

RISK AND PROGNOSTIC FACTORS FOR SEVERE
LEPTOSPIROSIS IN ADULT POPULATION IN INTENSIVE
CARE UNIT HOSPITAL RAJA PEREMPUAN ZAINAB II,
KOTA BHARU

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TABLE OF CONTENT

1. INTRODUCTION.....	1
2. LITERATURE REVIEW	2
2.1 MORPHOLOGY & TOXONOMIC CLASSIFICATION.....	2
2.2 EPIDEMIOLOGY.....	3
2.3 LEPTOSPIROSIS IN MALAYSIA	6
2.4 PATHOGENESIS	7
2.5 CLINICAL MANIFESTATION	9
2.5.1 ANICTERIC LEPTOSPIROSIS	10
2.5.2 ICTERIC LEPTOSPIROSIS	11
2.6. LABORATORY AND RADIOLOGICAL FINDING	12
2.7 DIAGNOSIS OF LEPTOSPIROSIS	13
2.7.1 WHO DIAGNOSIS	15
2.7.2 MALAYSIAN GUIDELINESS 2011.....	17
2.8 MORTALITY IN LEPTOSPIROSIS	18
2.8.1 PATIENTS CHARACTERISTIC	18
2.8.2 COMPLICATION	
2.8.2.1 PULMONARY COMPLICATION	19
2.8.2.2 RENAL INVOLVEMENT	20
2.8.2.3 WEIL'S DISEASE	21
2.9 NATURAL DISASTER AND OUTBREAK OF LEPTOSPIROSIS	22
2.10 RATIONALE OF THE STUDY.....	24
2.11 STUDY OBJECTIVE	25
2.12 REFERENCES FOR INTRODUCTION AND LITERATURE REVIEW.....	26
3.0 STUDY PROTOCOL	32
4.0 BODY [MANUSCRIPT READY FOR SUBMISSION] CONTENT	50
4.1 TITLE PAGE	
4.1.1 ARTICLE TITLE	50
4.1.2 RUNNING HEAD	50
4.1.3 AUTHORS' NAMES AND INSTITUTIONAL AFFILIATIONS	50
4.1.4 CORRESPONDING AUTHOR'S DETAILS	50
4.2 MAIN DOCUMENT	51
4.2.1.2 ABSTRACT (ENGLISH)	51

4.2.2	INTRODUCTION	53
4.2.3	METHODOLOGY	54
4.2.4	RESULT	56
4.2.5	DISCUSSION	58
4.2.6	LIMITATION OF THE STUDY	67
4.2.7	CONCLUSION	67
4.2.8	REFERENCE	68
4.2.9	TABLES FOR MANUSCRIPT	72
5.0	GUIDELINES FOR AUTHORS; JOURNAL FORMAT (MJMS)	80
6.0	DOCUMENT SUBMITTED FOR ETHICAL APPROVAL	98
7.0	APPENDICES	
7.1	DATA COLLECTION SHEET	99
7.2	ADDITIONAL TABLE	105

ABSTRAK (BM)

Latar Belakang:

Penyakit kencing tikus mempunyai julat presentasi yang luas daripada tahap sederhana ke tahap yang kritikal serta melibatkan kegagalan organ dan kematian. Terdapat banyak factor risiko dan factor prognosis bagi keterukan dan kematian seperti demografi, epidemiologi, presentasi klinikal dan keputusan makmal. Pengenalpastian factor-faktor ini membolehkan sokongan dan rawatan rapi (ICU) diberikan awal.

Tujuan:

Kajian ini dijalankan untuk mengenal pasti risiko dan factor prognosis untuk penyakit kencing tikus dan kematian.

Kaedah:

Kajian ini merupakan kajian kawalan kes ICU secara retrospektif yang dijalankan di unit rawatan rapi (ICU), Hospital Raja Perempuan Zainab II bermula 1 Januari 2013 sehingga 31 Disember 2016. Pesakit yang datang dengan kegagalan fungsi organ serta memerlukan rawatan dialysis, kemasukan tiub bantuan pernafasan dan bantuan pernafasan secara mekanikal, bantuan ubat untuk meningkatkan tekanan darah dan menguatkan jantung, transfusi komponen darah atau kematian dimasukkan ke dalam kes (case) kajian ini. Kes kawalan (control) pula diambil daripada pesakit yang mempunyai kegagalan organ yang minima tanpa bantuan sokongan organ atau dengan bantuan sokongan organ yang minima. Model kajian Chi-square, Fisher's exact test, Student T' test, Mann Whitney (yang mana sesuai) digunakan untuk menganalisa data.

Keputusan:

96 pesakit kencing tikus telah dimasukkan ke dalam kajian ini. Daripada jumlah tersebut, seramai 66 pesakit memenuhi kriteria bagi kes (case) kajian ini. 30 pesakit dalam kumpulan kawalan. Tujuh faktor risiko yang independen telah didapati signifikan iaitu nilai bacaan Aspartate Transferase (AST) (OR: 4.2 [1.608, 10.970], P=0.002); Alanine Transferase (ALT) (OR: 2.857 [1.153, 7.082], P=0.021); Urea (OR:2.895 [1.081,7.753], P=0.031); Prothrombin time (PT) (OR:4.797 [1.629,14.126], P=0.003); International Normalised Ratio (INR) (OR: 3.714[1.157, 11.920], P=0.021); Ratio (OR: 8.399); Creatine kinase MB (CKMB) (OR:7.0 [1.961,24.985], P=0.001). Enam factor risiko yang signifikan dengan kematian pesakit di dalam ICU ialah skor Simplified Acute Physiology Score II (SAPS II) (OR: 1.045 [1.007-1.083], P=0.019); PT (1.069 [0.00--], P=0.038); INR (OR: 4.48 [1.524-13.17], P=0.005), Activated partial thromboplastin time (APTT) (OR:2.933 [0.993-8.66], P=0.048), ratio (OR:21.87[2.52-189.86], P=0.001) dan elektrokardiogram (ECG) (OR1.13 [0.00--], P=0.048)

