UNIVERSITI PUTRA MALAYSIA

CLINICAL AND LABORATORY CHARACTERISTICS OF DENGUE PATIENTS WITH BACTERIA CO-INFCTION IN HOSPITAL SERDANG IN 2016

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By

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ABSTRACT

Introduction: Dengue is one of the most common human arboviral diseases in tropical and sub-tropical countries globally. The clinical course of dengue fever can be affected by bacterial co-infection, resulting in atypical dengue manifestations. Furthermore, the clinical presentations of dengue and some bacterial infections are overlapping. As a result, bacteria co-infections can be easily overlooked among dengue cases. **Objectives:** The aim of this study is to analyze and differentiate the clinical and laboratory parameters between dengue and dengue bacterial co-infection. The clinical and laboratory parameters will be also identified and compared between leptospiral co-infection and other bacterial-coinfected dengue patients. **Hypothesis:** There is a significant difference in the clinical and laboratory parameters between dengue and dengue bacterial co-infection group. There are also significant differences in the clinical and laboratory parameters between leptospiral-coinfected and other bacterial-coinfected dengue patients. **Methodology:** This is a retrospective study conducted in Hospital Serdang that only involves data collection. All the data were retrieved from the hospital database. Statistical analysis was performed by using the SPSS software. Categorical data, continuous parametric data and non-parametric data were analyzed by using Chi-square test, Student's t-test and Mann-Whitney U test respectively. Binary logistic regression analysis was further carried out to identify clinical predictors of dengue with bacteria co-infection and leptospiral co-infection. A p-value of less than 0.05 ($p < 0.05$) is considered as statistically significant. **Results:** The total sample size of dengue patients is 1,730. There are 72 and 26 cases of dengue with bacteria co-infection and dengue with leptospiral-coinfection respectively. The presence of warning signs, severe dengue, severe leakage and prolonged fever are less likely to be associated with dengue bacteria coinfection (OR < 1). Elevated creatine kinase (CK) levels may be predictive factors of dengue with bacteria co-infection. High C-reactive protein (CRP) level is less likely to be associated with leptospiral co-infection. **Discussion:** Bacteria co-infected dengue patients are prone to be critically ill with compensated shock and warning sign of haemoconcentration. Some studies observed prolonged fever in Salmonella co-infection and elevated CRP levels in leptospirosis co-infection but these contradicted our results. Studies reported high CK level detected in dengue with *Staphylococcus aureus* pneumonia co-infection. **Conclusion:** Discrimination between dengue and dengue with bacteria co-infection a crucial for accurate diagnosis and empirical antibiotics prescription.

Keywords: Dengue, bacteria co-infection, leptospiral-coinfection

CIRI-CIRI KLINIKAL DAN MAKMAL PESAKIT DENGGI DENGAN KOINFEKSI BAKTERIA DI HOSPITAL SERDANG PADA TAHUN 2016

JANET LO ZHEN LIN

ABSTRAK

Pengenalan: Denggi adalah salah satu penyakit arboviral manusia yang biasa dijumpai di negara-negara tropika dan subtropika di seluruh dunia. Tanda klinikal demam denggi boleh dipengaruh oleh koinfeksi bakteria dan menyebabkan manifestasi denggi yang kurang diberi perhatian. Tambahan pula, terdapat pertindihan manifestasi klinikal antara demam denggi dan sesetengah jangkitan bakteria. Kesannya, koinfeksi bakteria mudah diabaikan dalam kes-kes denggi. **Objektif**: Kajian ini bertujuan untuk menganalisis dan membezakan parameter klinikal dan makmal antara denggi dan denggi dengan koinfeksi bakteria. Parameter klinikal dan makmal juga akan dikenal pasti dan dibandingkan antara pesakit denggi dengan koinfeksi leptospirosis dan koinfeksi bakteria yang lain. **Hipotesis**: Terdapat perbezaan parameter klinikal dan makmal yang signifikan antara kumpulan denggi dan denggi dengan koinfeksi bakteria. Terdapat juga perbezaan yang signifikan dalam parameter klinikal dan makmal antara pesakit denggi dengan koinfeksi leptospirosis dan dengan koinfeksi bakteria yang lain. **Kaedah**: Ini adalah kajian retrospektif yang dilakukan di Hospital Serdang yang hanya melibatkan pengumpulan data. Semua data telah diambil dari pangkalan data hospital. Analisis statistik telah dilbuat dengan menggunakan perisian SPSS. Data kategori, data parametrik dan data bukan parametrik telah dianalisis dengan menggunakan ujian Chi-square, ujian Student's t dan ujian Mann-Whitney U. Selanjutnya, analisis binary logistic regression telah dilakukan untuk mengenal pasti parameter klinikal yang boleh meramal kemungkinan koinfeksi bakteria dan koinfeksi leptospirosis dalam kalangan pesakit denggi. Secara statistiknya, nilai p yang kurang daripada 0.05 ($p < 0.05$) dianggap sebagai signifikan. **Keputusan**: Jumlah sampel pesakit denggi ialah 1,730. Terdapat 72 dan 26 kes denggi dengan koinfeksi bakteria dan denggi dengan koinfeksi leptospirosis. Kehadiran tanda-tanda amaran denggi, denggi yang serius, kebocoran plasma yang serius dan demam yang berpanjangan adalah antara parameter klinikal yang kurang dikaitkan dengan koinfeksi bakteria ($OR < 1$). Peningkatan kadar kreatin kinase (CK) merupakan salah satu tanda yang boleh meramal kemungkinan koinfeksi bakteria. Peningkatan kadar protein C-reaktif (CRP) pula kurang dikaitkan dengan koinfeksi leptospirosis. **Perbincangan**: Pesakit denggi dengan koinfeksi bakteria adalah terdedah kepada penyakit yang lebih kritikal dengan sindrom renjatan dan tanda amaran hemokonsentrasi. Beberapa kajian mendapati bahawa demam yang berpanjangan ditunjukkan oleh pesakit denggi dengan koinfeksi *Salmonella* dan peningkatan CRP dalam koinfeksi leptospirosis, tetapi ini bertentangan dengan hasil kajian kami. Kajian juga melaporkan kadar CK yang tinggi dikesan dalam koinfeksi pneumonia *Staphylococcus aureus*. **Kesimpulan**: Diskriminasi antara denggi dan denggi dengan koinfeksi bakteria sangat penting untuk diagnosis yang tepat dan preskripsi antibiotik yang segera.

