Leptospirosis in Northeastern Malaysia: Misdiagnosed or Coinfection?

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Abstract

Background: For the past decade leptospirosis has been re-emerging as an important disease and can be a serious public health issue in a humid tropical and subtropical country such as Malaysia. Leptospirosis also known as "the Great Mimicker" and may be overlooked and under diagnosed due to its varied clinical presentations.
Objective: Since leptospirosis is a significant cause of undifferentiated fever and frequently not recognized, this study was conducted to determine the prevalence of this disease among febrile patients in northeastern Malaysia hospitals.

Design: A hospital-based cross sectional study was conducted among 999 of febrile patients admitted in 10 hospitals in northeastern Malaysia.

Materials and methods: An interviewer-guided proforma sheet on sociodemographic and final hospital diagnosis data was distributed to all adult patients with fever on admission. Serum sample for leptospirosis was screened by IgM Enzyme-linked Immunosorbent Assay test (IgM ELISA) and confirmed by Microscopic Agglutination Test (MAT). The cut-off point for positive MAT was ≥1:400 titre in single acute specimen.

Results: The seroprevalence of leptospirosis was 8.4% (95% CI: 6.8, 10.3) (n=84) by MAT. In our study, only 31.0% of the confirmed leptospirosis cases by MAT in northeastern Malaysia hospitals were diagnosed as leptospirosis for the final diagnosis on discharge. About 38.1%, 14.3% and 7.1% of the confirmed leptospirosis cases by MAT were diagnosed as dengue fever or dengue hemorrhagic fever, pneumonia and typhoid fever respectively.

Conclusion: This study showed that the prevalence of leptospirosis is probably high among febrile patients in northeastern Malaysia hospitals. Awareness and knowledge regarding this disease should be strengthened, especially among public and health care personnel due to the clinical symptoms of leptospirosis mimicking other tropical diseases.

Keywords: leptospirosis, IgM Enzyme-linked Immunosorbent Assay, Microscopic Agglutination Test

Introduction

Leptospirosis occurs worldwide and can be a serious public health issue in a humid tropical and subtropical country such as Malaysia. Leptospira are spirochaetes, Gram-negative bacteria which is a causal agent for this disease.

Leptospirosis may present with a wide variety of clinical manifestations. These may range from a mild "flu"-like illness to a serious and sometimes fatal disease. It may also mimic many other diseases, e.g. dengue fever and other viral hemorrhagic diseases [1, 2].

In the absence of clear characteristic clinical features, especially in mild leptospirosis, laboratory diagnosis is essential. The microscopic agglutination test (MAT) is the gold standard test but requires significant expertise to maintain and interpret. IgM detection using ELISA is more widely used in diagnostic laboratories [1, 3].

Leptospirosis also known as "the Great Mimicker" and may be overlooked and under diagnosed due to its varied clinical presentations. The clinical features in early leptospirosis of either the mild or the severe type are not pathognomonic and give no indication of the diagnosis to an inexperienced clinician [3]. Misdiagnosis has become a critical issue in Malaysia where dengue, malaria and other infectious diseases with overlapping clinical presentations are endemic.

Victoriano *et al.* (2009) stated that countries such as Bangladesh, India, Laos, Sri Lanka, and Thailand have high incidence of leptospirosis, with annual incidence more than ten per 100,000 population. China, Indonesia, New Zealand and Malaysia as moderate, with annual incidence one to ten per 100,000 population. Australia, Hong Kong, Japan, Taiwan and South Korea have low incidence of leptospirosis with annual incidence less than one per 100,000 population [4].

The overall disease burden is underestimated, since leptospirosis is a significant cause of undifferentiated fever and frequently not recognized. Barriers to addressing this problem have been the lack of an adequate diagnostic test and effective control measures. The major burden of leptospirosis is due to severe disease forms, Weil's disease and Severe Pulmonary Hemorrhagic Syndrome (SPHS) [5].

Leptospirosis has overlapping clinical features with other febrile illnesses, which lead the diagnosis of leptospirosis is often overlooked and resulting in delay in treatment. This study was conducted to determine the prevalence of this disease among febrile patients in northeastern Malaysia hospitals.

From literature search, most of the studies on leptospirosis were conducted among febrile patient or pyrexia of unknown origin (PUO) patient with other associated sign and symptoms such as jaundice; and patient with obvious foci of infection such as dengue and malaria were excluded from the study. In addition, most of the studies on leptospirosis were done among specific high risk occupation such as military or agriculture sector worker.
To the best of our knowledge, this was the first study on leptospirosis among inpatient febrile cases, with no added sign and symptoms for inclusion criteria of recruitment; and involving a large number of respondents in our setting. From the records of hospitals in northeastern Malaysia, fever cases contributed about ten to twelve percent of the cases for hospital admission. This criteria was selected in order to optimize the case detection and to make an earlier diagnosis for leptospirosis among febrile inpatient cases.