Kesimpulan:

Pesakit penyakit kencing tikus yang mempunyai kenaikan dalam enzim hati, uria, CKMB dan masalah pembekuan darah adalah berisiko tinggi untuk menghadapi penyakit kencing tikus yang parah. SAPS, masalah pembekuan darah dan perubahan dalam elektrokardiografi merupakan risiko-risiko untuk kadar mortality yang tinggi.

ABSTRACT (ENGLISH)

Background:

Leptospirosis has a wide range of clinical presentation from mild to severe disease with organ dysfunctions and death. There are risk and prognostic factors for severity and mortality including demographic, epidemiological, clinical presentations and laboratory results. Early recognition of risk factors enables early ICU care and organ supports.

Purpose:

The study was conducted to identify the risk and prognostic factors for severe leptospirosis and its mortality.

Methods:

This was a retrospective case-control study carried out in the general ICU in Hospital Raja Perempuan Zainab II from 1st January 2013 to 31st December 2016. Patients who presented with severe organ involvement which required dialysis, tracheal intubation and mechanical ventilation, vasopressors or inotropes, transfusion or death were grouped as cases. Controls were defined as patient with mild organ involvement without requirement or with minimal organ supports. Chi-Square test, Fisher's exact test, Student t-test or Mann Whitney test was used. A logistic regression model was used to select final prognostic factors.

Results:

Ninety six leptospirosis patients were included in the study. Among 96 patients enrolled in the study, 66 patients were in the severe group, 30 patients were in the control group. Seven risk factors independently associated with severe leptospirosis: Aspartate Transferase (AST) (OR: 4.2 [1.608, 10.970], P=0.002); Alanine Transferase (ALT) (OR:

2.857 [1.153, 7.082], P=0.021); Urea (OR:2.895 [1.081,7.753], P=0.031); Prothrombin time (PT) (OR:4.797 [1.629,14.126], P=0.003); International Normalised Ratio (INR) (OR: 3.714[1.157, 11.920], P=0.021); Ratio (OR: 8.399); Creatine kinase MB (CKMB) (OR:7.0 [1.961,24.985], P=0.001). Six independent risk factors were associated with mortality: Simplified Acute Physiology Score II (SAPS II) score (OR: 1.045 [1.007-1.083], P=0.019); PT (1.069 [0.00--], P=0.038); INR (OR: 4.48 [1.524-13.17], P=0.005), Activated partial thromboplastin time (APTT) (OR:2.933 [0.993-8.66], P=0.048), ratio (OR:21.87[2.52-189.86], P=0.001), Electrocardiography (ECG) (OR1.13 [0.00--], P=0.048)

Conclusion:

Leptospirosis patients with elevated liver enzymes, elevated urea, coagulopathy and elevated CKMB were risky to develop severe leptospirosis. SAPS II, coagulopathy and ECG changes were high risk for mortality.

(252 words)

Keywords: severe leptospirosis, risk and prognostic factors, intensive care unit, mortality

1. INTRODUCTION

Leptospirosis is an acute bacterial infection. The incidence of the disease varies from sporadic cases in temperate climates to endemic cases in tropical countries. It can lead to epidemics during climate changes for example monsoon seasons and floods which cause an increase in contaminated soil or surface water(1).

Malaysia is a tropic country and usually receives heavy rainfall during the monsoon period. In the late December 2014, during the northeast monsoon season, Kelantan (and other northern states in peninsular Malaysia) were struck by torrential rains. This led to rapid raise in the water level of tributaries of Kelantan River. With the intense and prolonged rainfall, the massive water volume surged into the main Kelantan River. Incessant rain wrecked further havoc when the Kelantan River overflowed its banks and submerged almost all the state's districts. Malaysian then witness to a devastating natural phenomenon in 2014- a massive flood known as "Bah Kuning" or the Yellow Deluge that inundated much of Kelantan in late December 2014.

Hospital Raja Perempuan Zainab II, a tertiary hospital in Kota Bharu was not spared from the predicament. As flood water rapidly rose and submerged parts of the hospital compound, all services and medical treatment inside the hospital was suspended. The flood water also brought icu services to a halt where the ICU was forced to shut down from 25 December 2014 until 07 January 2015. The icu patients were then evacuated and transferred to HUSM. Since the re-opening of the ICU, cases of leptospirosis were referred from various district hospitals and patients who warranted intensive care were admitted to ICU. Spectrum of presentation of leptospirosis is protean and varies from mild form to a severe one involving multiorgan system where patients eventually succumbed. Therefore, this study was

developed to answer the research question: what is/are the risk factor(s) associated with severe leptospirosis in ICU?

The expected outcome of the study could determine the risk and prognostic factors for severe leptospirosis. At the same time, it can also serve as a guideline intended to assist health care practitioners at primary and secondary health facilities in early recognition, prompt management and prevention of leptospirosis complications with early referral to a tertiary centre with ICU settings so that intensive care can be commenced as early as possible.