Kata kunci: Denggi, koinfeksi bakteria, koinfeksi leptospirosis

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ABG/VBG	Arterial blood gases/Venous blood gases
AKI	Acute kidney injury
ALT	Alanine transaminase
aPTT	Activated partial thromboplastin time
AST	Aspartate transaminase
BMI	Body mass index
C & S	Culture & sensitivity
CDC	The Centers of Disease Control and Prevention
CI	Confidence interval
CK	Creatine kinase
CKD	Chronic kidney disease
CNS	Central Nervous System
CRP	C-reactive protein
DBP	Diastolic blood pressure
DENV	Dengue Virus
DHF	Dengue Hemorrhagic Fever
DSS	Dengue Shock Syndrome
ELISA	Enzyme-linked Immunosorbent Assay
ESR	Erythrocyte sedimentation rate
FBC	Full blood count
Hb	Hemoglobin
HCO_3	Bicarbonate
HCT	Hematocrit
HR	Heart rate

IgG	Immunoglobulin G
IgM	Immunoglobulin M
INR	International normalized ratio
IQR	Interquartile range
K	Potassium
MAT	Microscopic Agglutination Test
MOH	Ministry of Health
MREC	Medical Research and Ethics Committee
Na	Sodium
NAATs	Nucleic Acid Amplification Tests
NMRR	National Medical Research Register
NS1	Nonstructural protein 1
OR	Odds ratio
O_2 sat	Oxygen saturation
$PaCO_2$	Partial pressure of carbon dioxide
PaO_2	Partial pressure of oxygen
PT	Prothrombin time
RT-PCR	Reverse transcription polymerase chain reaction
SBP	Systolic blood pressure
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
Urine FEME	Urine full and microscopy examination
WBC	White blood cell
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Research Background

With nearly 400 million infections yearly, dengue fever is one of the most common human arboviral diseases in the tropical and subtropical countries around the world, which include our country, Malaysia (Tan et al., 2018). According to the World Health Organization (WHO), dengue has become an important public health concern with about half of the world's population at risk (World Health Organization [WHO], 2019). Factors such as industrialization, climate change, travel and environmental cleanliness are contributing to the increasing number of dengue cases (Vogels et al., 2019).

Dengue fever is caused by flavivirus with four known serotypes – DENV-1, DENV-2, DENV-3, DENV-4 (Cheah, Ng, Marzilawati & Lum, 2014). Infected individuals normally present with as mild as flu-like signs and symptoms to severe, possibly lethal dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). Previously, dengue associated co-infections are scarcely reported (Chai et al., 2007; Siddiqui, Islam & Islam, 2019). They are normally presented as separate entity - individual case reports or case series (Panda, Mohta, Wig & Soneja, 2018; Srinivasaraghayan, Narayanan & Kanimozhi, 2015). Little is known on how the associated coinfections will affect the disease severity and clinical outcome. Recently, however, atypical dengue manifestations such as severe gastrointestinal bleeding, acute kidney injury (AKI) seizures and different types of inflammations which include encephalitis, myocarditis and hepatitis have raised concerns on possible dengue co-

infections (Ahlawat & Kalra, 2017; Natarajan, Cugati, Padi & Padmapriya, 2017). According to a study by Panda, Mohta, Wig and Soneja in 2018, more dengue patients are co-infected with bacterial infection in comparison to other organisms such as virus, fungus or parasites, leading to more severe outcomes. The example of the common bacteria being reported includes *Salmonella*, *Leptospira*, *Staphylococcus*, *Pseudomonas, Klebsiella* and *Rickettsia*. Meanwhile, gram negative bacteria have been shown to be associated with higher mortality due to bacteraemia (Panda et al., 2018).

On top of that, bacteria co-infections in dengue patients are now being increasingly reported (Trunfio, Savoldi, Viganò & d' Arminio Monforte, 2016). This is becoming a critical issue because the clinical course of dengue fever can be affected by bacteria co-infection, which may have led to the atypical dengue manifestations being reported such as encephalitis, rhabdomyolysis, severe gastrointestinal haemorrhage and others (Natarajan et al., 2017; Trunfio et al., 2016). In fact, infection by two agents at the same time can result in diagnosis dilemma as the diseases' symptoms overlap (Soegijanto, Nuryandari, Churrotin & Sucipto, 2018). Consequently, this can pose serious challenges especially during early clinical diagnosis where the physicians might miss or fail to make an accurate diagnosis. As a result, bacteria co-infection can be easily overlooked among dengue patients, especially in dengue-endemic regions or during an outbreak (Natarajan et al., 2017; See, Phua, Yip, Yeo & Lim, 2013).

To date, dengue associated co-infections are only suspected when the patients are presented with atypical clinical course or prolonged febrile illness (Soegijanto et al., 2018; Srinivasaraghavan et al., 2015). Therefore, this is especially crucial to determine the type of bacteria co-infections associated as it can affect disease severity, treatment and management as well as disease prognosis.

1.2 Problem Statement

Dengue fever is an endemic disease in Malaysia. Despite the various preventive efforts being promoted and carried out by the Ministry of Health and Municipals, the trend of dengue incidence is not showing any sign of decrease in the near future due to multiple other factors such as climate, vector capacity and integrity of the host immune system (Mudin, 2015; Zaki et al., 2019). Since 2001, the incidence of dengue exhibits a general upward trend up until 2014. In 2020, the incidence is expected to reach about 203, 901 cases compared to 108, 698 cases in 2014 (Bujang et al., 2017). Meanwhile, the total population size in Malaysia is estimated at approximately 32.4 million in 2020 (Department of Statistics Malaysia [DOSM], 2019). With the hot and humid weather all year round as well as the ever-expanding population, these conditions favour the reproduction and growth of *Aedes* mosquitoes and more people will be prone to dengue infection.

Other than that, dengue vaccines are still not available in Malaysia (Landau & Yusof, 2019). So far, the only vaccine available and authorized in some of the countries is Dengvaxia® (The Centers of Disease Control and Prevention [CDC], 2019). No specific treatment has been scientifically proven to be effective as well. Prevention such as fogging remains the primary way to control dengue infection.

Moreover, dengue diagnosis proves to be challenging as the clinical presentations during febrile and critical phases overlap with other diseases (Wong, Wong & Abu Bakar, 2020). Recently, reports on dengue with bacteria co-infection has been on the rise (Trunfio et al., 2016). Many of the bacterial infections mimic dengue fever which include leptospirosis, scrub typhus and typhoid (Bansal, Bansal & Tomar, 2015; Kaleem, Ain, Shahid & Essa, 2017; Mishra, Singhal, Sethi & Ratho, 2013; Mitra, Gautam, Jambugulam, Abhilash & Jayaseeelan, 2017; Pan, Roy, Kumar & Panwar, 2016; Sapkota, Bhandari, Sapkota & Hamal, 2017; Wijesinghe, Nanthini, Ranasinghe & Ragunathan, 2015). As a result, bacteria co-infection in dengue patients can be easily missed, especially where both dengue and the particular bacterial infection are highly prevalent in the region, leading to even more complications and higher mortality.

Currently, there are limited ways to identify dengue patients with bacteria co-infections. A clinical scoring system may be used to differentiate scrub typhus and dengue fever (Mitra et al., 2017). Meanwhile, a study by Basheer et al. demonstrated that normal white blood cell counts, prompt reduction in platelet numbers and hypoalbuminemia in dengue-infected patients may serve as possible clues to dengue-scrub typhus co-infection. As for dengue-typhoid co-infection, if fever persists for a longer duration, concurrent infection should be suspected (Bansal et al., 2015; Basheer et al., 2016). As for dengue with leptospirosis co-infection, the way to detect it is to be aware of any inconsistent clinical characteristics (Mishra et al., 2013). However, confirmatory tests need to be done in the laboratory setting to avoid misdiagnosis

(Kaleem et al., 2017). All in all, laboratory tests remain the most reliable tool in disease diagnosis (Mishra et al., 2013).

