negative (OR 10.667; CI 1.705-66.720; p=0.015). Clinical manifestation, MAT, and serologic marker are all correlated with mortality in Leptospirosis. However, statistically, clinical manifestation has an insignificant correlation.

1. Introduction

Leptospirosis is a widespread and potentially fatal zoonosis that is endemic in many tropical regions and causes large epidemics after heavy rainfall,and flooding.[1] Leptospirosis is a zoonotic infection caused by spirochaetes of the genus Leptospira, and humans are affected as incidental hosts.[2]Many mammals are hosts to the disease, but humans commonly acquire the disease from rodents, hence the name‘rat fever’. The disease is transmitted to humans through abraded skin or mucous membrane coming into contact with water or soil contaminated with rodent urine.[2]

It is a disease with significant mortality and morbidity. Recent epidemiological data show anincreasing trend of leptospirosis from all over Asia Pacific region with increased hospital admissions, posing a huge public health threat.[3] Clinically, leptospirosis infection has a range of manifestations, from a mild febrile illness to a severe and potentially fatal disease with acute kidney injury, liver dysfunction, pulmonary haemorrhage and acute respiratory distress syndrome, bleeding, and cardiac involvement.[4] Severe disease is estimated to occur in 5–15% of all human infections.[5] Mortality from severe forms of the disease is 5% to 40%.[6] Around 550,000 cases of leptospirosis are estimated to occur every year, with around 55,000 deaths,[1] though it is possible to be higher than reported.

Development of more severe outcomes depends on three major factors: epidemiological conditions, host susceptibility, and pathogen virulence. The laboratory confirmation of leptospirosis is often based on the microscopic agglutination test (MAT), isolation of the organism, or demonstration of leptospiral DNA by means of PCR.[4]

We designed this observational analytic study to present the findings of a study that aimed to perform leptospirosis mortality rate based on clinical manifestation, serology marker immunoglobulin M Enzyme-Linked Immunosorbent Assay (ELISA) and Microscopic Agglutination Test (MAT).

2. Methods

This cross-sectional analytic study was held at Dr. Moewardi Hospital by recruiting 26 hospitalized leptospirosis patients. All patients clinically suspected leptospirosis (n=26), admitted to Dr. Moewardi Hospital, Surakarta, Central Java between January and July 2014 were screened based on the inclusion criteria.[3] The inclusion criteria were: male or female older than 17 years, with clinical manifestation of Leptospirosis.

The diagnosis of Leptospirosis was based on clinical, laboratory and epidemiological data according to the World Health Organization (WHO) criteria.[7]A definitive case was classified based on the WHOLERGreport, by symptoms consistent with leptospirosis and a single MAT titer ≥1:400 or/and by detection of Leptospira DNA by PCR. A presumptive case was identified as symptoms consistent with leptospirosis and presence of IgM antibodies.[10] Diagnosis of leptospirosis was made based on the modified Faine's criteria (with amendment) 2012 using clinical data (Part A), epidemiological data (Part B) and laboratory data (Part C).[11]Faine's score was obtained for each patient using 3 observation: clinical, epidemiological and laboratory data,shownin Table 1. A score of 25 ormore for all parts or a score of 26 or more for A and B was considered as presumptive leptospirosis(Table 1).

Table 1. System of scoring using the modified Faine's criteria (with amendment) 2012 for the diagnosis of leptospirosis[11].

Part A: Clinical data	**Score**
Headache	2
Fever	2
Fever >39 °C	2
Conjunctival suffusion	4
Meningism	4
Myalgia	4
Conjuctival suffusion + Meningism + Myalgia	10
Jaundice	1
Albuminuria / Nitrogen retention	2
Haemoptysis/ dyspnoea	2
Part B: Epidemiological factors Score	
Rainfall	5
Contact with contaminated environment	4
Animal contact	1
Part C: Bacteriological and Laboratory Findings Score	
Isolation of leptospira in culture – Diagnosis certain	25
PCRa	
Positive serology	
ELISAIgM positive	15
SATc positive	15
Other rapid tests	15
MATe – single positive in high titer	15
MATe – Rising titer / seroconversion (paired sera)	25
A presumptive diagnosis of leptospirosis is made of:	
Part A or Part A & Part B score : 26 or more	
Part A, B, C (Total) : 25 or more	
A score between 20 and 25 suggests leptospirosis as a possible diagnosis.	

In every case, the diagnosis of leptospirosis was made by one of the following laboratory measures: lateral flow immunoassay by rapid immunochromatographic test (ICT); ELISA IgM; or a positive culture.[5]

The MAT method was performed as described by the World Health Organization.[8] Cases were defined as positive for leptospirosis if agglutination titer achieved ≥400 level to one or more Leptospiraserovars. All samples were screened by MAT to determine the serum is positive or negative for leptospirosis.[9]

Categorical data were presented as proportions. Chi-square test was used to test the difference of the proportion between the two groups. The onset of the disease was the date when the first symptom started, and the end of the analysis was the date when the patient died or was still alive at discharge. The event of interest was death from leptospirosis. Statistical analysis was performed using the Statistical Package for Social Science (SPSS) version 17 for Windows. P values below 0.05 were considered statistically significant.

