Malaysian Journal of Medicine and Health Sciences (eISSN 2636-9346)

CASE REPORT

Detection of Leptospiral DNA in Urine Sample Following Prolonged Hospitalization: A Case Report

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ABSTRACT

We described a case of positive molecular detection of leptospirosis in urine sample following prolonged hospitalization. Relevant clinical history had raised suspicion to leptospirosis infection. A significantly high level of creatinine kinase suggested possibility of rhabdomyolysis. Blood and urine samples collected on 4th day of admission were negative for leptospires culture and serological method showed no significant evidences of positive infection. Molecular detection of *Leptospira spp*. in blood sample was positive but not in urine sample. After seven weeks of infection, leptospiral DNA was detected in urine sample using molecular method.

Keywords: Leptospirosis, Detection, Molecular, MAT, Urine

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INTRODUCTION

Leptospirosis is an infectious zoonotic disease that can affect both humans and animals worldwide. Clinical manifestations of leptospirosis ranging from mild, flu-like illness to severe disease characterized by multiorgan system complication finally leading to death (1). Leptospires enter the host organisms via penetration into abraded skin, breaches of the surface integument, mucous membrane and genital track, after which they enter the blood circulation and disseminate to various tissues and organs (1). Although the pathogenesis of the leptospires that causing muscular damages leading to rhabdomyolysis is not fully understood, several virulence determinants like motility and chemotaxis, lipopolysaccharide (LPS), surface protein and secretory proteins have been characterized (2). Leptospires can be observed during early infection in the blood and cerebrospinal fluid (CSF) of the patients. This was followed by excretion of the leptospires through urine starting on the second week of infections. Cases of leptospirosis were often diagnosed with serological methods rather than culture or molecular methods. This has contributed to poor documentation of prolonged urinary shedding of leptospires in humans (3). We present a case of positive leptospires detection through molecular method in urine sample collected approximately seven weeks after infection.

CASE REPORT

A 30 years old Chinese man came to the emergency department of Hospital Serdang, Selangor after two days of fever. He had persistent vomiting, chills, rigors and felt lethargic. The patient had no comorbidity history. Upon physical examination, his vital signs were unremarkable and the preliminary diagnosis was possible dengue fever. Further history revealed that he had recreational activities at Port Dickson beach with another nine individuals. However, none of the rest presented with similar symptoms.

Early blood investigation upon admission to the ward showed leucocytosis with white blood cells count at 17.6 x 10^9/L, low platelet count (104 x 109/L) while prothrombin time and red blood cell count were normal. Liver function test and renal profile were within normal range. Creatinine kinase level was high (2548 U/L) suggesting heart related disease or the possibility of rhabdomyolysis. Blood aerobic test using BacT/ALERT FAN Aerobic Medium was positive for methicillin resistant Staphylococcus aureus (MRSA) and sensitive to vancomycin at concentration of 2.0 µg/ml via E-test. Investigations for dengue, malaria parasite, Treponema pallidum, Hepatitis B, Hepatitis C and HIV were non-reactive. This patient was on doxycycline upon admission and the patient complained of having fever with headache, arthralgia and myalgia on the next day. He also had persistent vomiting and diarrhoea for two days and dysuria. Episodes of diarrhoea continued, but no vomiting. On the 4th day of hospitalization, temperature spike was observed and history of

recreational activity with fever and leucocytosis increase the suspicion towards leptospirosis infection. This patient was diagnosed as acute leptospirosis with rhabdomyolysis. Further blood and urine samples were taken for leptospirosis investigation. Antibiotic treatment was switched to intravenous rocephine. The symptoms of diarrhoea and vomiting resolved on the 5th day and the fever subsided. Detection of IgM antibody performed using ImmuneMed Leptospira IgM Duo Rapid showed intermediate result using the serum sample collected 4th day of admission while microscopic agglutination test (MAT) was found to be negative. Subsequent clinical examination revealed murmur and further investigation showed vegetation sized 0.5 cm was seen at accessory mitral valve leaflet (AMVL). He was then referred to the cardiologist for further management. This patient was hospitalized for 54 days to manage all the complications.

LABORATORY RESULTS

This patient was recruited as one of the study subjects under a research project conducted by MyLepto research group with ethical approval (NMRR-15-2148-27536). Blood and urine samples were collected from this patient on the 4th day of admission and prior to discharge. *Leptospira* culturing in EMJH and rapid test with Leptocheck®-WB and enzyme-linked immunosorbent assay (ELISA) were performed. All the tests were negative. MAT was performed on samples collected at three stages; admission (4th day), during the middle of hospitalisation period (31st day) and prior to discharge (54th day). The result was inconclusive where the titre was 1:50 for *L. interrogans* serovar Sarawak for admission sample, 1: 100 and 1:50 for *L. biflexa* serovar Patoc for middle stage and discharge samples, respectively.