1.3 Significance of Study

The intention of this study is to identify the clinical and laboratory parameters associated with bacteria co-infection in dengue patients. As atypical dengue symptoms have made the clinical diagnosis challenging, understanding these parameters can assist the clinicians in identifying dengue patients with bacteria co-infection in the near future. This is especially vital for accurate diagnosis to prompt necessary treatment accordingly. One of the reasons why this study is important is due to the fact that dengue is an endemic disease and a major health threat in Malaysia with increasing infection rate. Therefore, early identification is crucial to avoid late antibiotic therapy for bacterial infection as well as fluid management for dengue. Lastly, there has been a lack of study on dengue patients with bacteria co-infection, especially large cohort studies. Most the findings are based on *in vitro* studies and animal models. The number of dengue with bacteria co-infection may have been under reported (Trunfio et al., 2016).

1.4 Research Objectives

The objectives of this study are:

i. To analyse the clinical and laboratory parameters of dengue and dengue with bacteria co-infection.

ii. To differentiate dengue and dengue with bacteria co-infection based on clinical and laboratory parameters.

iii. To compare the clinical and laboratory parameters of leptospiral-coinfected dengue patients and other bacterial-coinfected dengue patients.

1.5 Research Hypothesis

The hypothesis of this study is:

i. Dengue patients with bacteria co-infection will have a more significant clinical presentations or laboratory parameters compared to patients with dengue infection only.

ii. There is a significant difference in the clinical and laboratory parameters between leptospiral-coinfected dengue patients and other bacterial-coinfected dengue patients

CHAPTER 2

LITERATURE REVIEW

2.1 Dengue

2.1.1 History and Epidemiology of Dengue in Malaysia

The first reported case of dengue outbreak in Malaya was recorded in 15 November 1902 (Gill, 2017). An outbreak was discussed by Skae in Penang from December 1901 to March 1903. In November 1962, severe form of dengue - dengue haemorrhagic fever (DHF) was first seen in Georgetown, Penang. Later, dengue began to spread to the city areas of Penang as well as Kuala Lumpur. By early 1970, DHF has spread to all parts of Malaysia and led to serious disease burden to the Malaysian community.

The incidence of dengue fever shows an overall increasing trend in Malaysia for the past 22 years (Figure 2.1). There were several major outbreaks that had happened in 1974, 1978, 1982 and 1990 (Gill, 2017). Between 2014 and 2016, there was a notable spike in the number of dengue cases where the highest number was recorded in 2015 - 120,836 cases in total. The epidemic activity of dengue has been noticed to happen every five to eight years which may have been due to serotypes shift. One article showed that the dengue outbreak that happened from the year 2014 to 2016 is due to the serotype shift to DENV-1 genotype I, replacing the previously dominant DENV-2 before the outbreak (Suppiah et al., 2018). Global climate change in a particular region may play an important role as well (Mudin, 2015). The most recent dengue outbreak was reported in 2019 with 119,198 cases (Loh, 2019).

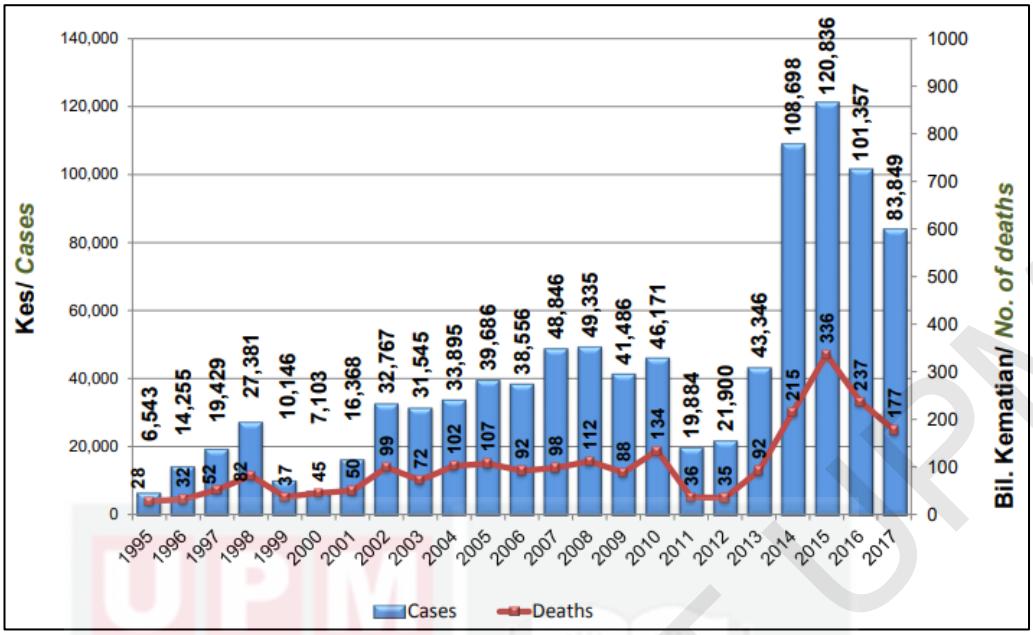

Figure 2.1. Dengue Cases and Deaths in Malaysia (1995 – 2017)

(Source: Ministry of Health [MOH], 2019)

Dengue is endemic in Malaysia and primarily a big city disease because the disease vector - the *Aedes* mosquitoes thrive in the tropics and high population density areas (WHO, n.d.). The highest recorded dengue cases were from Selangor, followed by Wilayah Persekutuan Kuala Lumpur and Putrajaya and Johor with 72,543, 15,424 and 10,873 accumulated dengue cases respectively in 2019 (Table 2.1).

Table 2.1. Dengue cases and deaths in Malaysia for 2019 (The Star, 2019)

States	Cases
Selangor	72,543 (56)
Kuala Lumpur/Putrajaya	15,424 (10)
Johor	10,873 (30)
Kelantan	6,003 (8)
Sabah	5,478 (25)
Penang	4,119 (9)
Perak	3,226 (4)
Pahang	2,873 (8)
Sarawak	2,648 (5)
Negeri Sembilan	2,305 (11)
Melaka	2,156 (7)
Kedah	1,587 (6)
Terengganu	542 (3)
Perlis	288 (0)

Labuan	36 (0)
Total	130,101 (182)

Note: ( ) number of deaths

2.2 Dengue Virus and Serotype Trends in Malaysia

Dengue infection happens when the dengue virus is being transmitted to a host via the female *Aedes* mosquitoes after feeding on an infected individual. The virus belongs to the genus Flavivirus of the *Flaviviridae* family. It is a single-stranded positive sense RNA virus with three structural proteins – capsid (C), membrane associated protein (M) and envelope (E). The viral genome has about 10,700 nucleotides and seven non-structural protein genes namely NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5, with NS1 being the main structure for disease pathogenesis (Bäck & Lundkvist, 2013). There are four known dengue virus serotypes (DENV) which are DENV-1, DENV-2, DENV-3 and DENV-4 (Cheah et al., 2014). All four of the serotypes can be found in Malaysia. A particular serotype can be dominant for at least two years before another serotype takes over (Figure 2.2). No particular serotypes stay dominant from year to year. As can be seen, the dominant serotype DENV-2 switched to DENV-1 two times in 2014, once in February and the other one in June (Figure 2.3). After that, an epidemic surge followed from July until September in the same year. Serotype shift is deemed to be the primary cause of rising dengue cases and deaths in a population because of decreased herd immunity against the new serotype (Mudin, 2015).