3. Results

This study reviewed characteristic data like age, gender epidemiological data, length of fever that shown in table 2. Twenty-six patients were included encompassing the study. The mean age of thestudy population was 46.65 (SD=15.74), 21 patients (80.8%) were male and 5 other were female (19.2%). The mean duration of fever was 5.58 days (SD=1.39). Clinical manifestation indicated by modified Faine's score. Possible leptospirosis (score 20-24) were note at 15 (57.7%) patients and presumptive leptospirosis (score ≥ 25) were 11 (42.3%) patients. Serology marker indicated by IgM ELISA, seropositive IgM ELISA were 18 patients (69.2%), and seronegative IgM ELISA were 8 patients (30.8%). Patients that are having MAT positive were 11 patients (42.3%) and 15 patients (57.7%) having MAT negative. Prevalence of outcome patients were 15 (57.7%), and dying patients were 11 (42.3%).

Table 2. Characteristic subject study.

Variable	N (%)	Mean ± SD
Age (year)		46.65 ±15.74
Gender		
Female	5 (19.2)	
Male	21 (80.8)	
Length of fever (day)		5.58 ± 1.39
Modified Faine's Score		
≥25	11 (42.3)	
20-24	15 (57.7)	
Ig M		
Positive	8 (30.8)	
Negative	18 (69.2)	
MAT		
Positive	11 (42.3)	
Negative	15 (57.7)	
Outcome		
Death	11 (42.3)	
Outpatient	15 (57.7)	

We can see the correlation of clinical manifestation with serology marker and MAT. We used thechi-square test to know thecorrelation between it. If there is no eligible to chi-square test, we used Fisher exact test (expected value less than 5). The odd ratio used to determine the risk posed.

Table 3. The association of clinical manifestation with the outcomes.

Modified Faine's Score	Outcome (%)		OR (95%CI)	P
	Death	Outpatient		
≥ 25	5 (45.5%)	6 (40.0%)	1.250 (0.259-6.029)	1.000
20–24	6 (54.5%)	9 (60.0%)		

Patients with modified Faine's score ≥ 25 tend to die (45.5 %) while modified Faine's score 20 – 24 tend to heal (60%) (Table 3). Odd ratio 1.250 (CI 0.259-6.029) show that modified Faine's score tends to be arisk factorfor mortality but not significant statistically (p=1.00).

Table 4. The association of serology marker with the outcomes.

Ig M	Outcome (%)		OR (95%CI)	P
	Death	Outpatient		
Positive	6 (54.5%)	2 (13.3%)	7.800 (1.162-52.353)	0.038
Negative	5 (45.5%)	13 (86.7%)		

Patients with seropositive IgM ELISA tend to die 54.5% while patients with seronegative IgM ELISA tend to heal 86.7% (Table 4). Odd ratio 7.800 (CI 1.162-52.353) show that seropositive IgM ELISA predicts mortality 7.8 times higher than seronegative IgM ELISA (p=0.038).

Table 5. The association of MAT with the outcomes.

MAT	Outcome		OR (95%CI)	P
	Die	Outpatient		
Positive	8 (72.7%)	3 (20.0%)	10.667 (1.705-66.720)	0.015
Negative	3 (27.3%)	12 (80.0%)		

Patients with MAT positive tend to die 72.7% while MAT negative tend to heal 80.0% (Table 5). Odd ratio 10.667 (CI 1.705-66.720) show that MAT positive predict mortality 10.667 times higher than MAT negative (p=0.015).

4. Discussion

The findings of this study give insight into association leptospirosis with mortality, indicated by clinical manifestation, serology marker, and MAT. To diagnose leptospirosis, we need to confirm with clinical manifestation and laboratory finding. The laboratory confirmation of leptospirosis is based on MAT, isolation of the organism or leptospiral DNA by finding in PCR examination.[4] Faine's criteria essentially use clinical,epidemiological and microbiological features to score the likelihood of leptospirosis. These criteria,with modifications, have been evaluated in various studies, giving varying degrees ofspecificity and sensitivity.[3]

Characteristic data in table 1 shows theproportion of subject study. Majority patient with Modified Faine's Score possible leptospirosis, IgM negative and MAT negative. Also, majority outcome from this study is patient get improve from leptospirosis. This, made us want to study about possible leptospirosis, IgM negative and MAT negative can make patient getting well. Then, this makesa presume that severe clinical leptospirosis, IgM positive and MAT positive can predict mortality risk.

This current study showsthat severe clinical manifestation tends to worsen leptospirosis and lead to mortality. Yet, it is statistically insignificant. Possibly related toa number of sample study, improvement of clinical manifestation when study start, also there is overlap with another disease.

Seropositive leptospirosis has a tendency to be severe manifestation and risk of mortality. Patient leptospirosis with IgM ELISA positive has arisk of mortality 7.8 times higher than other. MAT with

therisk of mortality also has asignificant correlation. Patient leptospirosis with MAT positive has arisk of mortality 10,667 times higher than other.

The total time duration under investigation was low. Furthermore, prospective data are requiredto make stronger recommendations. Studies including alarger number of participants with leptospirosis are needed to permit valid estimates of the association between clinical manifestation; Immunoglobulin M Enzyme-Linked Immunosorbent Assay (ELISA) and MAT with mortality, also anassociation of each variable.

5. Conclusion

Clinical manifestation, MAT,and serologic marker are all correlated with mortality in Leptospirosis. However,statistically, clinical manifestation has aninsignificant correlation.

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