2. LITERATURE REVIEW

Leptospirosis is a zoonotic disease, caused by infection with *leptospira interrogans*. This disease was first recognised as an occupational disease of sewer workers in 1886. It was then described by Adolf Weil in 4 men with manifestations of severe jaundice, haemorrhage with renal involvement. The disease was named after him (Weil's disease) for severe form of leptospirosis (2). It was only later in year 1917, where the role of rats as the source of human infection was first discovered (3). The subsequent studies have shown that other wild mammals can be potential carriers as well, such as dogs, swines, cattle and flying foxes.

2.1 Morphology and taxonomic classification

Leptospire are spirochaetes belonging to the order Spirochaetales, a member of the family Leptospiraceae and subdivided into two genera, *Leptospira* and *Leptonema* (4). The genus *Leptospira* is divided into two species based on serological classification: a. *Leptospira interrogans*, which comprises all the pathogenic strains and b. *Leptospira biflexa*, the environmental saprophytic strains. *Leptospira* can be further divided into 250 serovars

based on microscopic agglutination test (MAT). Serovars that have antigenic similarity are classified into serogroups (5). Identification and classification of the species of *Leptospira* is vital due to host specialities. The most frequent hosts are rodents, rats (e.g *Rattus norvegicus*, *Rattus rattus*), others such as livestock, cattle, dogs and cats. Particular serovars are associated with characteristic animal hosts; *L. icterohemorrhagiae/copenhageni* is the classical parasite of rats, *L. pomona* of pigs, *L. hardjo* of cattle and *L. canicola* of dogs. These bacteria can persist in the convoluted tubules of kidneys without causing any signs nor symptoms; they multiply and are shed into the environment via urine.

2.2 Epidemiology

The main sources of infection are urine of infected or carrier mammals, contaminated surface water, mud or soil. Human leptospiral infections result primarily from either direct or indirect contact with the urine of infected or carrier animals. The incidence of leptospirosis is significantly higher in warm climate countries than in temperate regions (6). This is because leptospires have a longer survival period in warmer humid environment. Tropical countries are also mostly developing countries, therefore, there are greater opportunities for exposure of human population to infected animals. In temperate countries, leptospirosis is a seasonal disease with peak incidence during summer and fall.

Leptospires can live in untreated water for months, even up to years. It is endemic in tropical countries, areas of heavy rainfall, or areas with high level of subsurface water. This disease occurs sporadically throughout the year. However, it may cause epidemics during seasonal incidences such as monsoons, flooding and unusual heavy rainfall.

Leptospire usually enter the body via abrasions or cuts in the skin or via the conjunctiva. It may penetrate the intact skin after prolonged immersion in water. Other modes of transmission such as ingesting contaminated food and water are also possible. Infection may also occur via inhalation of infected water through respiratory tract. Transmission via animal bites is rare. Direct human-human transmission is almost impossible due to the fact that humans are the end hosts of the infection.

Animals and humans can be divided into maintenance and accidental (incidental) hosts for the disease. A maintenance host is defined as a species in which infection is endemic and is usually from animal to animal via direct contact. The disease is maintained by chronic infection of the renal tubules of the maintenance hosts. The spirochetes then are excreted via the urine into the environment. Humans may become infected by indirect contact with the maintenance host via infected water and soil. The extent of the disease transmission depend on factors like climate, population density and the degree of contact between maintenance and accidental hosts.

Different species of maintenance host may harbour different leptospiral serovars; rats generally harbour serogroups icterohaemorrhagiae, dairy cattle may harbour serovars hardjo, pomona and grippotyphosa; swines may harbour pomona; tarassovi and brattislava; whereas sheep may harbour hardjo and pomona and dogs may harbour canicola. Knowledge of the prevalent serovars and their maintenance hosts is vital for the understanding the epidemiology of the disease in any region.

Humans may be infected through occupational, recreational and avocational activities. Occupation is a significant risk factor for infections either directly or indirectly. Direct contact with infected animals accounts for infections in farmers, veterinarians, meat

inspectors, and rodent control workers. Indirect contact usually occurs in sewer workers, miners, soldiers, septic tank cleaners and farmers as well.

There is a significant risk associated with recreational exposures following water sports such as swimming, canoeing and fresh water fishing. The potential for exposure of large numbers of individuals to the bacteria occurs during competitions. Outbreaks of leptospirosis among water sports enthusiasts have been reported following the athletic events such as the EcoChallenge in Sabah in year 2000 (7).

A number of sporadic cases of leptospirosis are acquired following avocational activities involving barefoot walking, gardening with bare hands, handling of rodents and contamination of drinking water.

Faine reported three epidemiological patterns of leptospirosis (8):

1. The first pattern occurs in temperate climates where human infection almost always occurs through direct contact with infected animal through farming of cattle and swine. Only a few serovars are involved. Control by immunisation of cattle and workers are potentially possible.
2. The second pattern occurs in wet tropical areas where human infection is not only by occupational exposure but also by environmental contamination especially during rainy seasons. Many serovars are involved in these areas infecting humans and animals alongside large numbers of reservoir species. Rodent control, proper drainage and sewage system, sanitation and occupational hygiene are all necessary for preventing leptospirosis. Large outbreaks of leptospirosis are likely to happen following floods, hurricanes or other natural disasters.
3. The third pattern involves rodent borne infection in urban environments where urban infrastructures have been destroyed by war or natural disasters.