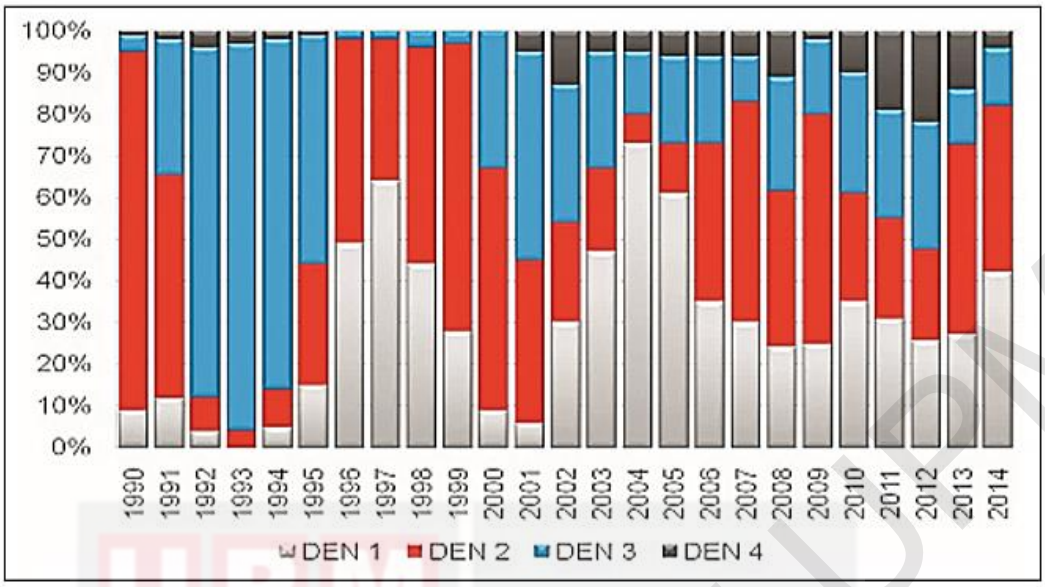

Figure 2.2. Dengue Serotypes in Malaysia (1990-2014)

(Source: Malaysia Health Technology Assessment Section [MaHTAS], 2015)

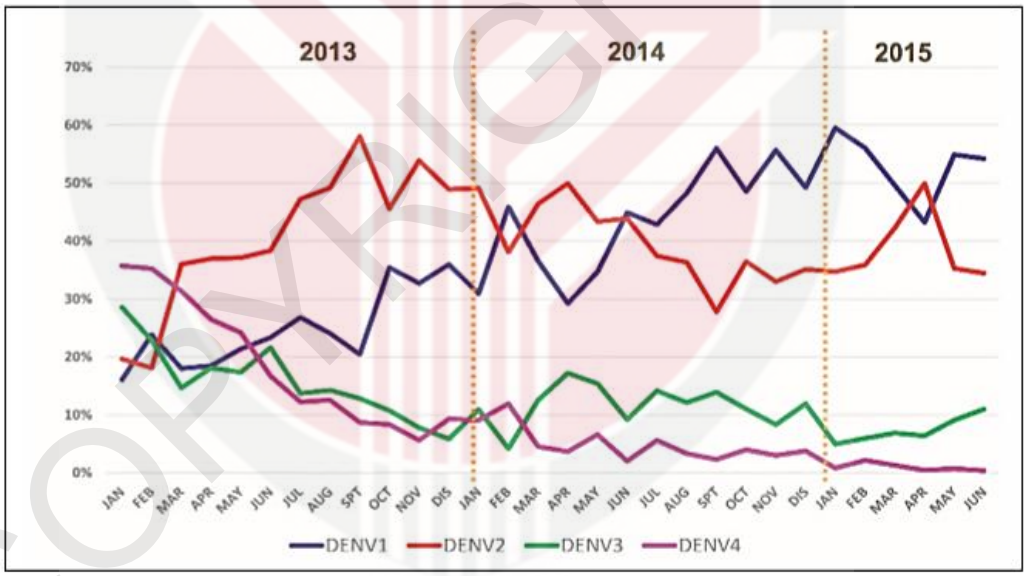

Figure 2.3. Dengue Serotypes in Year 2013, 2014 and 2015 (Jan-June)

(Source: MaHTAS, 2015)

After each occurrence of dengue infection, human body will develop long-term protection towards the similar serotypes, but provide only limited and temporary immunity towards other serotypes. As a result, secondary infection may lead to more severe dengue cases because of antibody-dependent enhancement (MaHTAS, 2015).

The pre-existing antibodies from the primary infection bind to the different infecting dengue serotype antigens which does not neutralize the new virus. On the contrary, the antibody-virus complexes that are formed bind to the Fcγ receptors on circulating monocytes (Rothman, 2011). This in turn helps the virus entry and infects the monocytes more efficiently, leading to increased replication of the virus and higher chance of developing severe dengue. Other contributing factors for increased dengue cases include climate change, urbanization, poor hygiene and waste management.

2.3 Mode of Transmission

The *Aedes aegypti* mosquitoes are the main vectors that carry the dengue viruses other than *Aedes albopictus*. *Ae. aegypti* have adapted to breed in artificial containers like vases, tyres, toilet tanks and others in urban areas. *Ae. aegypti* are most active during dawn and dusk. They can feed multiple times during the day in one gonotrophic cycle. Flight range studies propose that almost all female *Ae. aegypti* stay indoors and they normally fly a mean of 400 metres. In other words, humans instead of the mosquitoes help to spread the viruses quicker within and between populations (European Centre for Disease Prevention and Control [ECDC], 2016; WHO, n.d.).

Meanwhile, *Ae. albopictus* are less competent vector in comparison to *Ae. aegypti* as they feed on only one individual and on animals as well which reduces the chances of feeding on humans. As they adapt better in temperate regions than *Ae. aegypti*, they can now be found in other continents around the world other than Asia, where their spread is mainly facilitated by international second-hand tyres trading. As their eggs can sustain drier conditions, the eggs deposited still remain viable for

months without source of water in the tyres until they reach their destination. They have been linked to most dengue outbreaks where *Ae. aegypti* are absent (Rezza, 2012).

The virus is transmitted to other susceptible hosts through the bites of the infected mosquitoes. Once a person is infected, he/she becomes the primary carrier and reservoir for virus replication as well as source of virus for other uninfected mosquitoes. After incubation period of about 8 to 12 days, the infected mosquitoes are able to spread the virus for as long as they live. The infected person can spread the infection through *Aedes* mosquitoes, usually during day four to five or maximum up to day 12 after early symptoms appear (Ghebreab, 2017; WHO, n.d.).