Real-time polymerase chain reaction (qPCR) targeting *lipL32* gene, conventional PCR and loop-mediated isothermal amplification (LAMP) targeting *secY* gene were positive for blood sample but not for urine sample collected during 4th day of admission. The discharge samples taken 50 days later were positive by both conventional PCR and LAMP targeting *secY* gene for urine sample but not for the blood sample. The urine test was negative for *lipL32* gene. Summary of the results were tabulated in Table I. The identity of the *secY* PCR product was further confirmed by sequencing.

Table I: Test results for leptospirosis diagnosis

		Admission samples	Discharge samples
Leptocheck WB		Negative	-
ELISA		Negative	-
MAT		Inconclusive for *L. interrogans* serovar Serawak (1:50)	Inconclusive for *L. biflexa* serovar Patoc (1:50)
Culture	Blood	Negative	Negative
	Urine	Negative	Negative
qPCR (*lipL32*)	Blood	Positive	Negative
	Urine	Negative	Negative
PCR (*secY*)	Blood	Positive	Negative
	Urine	Negative	Positive
LAMP (*secY*)	Blood	Positive	Negative
	Urine	Negative	Positive

(-) indicate the test was not performed

DISCUSSION

Leptospirosis is a disease caused by pathogenic Leptospira. Although this disease is often related to non-specific clinical symptoms, risk factors and history of exposure could guide clinicians to initiate the therapy. Preliminary diagnosis of dengue is common due to its high endemicity and exclusion of the disease usually supported by negative serology result of dengue NS1 and IgM as in this case. Recent recreational activities were relevant determinant of leptospirosis as differential diagnosis. High level of creatinine kinase was most likely due to his heart condition but indicating possibility of rhabdomyolysis.

Laboratory tests for leptospirosis comprised of serological test for detection of IgM antibody using both rapid test (Leptocheck WB) and ELISA method. Early serum collection (4th day of admission) resulted in false negative as in this case because leptospires antibodies level is generally detected after 7 days of illness. Apart from that, MAT showed inconclusive result against *L. interrogans* serovar Sarawak with low antibody titre during 4th day of admission. The titre increased in the middle stage and reduced again during discharge with dissimilar detection of serovar which was *L. biflexa* serovar Patoc. This indicated that cross reactivity between the serovar occurred which was less specific.

It was found that none of the samples from this patient positive by culture method. Low sensitivity of culture method in diagnosis of leptospirosis has been reported due to fastidious characteristic and low level of leptospires in clinical specimens (4). However, it is apparent that the main reason for negative culture in blood specimen for this patient was due to the prior administration of doxycycline on the day of admission. Negative culture on urine sample might be due to the presence of insufficient viable leptospires. The leptospires usually present in urine on second week after the onset of clinical presentations (1,2).

Three different molecular detection methods targeting two Leptospira genes, *lipL32* and *secY* were utilised in this study to increase the positive detection rate for leptospiral DNA in the samples. The results obtained

showed that the presence of leptospiral DNA was detected from blood sample collected on 4th day of admission, indicating molecular techniques are generally more sensitive and suitable compared to serology and culturing for early diagnosis. Positive detection of leptospiral DNA in discharge urine sample after prolonged illness was possible although it is not common. Al *et al* (1994) reported detection of leptospiral DNA in the urine sample of asymptomatic individual after one year of infection and concluded that this could be due to past infection or recent asymptomatic reinfection (5). Precautions in relation to this issue should be taken seriously as this could contribute to the transmission of the disease due to the behavioural act of humans as carriers. This includes poor sanitation practice and urinating at public places such as in the river during recreational activity which may eventually leads to transmission of leptospirosis through rodents or possible of human-to-human transmission.

CONCLUSION

Detection of Leptospira using molecular method showed a promising result compare to serology and culture. We showed direct relationship of leptospirosis and the presence of leptospiral DNA in urine after 54 days of infection. This could become the evidence that humans may be the potential carrier of this pathogen and extra precaution measures should be taken to control the infection of the disease.

ACKNOWLEDGMENT

We would like to thank the Director General of Health Malaysia for his permission to publish this article and Ministry of Higher Education Malaysia for this research funding through the Long-Term Research Grant Scheme (UPM/700-2/1/LRGS/5526400).

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