2.3 LEPTOSPIROSIS IN MALAYSIA

The history of leptospirosis dates back to 1925 when the first case of leptospirosis was reported by Fletcher. In 1928, Fletcher isolated leptospira from black rats (*Rattus rattus*) (9). Subsequent investigations by Robinson and Kennedy have demonstrated a high prevalence of infection where 31 cases of leptospirosis were reported among British army personnel in Malaya (10). Later, in between 1953 to 1955, Alexander had successfully identified 30 pathogenic leptospiral serovars from both military personnel and civilians (9). These studies demonstrated a high seroprevalance in the Malayan population. The highest distribution of seroprevalance was found in labourers working in rubber estates, sewage workers and workers involved in drainage, forestry and town cleaning sectors (9).

In the 1950's and 1960's, a comprehensive study was conducted involving testing of various mammals from a range of environments and its occupational risks to humans. The study suggested that rats were the main reservoir for leptospirosis. One hundred and four strains were isolated and identified (11). The cross sectional serological survey conducted by Bahaman et al (9) showed that approximately one quarter of the domestic animals in peninsular Malaysia had agglutinating antibodies to *L. interrogans*. Cattle and buffaloes were observed to have high seroprevalence, which was approximately 14.4%.

There have been recent reports on leptospirosis in Malaysia. An outbreak of leptospirosis was reported among participants of the Eco-Challenge which took place in Sabah in year 2000 (7). This event involved water recreational activities and sports. Eighty out of 189 participants (42%) who were contacted after the event fulfilled the case definition of leptospirosis. Twenty nine cases were hospitalised. Fortunately there were no deaths. In July 2010, cases of leptospirosis were reported when eight people who took part in a search and rescue operation in Lubok Yu, Maran, Pahang died from the disease (12).

The incidence rate of leptospirosis in Malaysia is estimated to be between 2-5 per 100000 population. However, leptospirosis cases are on the rise. The Ministry of Health Malaysia reported that the number of confirmed cases of leptospirosis had increased from 263 in 2004 to 1418 in 2009. The male to female ratio is 4:1. The majority of patients are between 20-60 years old. The case mortality rate is estimated to be approximately 10%.

In Malaysia, there are 38 serovars which are divided into 13 serogroups, namely *australis*, *autumnalis*, *bataviae*, *canicola*, *elledoni*, *grippotyphosa*, *hebdomanis*, *icterohaemorrhagiae*, *javanica*, *pomona*, *pyrogenes*, *sejroe* and *tarassovi* (9).

2.4 PATHOGENESIS

The pathogenesis of leptospirosis is not completely understood. However, vasculitis is believed to be its main mechanism of organ tissue injury. There are 2 distinct phases of leptospirosis: the initial septicaemic phase followed by an immune phase. The progression of vasculitis in these two phases is different. During the septicaemic phase, spirochetes are found in the wall of medium to large vessels and in capillaries causing a direct toxic effect to the vessels. However in the second phase, the host immune response and the deposition of immune complexes are believed to be the cause of vasculitis (13).

During the first phase, the leptospire penetrates and invades the host tissues via a mechanical burrowing motion produced by its filaments with release of hyaluronidase. This leads to dissemination of the organism causing systemic illness with a broad spectrum of clinical presentation. It also causes generalised petechiae, ecchymosis and even massive bleeding in internal organs like pulmonary haemorrhage as an example. Main organs commonly affected are the kidneys, liver, brain, meninges and lungs. Histopathologically,

renal tubular necrosis is a prominent feature of leptospiral infection. Liver might show hepatocytes degeneration, hyperplasia of Kupffer cells, infiltration of inflammatory cells and cholestasis. Focal necrosis may also be present in the liver. Invasion of the leptospire to the lungs cause severe lung congestion, focal intraalveolar haemorrhage or massive pulmonary bleed if severe, other organs including brain, eyes, heart and muscles can also be affected.

In the kidneys, leptospire migrate to the interstitium, renal tubules, and tubular lumen, causing interstitial nephritis and renal tubular necrosis. Hypovolemia due to dehydration and altered capillary permeability contribute to the development of acute renal failure. Vasomotor nephropathy causing capillary leakage with loss of fluid and protein leads to intravascular depletion. Renal involvement is the most serious complication in leptospirosis and is the commonest cause of death (14). Two mechanisms have been postulated in the development of acute renal failure: (i) direct nephrotoxicity and (ii) anoxic effect due to disturbances in renal circulation. The typical lesion is tubulointerstitial nephritis, characterized by interstitial oedema and dense focal infiltration of inflammatory cells predominantly mononuclear cells. Degenerative tubular changes are common, principally involving the proximal tubules (14).

In the liver, centrilobular necrosis with proliferation of Kupffer cells may be found. Elevated liver enzymes and jaundice often appear in leptospirosis with multi organ involvement.

Pulmonary involvement is the result of intraalveolar haemorrhage and not the inflammation. It may lead to acute respiratory distress syndrome, hypoxia and death if prompt oxygen therapy via ventilatory support is not provided.

Invasion of skeletal muscles by leptospire results in swelling, vacuolation of the myofibrils and focal necrosis. This leads to release of intracellular substances like myoglobin

and potassium. In severe leptospirosis, it may even lead to rhabdomyolysis which may bring about acute renal failure.

Leptospire affect the heart by causing myocarditis and endocarditis (15). The etiology for the cardiac involvement is not fully understood. Cardiovascular disturbance is one the grave complications of leptospirosis. It has been reported that serologically positive patients with leptospirosis showed electrocardiographic abnormalities with atrial fibrillation being the most common arrhythmia. Conduction system abnormalities, T wave changes and atrioventricular blockade were also reported in the same study (16).