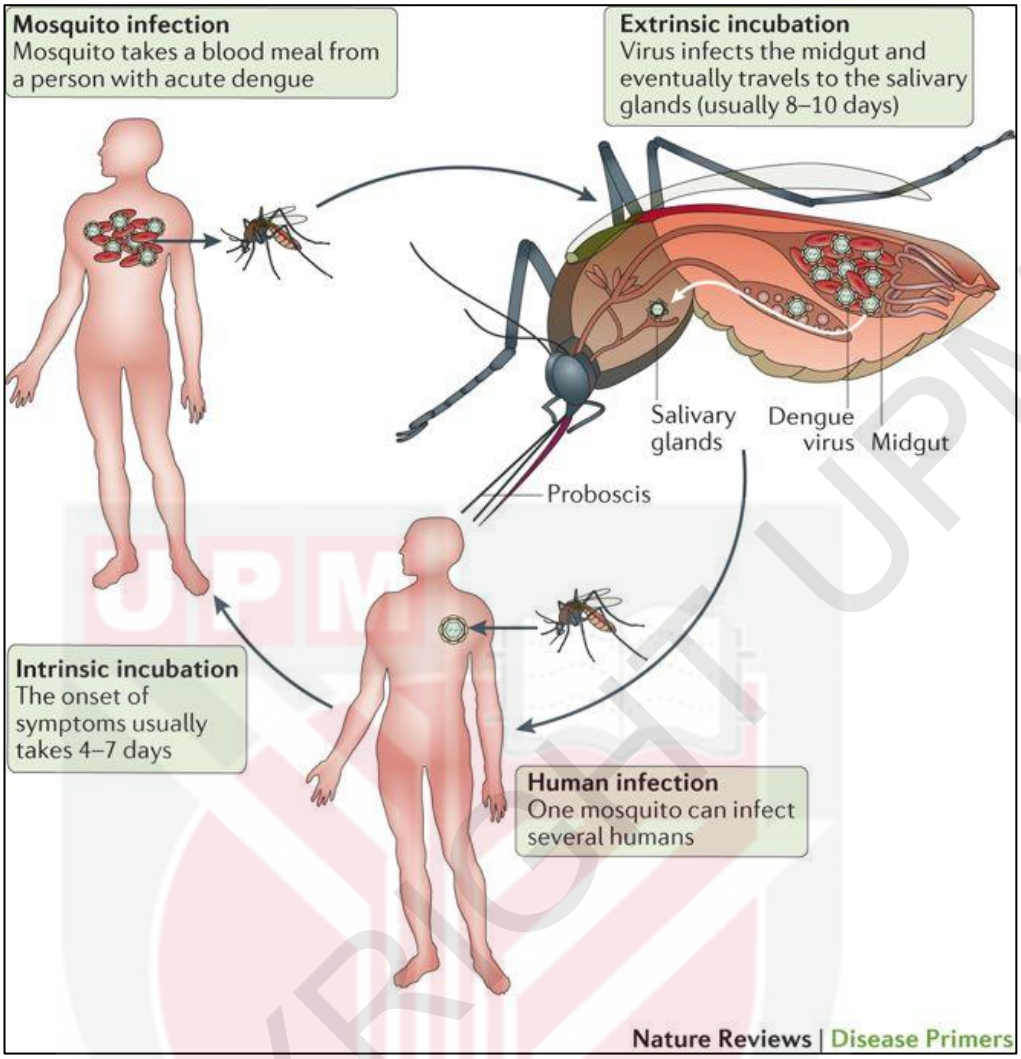

Figure 2.4. The urban dengue virus cycle in humans and mosquitoes

(Source: Guzman, Gubler, Izquierdo, Martinez & Halstead, 2016)

2.4 Clinical Manifestations and Pathophysiology

The clinical presentations of dengue fever vary from person to person. For example, a person can appear to be asymptomatic or present with different forms of severity, such as from mild self-limiting fever to potentially deadly severe dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS) (Pang & Loh, 2016; Syue et al., 2018). 50% of the dengue infections are symptomatic with three distinct phases – febrile, critical and recovery. Most of the patients will improve after the febrile stage, however 5% will progress to the critical phase (Patterson, Sammon & Garg, 2016).

The most common signs and symptoms of dengue include sudden high-grade fever that usually lasts for two to seven days, headache, myalgia, arthralgia as well as nausea and vomiting (MaHTAS, 2015).

2.4.1 Febrile Phase

The incubation period of dengue fever lies between 3 to 14 days. After that, patients usually start to develop acute and high, sometimes biphasic fever - 40°C that generally lasts for two to seven days. Frequently, other signs and symptoms come together such as rashes, vomiting, headache, retro-orbital pain as well as joint and muscle pain. Sometimes, infected individuals experience pharyngitis (sore throat), conjunctival hyperemia, mild bleeding manifestations such as petechia, gum bleeding or positive tourniquet test. Vaginal and gastrointestinal bleeding may happen but they are not common.

In terms of laboratory tests, continuous drop in total leukocyte count followed by platelet numbers are the most apparent deviation in complete blood count (CBC) (CDC, 2019; MaHTAS, 2015).

2.4.2 Warning Signs

There are signs that may indicate possible progression into critical phase after fever begins to subside between day three and eight. For example, non-stop vomiting, capillary leak, mucosal bleed, abdominal pain, tiredness or restlessness, fluid retention, enlarged and tender liver and haemoconcentration. Therefore, it is crucial to monitor defervescence and watch out for these warning signs (Patterson et al., 2016).

2.4.3 Critical Phase

When the fever subsides, patients may recover and enter the recovery phase. However, sometimes, patients with increased capillary permeability are at risk of presenting the warning signs aforementioned and subsequently developing severe dengue or dengue shock syndrome (DSS). The phase typically lasts for one to two days.

At first, the body is able to maintain the normal circulation through the physiological mechanism as diastolic blood pressure increases. Patients may look physically well in spite of initial signs of shock. Unfortunately, when hypotension comes about, systolic blood pressure will drop almost instantly, leading to irreversible shock and death. If the shock prolongs, critical bleeding manifestations can happen like dysentery, hematemesis or menorrhagia for female. Impaired organ functions are often but not exclusive.

Notably, thrombocytopenia and haemoconcentration can be identified during this phase. The level of haematocrit (HCT) corresponds with the plasma leakage and severity of the disease. Nonetheless, the HCT result can be hard to analyse due to blood volume loss or fluid replacement therapy. Others include leukopenia, transaminitis (elevated aspartate transaminase (AST) and alanine aminotransferase (ALT) levels), hypoproteinemia and hypoalbuminemia (CDC, 2019; MaHTAS, 2015).

2.4.4 Recovery Phase

This phase is also known as convalescent phase where the patient stabilizes and reabsorption of extravasated fluid happens. Laboratory findings will show improvement in white blood cells count followed by normal platelet numbers. Despite so, some patients may show rashes with generalised itchiness. New complications may arise as a result of fluid reabsorption which include acute pulmonary edema (CDC, 2019; MaHTAS, 2015).

Phase	Febrile phase	Critical phase	Recovery phase
Time frame	3 – 7 days	1 – 2 days	3 – 5 days
Signs & Symptoms	Fever is present	Fever resolves	
	• Headache • Nausea • Myalgia • Rash Warning signs	• Capillary leak • Shock • Hypotension • Severe hemorrhage • Severe organ involvement	• Diuresis • Fluid reabsorption
Laboratory findings	• Hyponatremia • Leukopenia • Thrombocytopenia • Transaminitis (aspartate transaminase & alanine transaminase) • Normal or slight increased haematocrit level	• Thrombocytopenia • Rise in hematocrit level • Leukopenia (24 hours), before platelets drop	

Figure 2.5. Clinical course of dengue fever

(Source: Patterson et al., 2016)

2.4.5 WHO Case Definition of Dengue

The Ministry of Health (MOH) Malaysia uses the same case definition by WHO in 2009. The classification illustrates the degree of severity for better management and treatment of dengue infection (Figure 2.5).