Materials and Methods

This was a hospital-based cross sectional study done among febrile cases in northeastern Malaysia. The study was conducted in 10 health facilities, namely Hospital Universiti Sains Malaysia (HUSM), Hospital Raja Perempuan Zainab 2 (HRPZ II), Hospital Pasir Puteh, Hospital Machang, Hospital Tumpat, Hospital Pasir Mas, Hospital Tanah Merah, Hospital Jeli, Hospital Kuala Krai and Hospital Gua Musang. A proportionate sampling method based on the proportion of number of beds in each study hospital was used and a total of 1200 respondents were estimated.

The duration undertaken to complete data collection was 7 months, from August 2010 till February 2011.

All adults (18 years old and above) with fever and admitted to medical ward, were considered for entry into the study. Type of fever cases that could be included as a respondent in this study was based on differential diagnosis of leptospirosis by WHO (2003). HIV patient, patient on immunosuppressive drugs and patient with autoimmune disease were excluded from this study. After providing written informed consent, the patients were interviewed and the data were recorded on patient proforma sheet which consists of sociodemographic data and hospital diagnosis on discharge.

Single venous samples of 5-8 ml were collected from subjects. The samples were centrifuged and stored at -20^{o} Celsius. The PanBio Leptospira IgM ELISA test (PanBio, Queensland, Australia) was used for the qualitative detection of IgM antibodies to leptospira. All serology samples were analyzed in Microbiology Laboratory in Universiti Sains Malaysia. One operator trained laboratory technician performed the ELISA test to avoid bias. A score of less than 9 units indicates a negative result or no detectable IgM antibody, 9-11 units as indeterminate and more than 11 units as positive result, indicating the presence of leptospira-specific IgM antibodies.

The Microscopic Agglutination test (MAT) was performed to determine the presence of leptospira antibodies in the positive and indeterminate samples of ELISA test. The frozen sera were sent to the Institute of Medical Research (IMR) for the MAT. A panel battery of 18 live

reference serovars, representing 18 serogroups were used as antigen, as recommended by WHO. In this study, there were three other local pathogenic serovars were included in the panel, but the serovars could not be mentioned due to it is still under study.

A laboratory confirmed case of leptospirosis was defined as the presence of the titre of ≥1:400 in a single sample. This cut-off MAT titre also was used in several studies on leptospirosis [6-12].

Informed consent was obtained from all subjects based on approved study protocol by the Research Ethics Committee (Human), Universiti Sains Malaysia (Ref:USMKK/PPP/JEPeM[219.3.(03)]) on 3rd of December 2009 and National Medical Research Registry, National Institute of Health (NMRR-10-318-5389).

Data were entered and analyzed using PASW Statistics 18. All continuous variables were described using mean (SD) and for categorical data as frequency (%). Seroprevalence was computed using positive MAT over total samples and presented as percentage with 95% confidence interval.

Results

A total of 1200 patients were eligible for the study after considering the inclusion and exclusion criteria. However, only 999 respondents were recruited to participate in this study. A total of 88 of IgM ELISA test were positive and 23 of IgM ELISA test were indeterminate among respondents. Only 84 result confirmed positive by MAT. The seroprevalence of leptospirosis was 8.4% (95% CI: 6.8, 10.3) (n=84) by MAT.

Majority of respondents were Malay and male respondents contributed about 54.4% of the total number of respondent. The mean (SD) age was 39.4 (17.6) years. Table 1 shows the sociodemographic characteristics of respondents.

Table 1: Sociodemographic characteristics of respondents (n=999)

Variable	Frequency (%)	Mean (SD)
Age (year) [range:18-94]		39.4 (17.6)
Sex		
Female	456 (45.6)	
Male	543 (54.4)	
Race		
Non-Malay	53 (5.3)	
Malay	946 (94.7)	

SD: standard deviation

Only 31.0% of the confirmed leptospirosis cases in northeastern Malaysia hospitals were diagnosed as leptospirosis for the final diagnosis on discharge. About 38.1%, 14.3% and 7.1% of the leptospirosis cases were diagnosed as dengue fever or dengue hemorrhagic fever, pneumonia and typhoid fever respectively. Table 2 shows the diagnosis on discharge for leptospirosis case in northeastern Malaysia hospitals.

Table 2: Final hospital diagnosis on discharge of leptospirosis case confirmed by MAT (n=84)

Variable	Frequency	%
Leptospirosis	26	31.0
Influenza like illness	0	0.0
Dengue fever / Dengue hemorrhagic fever	32	38.1
Malaria	1	1.2
Acute gastroenteritis	1	1.2
Typhoid	6	7.1
Hepatitis	1	1.2
Acute renal failure	0	0.0
Pyrexia of unknown origin	0	0.0
Pharyngitis	1	1.2
Pneumonia	12	14.3
Upper respiratory tract infection	0	0.0
Viral fever	2	2.4
Other	2	2.4

Discussion

During early clinical presentation, leptospirosis is often indistinguishable from other common causes of acute febrile illnesses in the tropics such as dengue fever, malaria, typhoid and others [13, 14]. In our study, only 31% of confirmed leptospirosis case by MAT was diagnosed as leptospirosis for final hospital diagnosis on discharge. Another 38% of confirmed leptospirosis case by MAT was diagnosed as dengue fever or dengue hemorrhagic fever.