When the antibodies are formed, the systemic immune response takes place with the formation of antibodies. Leptospire are eliminated from all sites in the host except the eyes, the proximal renal tubules and perhaps the brain, where they may persist for weeks or up to months. The persistence of leptospire in the aqueous humor can cause chronic or recurrent uveitis. The rise in antibodies coincides with the development of meningitis.

2.5 CLINICAL PRESENTATION

Leptospirosis has a biphasic clinical presentation. The first phase, acute or also known as septicaemic phase lasts about a week, and is characterised by dissemination of leptospire into various organ tissues and is followed by the second or immune phase which is characterised by antibodies production and excretion of leptospire via urine. Clinical presentation can vary from mild disease symptoms such as influenza-like illness such with fever, headache, myalgia or arthralgia, to severe leptospirosis which may manifest as multiple organ failures or even death. These clinical symptoms can be grossly classified into mild anicteric leptospirosis and icteric leptospirosis, which is also named Weil's disease.

More than 90% of symptomatic cases are the relatively mild and usually anicteric form of leptospirosis, with or without meningitis. Severe leptospirosis with profound jaundice develops in 5 – 10% of patients. It has been suggested that the distinct clinical syndromes may be associated with specific serogroups.

The incubation period for leptospirosis is usually 1-2 weeks but may range from 2-20 days.

2.5.1 ANICTERIC LEPTOSPIROSIS

The majority of leptospirosis cases are subclinical to mild disease and presents as an acute flu- like illness with fever, chills, headache, myalgia, arthralgia, nausea and vomiting. Muscle pain especially affecting the calves, back, and abdomen is an important feature of leptospirosis. Sometimes it includes sore throat, rash, intense headache mainly frontal or retroorbital, photophobia and mental confusion. Pulmonary involvement is manifested in most cases by cough, chest pain and haemoptysis.

The most common finding on physical examination is fever with conjunctival suffusion. Others findings may include muscle tenderness, lymphadenopathy, pharyngeal injection, rash, hepatomegaly, splenomegaly and mild jaundice. The rash may be macular, maculopapular, erythematous, urticarial or hemorrhagic.

Most people become asymptomatic and afebrile within 1 week which coincides with the development of antibodies. After an interval of 1-3days, the illness recurs in a number of cases with new onset of fever and other symptoms. The start of this second (immune) phase coincides with the development of antibodies. Symptoms are more variable during this phase, fever and myalgia is less profound in this period. An important event during the immune phase is the development of aseptic meningitis with CSF pleocytosis. No more than 15% of

all patients have symptoms and signs of meningitis. Some other studies showed that aseptic meningitis may be found in less than 25% of all leptospirosis cases. Meningeal symptoms may persist from days to weeks. Aseptic meningitis is more common among children than adults. Alston and Broom reported that 62% of children aged 14 years old or less presented with aseptic meningitis whereas only 31% of patients aged 15 to 29 and just only about 10% of patients aged over 30 did so. Mortality in anicteric leptospirosis is almost none.

2.5.2 ICTERIC LEPTOSPIROSIS

It is also known as Weil's syndrome, and is characterized by jaundice, renal dysfunction, bleeding tendencies, and a mortality rate ranging from 5-15%. This syndrome is frequently but not exclusively associated with serovar icterohaemorrhagiae/copenhageni infection. The onset of the disease is similar to that of anicteric form, however, after 4-9 days, jaundice as well as renal and vascular dysfunction generally develop. Unlike the anicteric leptospirosis, Weil's syndrome lacks a biphasic pattern. The jaundice in Weil's may be profound but is usually not associated with severe hepatic necrosis. Death is rarely due to hepatic failure. Hepatomegaly and tenderness in the right upper quadrant are usually detected. Splenomegaly is found in 20% of cases.

Renal failure may develop, often during the second phase of the disease. Renal insufficiency manifests in 40 to 70% of patients (17, 18). Hypovolemia and decreased renal perfusion contribute to the development of acute tubular necrosis with oliguria or anuria. A fair number of cases can be managed without dialysis. However, dialysis or other forms of renal replacement therapy may be required. Renal function may be completely recovered.

Pulmonary involvement occurs frequently. It occurs in 20-70% of cases (19). In some of the cases, it is reported as a major manifestation, resulting in cough, dyspnea, chest pain, blood stained sputum, haemoptysis and respiratory failure secondary to acute respiratory distress syndrome (ARDS) which may bring about 50% mortality (20).

Other symptoms like rhabdomyolysis, acute haemolysis, myocarditis, pericarditis, congestive heart failure, cardiogenic shock, necrotizing pancreatitis and multiorgan failure have all been described during severe leptospirosis.

2.6 LABORATORY AND RADIOLOGICAL FINDINGS

Routine laboratory tests show non-specific findings in leptospirosis. The total white cell count can be low, normal or elevated and it is usually associated with a shift to the left. Thrombocytopenia is a common feature. Mild anaemia is not uncommon. Some studies demonstrated that leucocytosis is associated with increased mortality (21). Thrombocytopenia is found in 50% of cases and is significantly associated with renal failure (22). A platelet count that is below 50,000 per microliter is associated with poor prognosis as reported by Tubiana (23).

The kidneys are invariably involved in leptospirosis. Related findings range from urinary sediment changes and mild proteinuria in anicteric leptospirosis to acute renal failure in severe disease. Haematuria, proteinuria, pyuria and hyaline or granular casts are common findings on urine analysis. Derangement of renal profile is primarily due to tubular damage. Hyperkalemia is an important parameter for development of acute renal failure and mortality in leptospirosis. Hyperkalemia has been shown to be an independent risk factor for mortality.