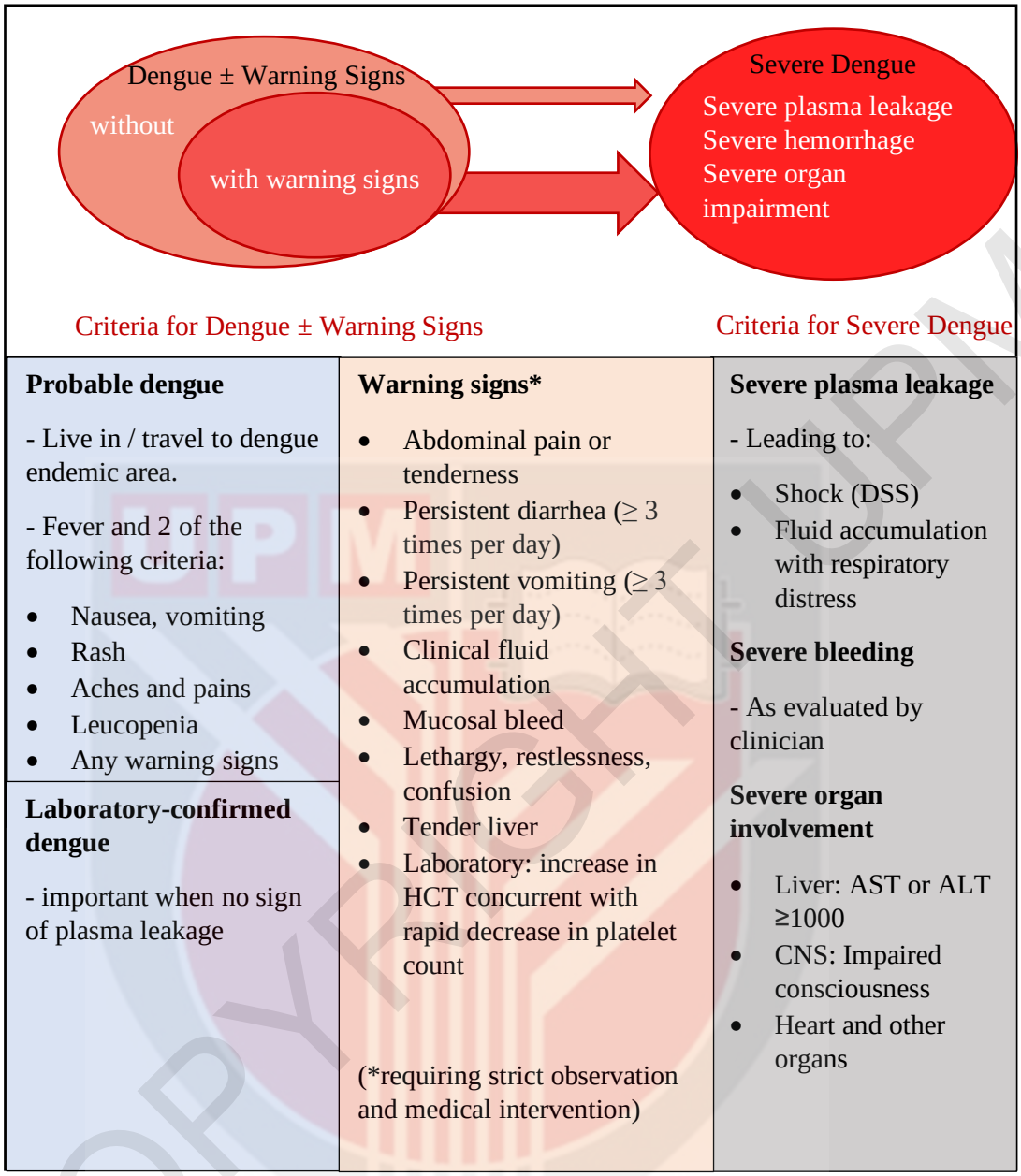

Figure 2.6. 2009 WHO Dengue Classification and Level of Severity

(Source: WHO, 2009)

2.4.6. Disease Monitoring Parameters

The most common tests being monitored are the leukocyte and platelet counts. During the early febrile phase, both of the counts are usually within normal range but they might drop quickly as the disease continues to develop. Typically, as the white cell counts decreases, platelet count also drops. Similarly, the white cell counts

recovery is accompanied by the recovery of platelet number. However, the platelet count is not directly associated with the severity of the disease.

Another parameter to be monitored is the haematocrit (HCT) level. The increased in the HCT level is an indicator of plasma fluid loss. The median of normal HCT levels of Malaysian population are as follow:

- Male <= 60 years – 46%
- Male > 60 years – 42%
- Female (all age groups) – 40%

Other equally important tests include renal profile, liver function tests, coagulation profile, lactate and blood gases (MaHTAS, 2015).

2.5 Diagnostic Tests

Accuracy and sensitivity are crucial when it comes to laboratory diagnosis methods. False positive and false negative results are minimized at all costs to avoid any unwanted consequences. The tests being carried out can include molecular testing, serological tests, or a combination of both. A few examples of the diagnostic tests are nucleic acid amplification tests (NAATs) with real time RT-PCR, IgM/IgG ELISA and NS1 antigen test (CDC, 2019; Ghebreab, 2017; MaHTAS, 2015).

2.5.1 Nucleic Acid Amplification Tests (NAATs)

NAATs are useful methods to detect the virus during the early stage of infection. It can only detect the viral RNA in serum up to seven days or less when the first symptoms appear. The result obtained takes only one to two days and is faster compared to viral isolation in cell culture. However, the test is costly and requires handling by experienced personnel. Positive test of the virus by real-time RT PCR in

a single laboratory specimen is regarded as confirmatory of dengue infection. Currently, the test sensitivity and specificity lie between 80% to 90% and more than 95% respectively (CDC, 2019; Ghebreab, 2017; MaHTAS, 2015).

2.5.2 Non-Structural Protein-1 (NS1 Antigen)

Another confirmatory test to detect acute phase of dengue infection is NS1 antigen test. NS1 antigens are glycoproteins secreted by the virus which are essential for its survival. False positive results may arise if the person is infected with yellow fever or West Nile viruses. This test is normally done on serum samples, though whole blood samples can also be used. Unlike NAATs, this test does not provide serotype information upon positive dengue infection. A negative NS1 test does not eliminate dengue infection, instead test should be done to detect the presence of IgM antibodies for any probably recent dengue exposure (CDC, 2019; MaHTAS, 2015; Mayo Clinic Laboratories, n.d.).

2.5.3 IgM/IgG ELISA

After about five days from the onset of symptoms, the viruses and antigens become undetectable. Therefore, any test to be done after that relies on the detection of antibodies instead. IgM antibody test is the most commonly used immunoassay. During primary dengue infection, IgM antibody titre is notably higher in comparison to secondary infection. It is detectable in majority (93% to 99%) of the cases after day five of the infection. A positive IgM test result is considered as unconfirmed, recent infection. In the case where negative IgM result occurs, repeat serum ought to be taken. Whereas during secondary infection, the IgM antibody titre is relatively lower, leading to the possibility of undiagnosed infection.

On the other hand, IgG antibodies will show significantly higher level by ELISA during secondary dengue infections. However, it is not a reliable diagnostic method as its level stays detectable forever if the individual has been exposed to dengue infection before (CDC, 2019; MaHTAS, 2015; WHO, 2009).