Our finding was about similar with a prospective, hospital-based, study of children with acute febrile illnesses conducted in Thailand, by Libraty *et al.* (2007). In their study, none of the clinical discharge diagnoses included leptospirosis and more than one third of the leptospirosis cases were erroneously diagnosed as scrub typhus or dengue [15].

Many other studies showed lower percentage of misdiagnosis than our study. A study by Sanders *et al.* (1999) showed there was an increase to 7% and 27% of leptospirosis cases in dengue-negative patients, during pre hurricane and post hurricane respectively in Puerto Rico in 1996 [12]. Leptospirosis has rarely been reported in Puerto Rico and in their study the respondents were enrolled via dengue laboratory-based surveillance system, within two months pre and after

Hurricane Hortense, to determine the increase in leptospirosis. These few factors contributed to the difference of the result with ours.

Comparison with a few studies done in Puerto Rico, Jamaica, Barbados, Hawaii and Bangladesh showed the seroprevalence among dengue-negative sera range from 4.5% to 18 % was positive for Leptospira IgM antibodies respectively [14, 16-19].

According to Bruce (1995), post mortem testing for leptospirosis on 12 available specimens from suspected dengue-related fatalities; 10 (83%) tested positive for leptospirosis [16]. It showed that overlapping a clinical feature with other febrile illnesses made the diagnosis of leptospirosis is often overlooked, resulting in delay in treatment and increased mortality.

A study by Perani *et al.* (1998) in Hawaii found the prevalence of leptospiral infection among patient with community acquired pneumonia was 5.7%, which was lower than ours [20]. These might be due to different study population, which the respondent in his study was enrolled among inpatient and outpatient units, whose was diagnosed as community acquired pneumonia and not respond to the antibiotics.

Even pulmonary involvement with cough, hemoptysis, respiratory failure and pulmonary hemorrhage had been described in recent studies [21-23]. A study in Turkey by Erdinc *et al.* revealed there was a case of leptospirosis which was admitted to the hospital with clinical presentation of pneumonia [24]. It was supported by Thorsteinsson *et al.* [25].

Only one leptospirosis case was diagnosed as malaria in our study. Malaria and leptospirosis are both common in the tropics. Co-infections are expected to be common. However such a scenario has seldom been reported [26-28]. It most probably because clinical features only are difficult to separate single infections from dual infections, and diagnostic test for leptospirosis are usually unavailable in many settings. It is common practice in a malaria-endemic area such as in our study setting, that if an acutely febrile patient is found to be malaria-positive, malaria is naturally assumed as the sole cause of the fever.

To date, only a few cases of co-infection with dengue and leptospirosis have been described [9, 18, 29]. The possibility of mixed infection more than these two common diseases also had been described because water is the vehicle of transmission for both hepatitis E virus and leptospiral. In addition, dengue outbreak more common to occur after rain fall [30, 31].
Leptospirosis and other tropical diseases were very difficult to distinguish and commonly confused especially at the critical early stages of illness. Additionally, our study was done in northeastern of Malaysia, which is one of the tropical countries and the dengue outbreak was common. A lack of affordable and accurate diagnostic tests in many settings also contributes to the diagnostic confusion. Knowledge of this infection's protean manifestations and high index of suspicion enable the proper diagnosis to be made.

In our study, possibility of mixed infection with dengue, malaria, hepatitis or typhoid and leptospirosis could not be estimated because the final diagnosis of the respondent was based on the hospital diagnosis on discharge only, which most of the confirmation result of the diagnosis was unavailable during discharge of the patients, due to most of the diagnostic laboratory

investigations were done only in two tertiary hospitals in northeastern Malaysia. Besides, analysis of mixed infection was not our study objective.

In northeastern Malaysia, the differential diagnosis of leptospirosis includes dengue fever, typhoid, and malaria. Because the specific treatment of these infections differs, rapid and accurate diagnosis is of clinical significance. Health care provider should be prepared to initiate appropriate antibiotic therapy when indicated.

Conclusion

About 38% of the leptospirosis cases were misdiagnosed as dengue fever or hemorrhagic fever due to the clinical symptoms mimicking other tropical diseases in northeastern Malaysia. Awareness and knowledge regarding this disease should be strengthened, especially among health care personnel and diagnostic laboratory test for leptospirosis should be available, to initiate prompt treatment because the management of these diseases are different.

Because of leptospirosis has protean and nonspecific clinical manifestations, it remains difficult to diagnose without serologic test. Further studies are needed to evaluate the current knowledge and sensitivity of clinically based diagnosis before serological results are known, which reflect the level of awareness regarding this disease among medical personnel. Besides, the availability of confirmation result of diagnosis on hospital discharge could help to identify any co-infection or prevalence of leptospirosis among dengue-negative sera in our setting. In addition, other unit such as surgery, pediatrics, intensive care unit and outpatient department should be included in further studies to reflect severity of the disease. In our study, we might underestimate the seroprevalence of leptospirosis due to only single sera were taken from the respondent. It was difficult to take the convalescent sera due to large geographical coverage in our study area.

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Conflict of interest statement: We declare that we have no conflict of interest.

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