Marotto et al found that a potassium level of over 4 mmol/L has an increased risk for mortality (24).

Liver function test in patients with anicteric leptospirosis typically show mildly elevated or normal serum levels of bilirubin and alkaline phosphatase, lactate dehydrogenase as well as small rise in serum levels of aminotransferases i.e aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Serum creatine kinase (CK) is usually raised due to muscle involvement. In icteric leptospirosis or Weil's syndrome, the liver function test usually shows a significant rise in bilirubin level with lesser increases in transaminases and alkaline phosphatase. Prothrombin time may be prolonged. The increment in bilirubin level is generally out of proportion to the other liver function parameters.

In severe leptospirosis, pulmonary radiographic abnormalities usually develop 3 to 9 days after the onset of illness. The most common radiographic finding is bilateral patchy alveolar infiltrates which corresponds to scattered alveolar haemorrhage; and mostly affect the lower lobes in the peripheral lung fields. In severe leptospirosis with pulmonary haemorrhage, it may present radiographically as large snowflakes, pulmonary edema, bilateral homogenous opacity with areas of consolidation. Due to the nonspecific nature of chest X-ray findings and laboratory tests, these can only suggest a diagnosis of leptospirosis. In order to confirm the diagnosis, specific microbiological tests are necessary.

2.7 DIAGNOSIS OF LEPTOSPIROSIS

Diagnosis of leptospirosis is based on 3 methods i.e;

1. Isolation of organism
2. Serological test

3. Detection of specific DNA

Leptospire can be identified using dark field examination of the patient's blood. It can be cultured on a semisolid medium like Fletcher's Ellinghausen-McCullough-Johnson-Harris (EMJH) if blood is cultured before the tenth day of illness. However, the culture will take 1-6 weeks to become positive rendering this method clinically unapplicable when quick diagnosis is crucial and early treatment should be initiated promptly. Isolation of the organism from the tenth day and onwards can be achieved via examination of urine under dark ground microscopy.

In clinical practice, diagnosis of leptospirosis is usually made by means of serological tests. There are microscopic agglutination tests (MAT) and ELISA IgM and Slide agglutination test (SAT). MAT usually yields positive results after 7-10 days of illness; peaks at 3-4 weeks and may persist for years. Therefore, to make a diagnosis, a fourfold or greater rise in titre must be observed. ELISA IgM and SAT are simple, sensitive tests that can be rapidly performed tests to measure IgM antibodies at the very early stage of the disease. It is currently used to diagnose leptospirosis and a single sample is sufficient. The ELISA test can be positive in as early as 2 days into the disease when the clinical presentation is still not specific. It was found to be 100% sensitive and 93% specific in a study reported by Kaur et al (25).

Detection of leptospiral DNA can be achieved using polymerase chain reaction test (PCR test). It appears to be sensitive, specific and able to detect leptospiral DNA early in blood, cerebrospinal fluid and aqueous humor. This method has been used to determine that leptospiremia of 10,000 or more bacteria per millilitre of blood or milligram of tissue in severe pulmonary haemorrhagic syndrome (SPHS) patients (26). It confirmed that the critical

threshold appears to be 10,000 or more bacteria per millilitre of blood for developing SPHS and death (27). The disadvantage of this test is that it is genus-specific, not serovar specific.

In Malaysia, the diagnosis of leptospirosis is made via the Microscopic Agglutination Test (MAT); polymerase chain reaction (PCR) test and Enzyme-linked Immunosorbent Assay (ELISA). The MAT is carried out at the Institute for Medical Research (IMR) Kuala Lumpur, at Makmal Kesihatan Awam Kebangsaan (MKAK) Sungai Buloh and also at some universities undertaking research on leptospirosis. ELISAs are available at local tertiary hospitals with the necessary facilities, IMR and also MKAK. Lepto PCR and culture of leptospire are performed only in IMR.

2.7.1 WHO DIAGNOSIS

A. Clinical description

The usual presentation is an acute febrile illness with headache, myalgia (particularly calf muscle) and prostration associated with any of the following symptoms/signs:

- conjunctival suffusion;
- anuria or oliguria;
- jaundice;
- cough, haemoptysis and breathlessness;
- haemorrhages (from the intestines; lung bleeding is notorious in some areas);
- meningeal irritation;
- cardiac arrhythmia or failure; and

- skin rash.

*Note. Other common symptoms include nausea, vomiting, abdominal pain, diarrhoea and arthralgia. The clinical diagnosis is difficult where diseases with symptoms similar to those of leptospirosis occur frequently.

B. Laboratory criteria

1. Presumptive diagnosis:

- A positive result of a rapid screening test such as IgM ELISA, latex agglutination test, lateral flow, dipstick etc.

2. Confirmatory diagnosis:

- Isolation from blood or other clinical materials through culture of pathogenic leptospires.
- A positive PCR result using a validated method (primarily for blood and serum in the early stages of infection).
- Four-fold or greater rise in titre or seroconversion in microscopic agglutination test (MAT) on paired samples obtained at least 2 weeks apart. A battery of *Leptospira* reference strains representative of local strains to be used as antigens in MAT.

C. Case classification (humans)

1. Suspected: A case that is compatible with the clinical description and a presumptive laboratory diagnosis.

2. Confirmed: A suspect case with a confirmatory laboratory diagnosis

2.7.2 MALAYSIAN GUIDELINES 2011

Leptospirosis is difficult to distinguish from a number of other diseases on clinical grounds alone. History of possible exposure is paramount to aid clinical diagnosis.