2.6 Diagnostic Challenges

The diagnosis of dengue fever can be challenging as it is often confounded by other diseases such as other types of flavivirus infections – Zika, West Niles, yellow fever, Japanese encephalitis – or other infections that also cause fever like typhoid, malaria, leptospirosis, measles and many more (WHO, 2009). Among the many different types of co-infections seen in dengue patients, bacterial infection is the most common one (Panda et al., 2018).

2.6.1 Bacteria Co-infection

Many bacterial infections seem to mimic dengue infections. Reports on co-infection in dengue patients are still scarce and limited. Most of the findings are only presented in the form of case reports or case series. Depending on the types of studies and dengue severity, the frequency of dengue/bacteria co-infection has been reported between 0.3 - 4% (See et al., 2013; Thein et al., 2015). The different types of bacteria found to be associated with dengue fever include *Leptospira*, *Salmonella*, *Staphylococcus*, *Pseudomonas* and *Rickettsia*. Several studies have demonstrated the similar clinical manifestations for dengue and leptospirosis infections during both the initial and severe phases (Mishra et al., 2013; Pan et al., 2016; Wijesinghe et al., 2015). For example, both infections present with acute fever, retro-orbital pain, jaundice, rash, thrombocytopenia and others. A number of other studies also demonstrated other

bacterial infections that show overlapping manifestations with dengue fever such as scrub typhus and typhoid fever (Table 2.2).

Table 2.2. Overlapping Manifestations of Dengue with Bacteria Co-infections

Types of Bacteria Co-infections	Findings
Dengue with Leptospirosis co-infection (Mishra et al., 2013; Pan et al., 2016; Wijesinghe et al., 2015)	➢ Similar manifestations during initial and severe phase ➢ Acute febrile illness, headache, myalgia, abdominal pain, retro-orbital pain, photophobia, jaundice, rash, thrombocytopenia, bleeding
Dengue with Scrub Typhus Co-infection (Mitra et al., 2017; Sapkota et al., 2017)	➢ Virtually indistinguishable at presentation ➢ Acute febrile illness, rash, thrombocytopenia, hepatic dysfunction
Dengue and Typhoid Co-infection (Bansal et al., 2015 Kaleem et al., 2017)	➢ Early clinical signs non-specific and similar ➢ Fever with complaints like headache, nausea, diarrhea, vomiting, loss of appetite, skin spots

Leptospirosis co-infection in dengue patients may be of particular interest in Malaysia, considering its increasing prevalence in the country (Mohd Taib et al., 2020). To date, the prevalence of dengue/leptospirosis co-infection has been reported between 0.9 – 8% from different countries. Like dengue fever, leptospirosis is also an

endemic disease in Malaysia. Both diseases will peak especially during monsoon and post monsoon seasons. Outbreaks of leptospirosis become prominent following heavy rainfall as well as floods (Faine, n.d.; Sachu et al., 2018; Suppiah et al., 2017).

Leptospirosis is a type of zoonotic disease which poses important health risk particularly in the tropical and subtropical countries. Humans are commonly exposed through direct mucosal contact with water or soil contaminated by urine of the infected animals, or indirectly through ingestion of contaminated water or food. Outdoor and agricultural workers or people who are frequently involved in leisure activities such as jungle trekking and freshwater activities are also at higher risk of getting leptospirosis infection. As both leptospirosis and dengue infections share related signs and symptoms, differential diagnosis becomes difficult when they co-exist in a patient (MOH, 2011; Radi et al., 2018)

Therefore, we designed a retrospective study, focusing on determining possible clinical and laboratory parameters that can possibly differentiate dengue and dengue with bacteria co-infection.

CHAPTER 3

METHODOLOGY

3.1 Study Type and Design

A retrospective study conducted in Serdang Hospital that involved only data collection. Clinical, laboratory and demographic data of the patients were extracted from the hospital database.

3.2 Study Population

All subjects regardless of their age, gender and medical background history who were admitted to the Serdang Hospital from 1 January 2016 to 31 December 2016. All the subjects were selected based on the inclusion and exclusion criteria.

3.2.1 Inclusion Criteria

Patients with confirmed diagnosis of dengue fever from 1 January 2016 to 31 December 2016, where they were tested positive for either dengue IgM serology tests, NS1 antigen rapid test or dengue RT-PCR test. Then, those dengue-positive patients with confirmed bacteria-coinfection(s) based on positive blood culture and sensitivity test were also identified in this study. Leptospiral co-infection dengue patients were confirmed through positive for either leptospiral IgM ELISA, MAT or PCR (Hishamshah et al., 2018; Suppiah et al., 2017).

3.2.2 Exclusion Criteria

Patients with only suspected dengue fever infections were not selected. Patients with negative results for dengue combo tests were also excluded.

3.3 Leptospiral Co-infection

From the dengue-confirmed cases, those with leptospiral co-infections were determined from blood culture and sensitivity tests. Those where blood culture tests were not available were based on the clinical diagnosis as below:

Table 3.1. Case Classification of Leptospirosis (MOH, 2011)

Case Classifications	Clinical Descriptions
Clinical Case	Sudden fever with history of exposure to water and/or environment possibly contaminated with urine of infected animals **and** present with ANY of the symptoms stated below: • Headache • Myalgia especially at the calf muscles and lumbar region • Arthralgia • Conjunctival suffusion • Meningeal irritation • Jaundice • Anuria or oliguria and/or proteinuria • Haemorrhages (from lungs and intestines) • Cardiac arrhythmia or failure • Skin rash • Gastrointestinal symptoms (nausea, vomiting, diarrhea, abdominal pain)

3.3.1 Inclusion Criteria

Leptospiral co-infection dengue patients were selected if they were tested positive for either leptospiral IgM ELISA, MAT with four-fold titre high in paired sera or leptospiral PCR were included in the study. Those that fall in the clinical case were also included if none of the leptospiral tests were available.

3.3.2 Exclusion Criteria

Dengue patients who were tested negative for leptospirosis were excluded.

3.4 Study Location

The study was based on patients admitted for dengue fever in Serdang Hospital.

3.4.1 Data Collection

Demographic, clinical and laboratory data of the patients positive of dengue infection were collected from the Information Technology Unit. The data collected was as follow.

Table 3.2. Demographic, Clinical and Laboratory Data Collected from Patients (Lee, Liu & Yang, 2005; Mohan et al., 2020; Soegijanto et al., 2018; Suppiah et al., 2017; Suppiah et al., 2018)

Demographic data	• Age • Sex • Ethnicity • Height • Weight • Body mass index (BMI) • Comorbidity
Clinical presentations	• Usage of antibiotics (type/group) • Source of infections (site of organ) • Phases (febrile, defervescence, recovery) • Days of fever (days) • Lethargy • Persist vomiting (more than 3 times per day) • Epistaxis • Presence of warning signs • Abdominal pain • Ascites • Gum bleed/mucosal bleeding • Diarrhea (more than 3 times per day) • Headache • Retro-orbital pain • Chills • Rigor • Cough • Sore throat • Rhinorrhea