A. Clinical case

A case that is compatible with the following clinical description:

Acute febrile illness with history of exposure to water and/or environment possibly contaminated with infected animal urine **with** ANY of the following symptoms:

- Headache
- Myalgia particularly associated with the calf muscles and lumbar region
- Arthralgia
- Conjunctival suffusion
- Meningeal irritation
- Anuria or oliguria and/or proteinuria
- Jaundice
- Hemorrhages (from the intestines and lungs)
- Cardiac arrhythmia or failure
- Skin rash
- Gastrointestinal symptoms such as nausea, vomiting, abdominal pain, diarrhea

B. Probable Case

A clinical case AND positive ELISA/other Rapid tests.

C. Confirmed case:

A confirmed case of leptospirosis is a **suspected OR probable** case with any one of the following laboratory tests:

- Microscopic Agglutination Test (MAT),

For single serum specimen - titre 1:400

For paired sera - four fold or greater rise in titre

- Positive PCR (samples should be taken within 10 days of disease onset)
- Positive culture for pathogenic leptospire (blood samples should be taken within 7 days of onset and urine sample after the 10th day)
- Demonstration of leptospire in tissues using immunohistochemical staining (example in post mortem cases)

2.8 MORTALITY IN LEPTOSPIROSIS

2.8.1 PATIENTS CHARACTERISTIC

A recent systematic review of the mortality from untreated leptospirosis revealed that median series mortality was 2.2% (range 0.0-39.7%), but the mortality was high in jaundiced patients (19.1%) (range 0.0 – 39.7%) , those with renal failure 12.1% (range 0-25%) and in patients aged over 60 (60%) (range 33.3-60%) but low in anicteric patients (0%) (range 0-1.7%) (28).

Mortality of leptospirosis varies in different forms of leptospirosis. 2 classic forms of leptospirosis have been described: the anicteric form, which is mild and rarely associated with severe complications or mortality, where the mortality rate, is almost nil. Whereas, the icteric form may cause multiorgan involvement, has a mortality varying from 5-20%.

The causes of death include pulmonary haemorrhage, acute renal failure, and multiorgan failure. Cardiac involvement is probably more common than reported. Prognostic factors vary among reported studies. In 1997, a retrospective study by Herve Dupont (21)

which involved 68 leptospirosis patients showed 18% mortality. Multivariate logistic regression demonstrated five factors that were independently associated with mortality: dyspnea, oliguria, white blood cell count $>12,900/\text{mm}^3$, repolarization abnormalities on ECG, and alveolar infiltrates on chest radiograph. In 1999, a retrospective study by Elizabeth Daher (29) involving 110 patients with Weil's disease in Brazil showed that the only independent risk factor associated with death was oliguria. Mortality in this study was 22%. Albert Ko, year 1999 showed that altered mental status, age over 37 years, renal insufficiency and respiratory insufficiency were independent risk factors for mortality. This study involved 193 patients with 15% mortality (30). Panaphut conducted a prospective cohort study in Thailand involving 121 patients with a mortality of 14%, showed independent risk factors of death were hypotension, oliguria, hyperkalaemia and presence of pulmonary rales (31). Anne Sphichler in year 2008, conducted a retrospective case control study involving 378 patients with a mortality of 23.5% showed the independent risk factors were: age >40 year, thrombocytopenia (platelet $<70000/\text{uL}$), oliguria, creatinine $> 3 \text{ mg/dL}$ and pulmonary involvement (32).

2.8.2 COMPLICATION

2.8.2.1 PULMONARY COMPLICATION

Pulmonary manifestations in leptospirosis are considered a major complication and are related to poor prognosis. In 1995, Nicaragua outbreak of leptospirosis raised the awareness for leptospirosis as the cause of severe pulmonary haemorrhagic syndrome (SPHS) (33). Severe pulmonary haemorrhagic syndrome (SPHS) is associated with a mortality of $>50\%$ and was reported to be as high as 74% in leptospirosis outbreaks in Salvador, Brazil in between 2003-2005 (20).

The study done by F. Paganin in 2011 showed that dyspnea and oliguria/anuria were the two independent risk factors related to pulmonary involvement whereas mechanical ventilation requirement and AST greater than 150IU/L were two independent risk factors associated with mortality (34).

2.8.2.2 RENAL INVOLVEMENT

Renal involvement as acute renal failure is a prominent feature of both mild and severe leptospirosis. It manifests in 44-67% of patients (35). It usually presents as a part of multiorgan involvement as recorded in the retrospective study done by Adrian Covic (36): ARF in hepatic failure in 72% of cases, respiratory failure in 38% of cases, circulatory failure in 33% of cases, pancreatitis in 25% and rhabdomyolysis in 5% of cases. In the same study, all the deceased patients had besides ARF, at least two other organ failures. Mortality in the study was 33%. The haemorrhagic diathesis and cerebral involvement are markers for unfavourable patient and renal outcomes (36). The reported prevalence of ARF in leptospirosis has a wide range, from 10-60% (18). Such wide range could be explained by various factors, such as patient age, ARF definition, sporadic cases or an outbreak, disease severity and selection of cases.

In the absence of ARF, death due to leptospirosis is very uncommon, but the reported mortality due to leptospirosis with ARF is high: 36% in Barbados, 26% in Sri Lanka, 17% in Turkey. Seguro et al (37) reported a 50% mortality among oliguric patients with Weil's disease and 5% among the non oliguric. Dupont and others also found oliguria, dyspnea and alveolar infiltration to be independent factors associated with mortality in hospitalised patients with leptospirosis. Ramachandran and others found bilirubin level of 15 mg/dL among the leptospirosis oliguric patients who died and 5 mg/dL among the oliguric and non-oliguric survivors.

Effect of dialysis on treatment of AKI caused by leptospirosis plays an important role. Andrade (17) et al compared the ‘delayed alternate day’ and the ‘prompt and daily dialysis’ in patients with Weil’s disease. It showed that the mortality was significantly lower in the ‘prompt and daily dialysis’ group (17% versus 67%, $p = 0.01$). However, there was no difference in terms of the time taken to the recovery of renal function. In Brazil, a study done by Daher et al in 2009, described that the mortality was related to the types of dialysis. The type of dialysis during the period of 1985 to 1996 was intermittent peritoneal dialysis and the mortality was 22%. During the period of 1996 to 2006 daily hemodialysis was performed and the mortality decreased to 10% ($p < 0.02$). This is due to acute kidney injury in leptospirosis is generally hypercatabolic and require frequent dialysis. Intermittent peritoneal dialysis is inadequate in removing the urea formed during this hypercatabolism. The need for dialysis reported in literatures ranges from 6% to 49% (29).

2.8.2.3 WEIL’S DISEASE

Weil’s disease is the severe form of icteric leptospirosis which characterized by jaundice, renal failure and bleeding diathesis. The clinical course usually progresses rapidly. Severe cases often present late and this contribute to the higher mortality rate, which ranges from 5-15%. Worldwide, the case fatality rate may be as high as 22% (38). Fulminant Weil’s disease can result in cardiovascular collapse and pulmonary haemorrhagic pneumonitis where the mortality rate could reach up to 70% (20).

2.9 NATURAL DISASTERS AND OUTBREAK OF LEPTOSPIROSIS

Leptospirosis is highly prevalent in developing countries. Outbreaks are commonly related to normal daily activities, overcrowding, poor sanitation and climatic conditions (39). Outbreaks usually occurs after floods caused by heavy monsoon, cyclones and hurricanes. Natural disasters increase the risk for exposure to leptospirosis through contact with contaminated water and mud (40). Numerous outbreaks of leptospirosis have been reported following extreme weather events around the world, in geographically diverse areas.

A Taiwan study collected information regarding 16 typhoons from year 2000 through 2009 to evaluate the effects of typhoon level, rainfall level, and maximum cumulative rainfall amounts on case numbers of leptospirosis and melioidosis after a typhoon (41). It showed that the frequencies of leptospirosis and melioidosis cases before the typhoon were significantly lower than those after the typhoon. Furthermore, more leptospirosis and melioidosis cases were observed during the post-typhoon period in 2009 than during 2006–2008. Effects of typhoons on numbers of leptospirosis and melioidosis cases in the late stage of a typhoon were further analysed by using records of typhoon level and maximum 24-hour cumulative rainfall during 2000–2009. It was found that typhoon level with higher weight was significantly correlated with more cases of leptospirosis and melioidosis. The results further suggested that, when the 24-hour cumulative rainfall was >500 mm, significantly more melioidosis ($p<0.05$) and leptospirosis cases were observed.

Several other studies have shown that there is an increase in leptospirosis cases after climate changes. In rural Nicaragua October 1995, epidemic “hemorrhagic fever,” without jaundice or renal manifestations, was reported following heavy floods; 2259 residents were evaluated for non-malarial febrile illnesses. 15 (0.7%) died with pulmonary hemorrhage. It was later confirmed as a leptospirosis epidemic (33).

In Puerto Rico in October 1996, following rainfall and a period of flooding caused by a hurricane, four of 72 prehurricane and 17 of 70 posthurricane patients, who all were tested negative for dengue; had laboratory confirmed cases of leptospirosis (relative risk=4.4, 95% confidence interval = 1.6-12.4) (42).

Outbreak of leptospirosis after the cyclone in Orissa, India in 1999 (43) and after the great flood in Hat Yai, Thailand in 2000 (44) showed that 19.2% and 32.5% of the study subjects had serological evidence of leptospirosis, respectively. In Mumbai, India, an eight-fold increase in disease incidence was noted after severe flooding in 2005 (45). In Philippines, after a typhoon in September 2009, an outbreak of leptospirosis occurred in Metro Manila; 471 patients were hospitalised and 51 (10.8%) died (46).

The reported case fatality rates of leptospirosis after floods range widely with literatures published from different parts of the world. A retrospective study done on mortality analysis of patients with acute febrile illness during monsoon season in Mumbai, India which involved 2214 patients were admitted to a tertiary teaching hospital in which 160 patients died. Proportional mortality rate (PMR) showed that 22.5% was due to malaria, 21.88% was due to leptospirosis and 1.88% was due to dengue fever whereas 54% was undetermined (47).

2.10 RATIONALE OF THE STUDY

Leptospirosis has a wide range of clinical presentation from mild illness to severe disease or even death. Several studies had attempted to identify the risks and prognostic factors for disease severity and its mortality. However, the results varied across different studies and might not be conveniently extrapolated into local settings in view of different geographical factors, climate, serotypes of etiologic agents, population structure and local cultures. In view of high mortality of the severe disease especially with the limitation of medical resources in district hospitals, a guideline is needed for physicians to identify severe and potential high risk cases which need early admission or transfer to tertiary hospital for intensive care, organ supports and closed monitoring of disease progress. Therefore, this study is aimed to determine the risk and prognostic factors independently associated with severe leptospirosis in laboratory confirmed cases in adult population.