		• Myalgia • Arthralgia • Severe dengue • Severe bleeding • Severe plasma leakage • Rash • Severe organ failure • Heart Rate on Presentation (HR) • Systolic Blood Pressure on Presentation (SBP) • Diastolic Blood Pressure on Presentation (DBP) • Pleural effusion • Duration of stay in the hospital
Laboratory parameters	Renal profile	• Urea • Creatinine • Potassium (K) • Sodium (Na)
	Liver function tests	• Aspartate transaminase (AST) • Alanine transferase (ALT) • Albumin
	ABG/VBG	• pH • Partial pressure of carbon dioxide ($PaCO_2$) • Partial pressure of oxygen (PaO_2) • Bicarbonate (HCO_3) • Oxygen saturation (O_2 sat) • Lactate
	Full blood count (FBC)	• Haemoglobin level (Hb) • Haematocrit level (HCT) • Platelet count • White blood cell (WBC) • Erythrocyte Sedimentation Rate (ESR)
	Others	• C-reactive protein (CRP) • Creatinine kinase (CK) • Prothrombin time (PT) • Activated partial thromboplastin time (aPTT) • International Normalized Ratio (INR) • Urine FEME (if available) • Presence of blood in urine • Presence of protein in urine • Presence of leukocyte in urine

	Infection status (primary/secondary) IgG/IgM	• IgG (Y/N) • IgM (Y/N) • NS1 Ag (Y/N)
	Bacterial infection	• Site of infection • Blood Culture and Sensitivity test (Y/N) • Type of organism (species)

3.5 Statistical Analysis

The data analysis was carried out using IBM Statistical Package for the Social Sciences (SPSS) version 24. Categorical variables such as the clinical presentations (present/absent) such as lethargy, epistaxis, sore throat and so on and so forth were expressed as frequencies (percentages). Chi-square test was performed to analyze the significance of the categorical variables. On the other hand, continuous variables which refer to laboratory parameters results categorized under renal and liver function tests, ABG/VBG, FBC and others. Continuous variables were checked for their normality using Shapiro-Wilk test as it is more sensitive (Yap & Sim, 2011). Normally distributed continuous variables were expressed as mean and standard deviation (SD) and analyzed using Independent-samples T test (McCluskey & Lalkhen, 2007; McHugh, 2013). Non-normally distributed continuous dependent variables were presented as median and interquartile range (IQR) and analyzed using Mann-Whitney U test. All analyses were performed based on 95% confidence interval. A p-value less than 0.05 ($p < 0.05$) was considered statistically significant (Hishamshah, 2018; Suppiah et al., 2017; Suppiah et al., 2018). Binary logistic regression analysis was performed to determine the significant prognosis parameters among the statistically significant parameters with $p < 0.05$ from the Chi-square test and Independent-samples T test (refer to Table 4.1, 4.2 and 4.3).

3.6 Study Ethics

The study was conducted in compliance with the ethical principles outlined in the Declaration of Helsinki and Malaysia Good Clinical Practice Guideline. Ethical approval has been obtained from the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia (Reference number: NNMR-19-3445-51176). All personal information will remain strictly confidential and will not be disclosed to any party. All subjects will remain anonymous throughout the study.

CHAPTER 4

RESULTS

The total number of subjects with confirmed dengue diagnosis was found to be 1,730 cases from 1 January 2016 to 31 December 2016. From the 4,899 notified dengue cases, 2,525 cases were omitted from not fulfilling the inclusion criteria. 644 cases had incomplete or missing clinical information were also excluded. 72 cases were diagnosed with bacteria co-infection where 26 cases were leptospiral co-infection. The total number of patients under each clinical presentations and laboratory parameters were different due to incomplete or missing clinical information. All missing data were not included in the statistical analysis. Chi-square analysis done on the clinical presentations between two groups of dengue patients are shown in Table 4.1.

Table 4.1 Clinical Presentations of Dengue Groups

	Groups of patients		
	Dengue patients without bacteria co-infection (n = 1635)	**Dengue patients with bacteria co-infection (n = 72)**	**P-values**
	% (n)	% (n)	
Clinical presentations			
Lethargy	17.2 (281)	22.2 (16)	0.270
Persistent vomiting (3 times or more/day)	32.8 (536)	41.7 (30)	0.117
Epistaxis	3.1 (51)	0.0 (0)	0.107
With warning signs	63.8 (1043)	77.8 (56)	**0.015***
Abdominal pain	32.0 (524)	38.9 (28)	0.225
Ascites	0.7 (11)	0.0 (0)	0.622
Mucosal/gum bleeding	15.1 (247)	6.9 (5)	0.056
Persistent diarrhea (3 times or more/day)	23.1 (377)	31.9 (23)	0.081
Headache	49.5 (810)	48.6 (35)	0.877
Retro-orbital pain	15.8 (259)	16.7 (12)	0.851

Chills	26.9 (440)	37.5 (27)	**0.049***
Rigor	21.6 (353)	30.6 (22)	0.072
Cough	18.3 (299)	13.9 (10)	0.343
Sore throat	8.9 (146)	8.3 (6)	0.862
Rhinorrhea	7.0 (115)	4.2 (3)	0.252
Myalgia	64.2 (1050)	61.1 (44)	0.590
Arthralgia	60.5 (989)	62.5 (45)	0.733
Severe dengue	0.1 (2)	2.8 (36)	**0.000***
Severe bleeding	0.1 (1)	0.0 (0)	0.958
Severe leakage	0.4 (7)	4.2 (3)	**0.007***
Rash	9.6 (157)	4.2 (3)	0.121
Organ failure	0.1 (2)	1.4 (1)	0.121
Pleural effusion	0.9 (14)	1.4 (1)	0.478
Dengue infection phase			
Afebrile	0.1 (1)	0 (0)	
Febrile	45.4 (416)	61.2 (41)	0.103
Defervescence	50.8 (465)	35.8 (24)	
Recovery	1.7 (16)	0 (0)	
Critical	2.0 (18)	3.0 (2)	

***** Indicates variables with *P* value < 0.05 which were selected for binary logistic regression analysis

Only a few of the clinical presentations showed significant association with the groups of patients. Dengue patients with bacteria co-infection were slightly more prone to present with warning signs (p = 0.015) with 77.8% compared to 63.8% in dengue without bacteria co-infection patients. Besides, chills (p = 0.049) seemed to be observed at higher percentage among dengue patients with bacteria co-infection (37.5%), compared to patients with dengue infection only (26.9%). Severe dengue (p < 0.001) and severe leakage (p = 0.007) were both observed to be higher in percentages in dengue with bacteria co-infection.

Table 4.2. Laboratory Parameters of Dengue Groups

Laboratory parameters	Groups of patients		P-value
	Dengue patients without bacteria co-infection (n = 1641)	Dengue patients with bacteria co-infection (n = 72)	
Renal profile			
Urea (mmol/L), mean (SD)	3.89 (1.80)	5.3 (4.33)	**0.006***
K (mmol/L), mean (SD)	3.7 (0.5)	3.7 (0.6)	0.729
Na (mmol/L), mean (SD)	133.21 (4.04)	132.61 (4.28)	0.289
Creatinine (µmol/L), mean (SD)	71.6 (25.2)	107.6 (76.2)	**0.000***
Liver function tests			
AST (U/L), mean (SD)	142.7 (199.9)	239.5 (529.6)	0.058
ALT (U/L), mean (SD)	73.9 (78.7)	128.5 (214.4)	**0.005***
Albumin (g/L), mean (SD)	36.4 (4.8)	34.7 (6.1)	**0.045***
ABG/VBG			
pH, mean (SD)	7.389 (0.079)	7.373 (0.084)	0.208
$PaCO_2$ (mm/Hg), mean (SD)	40.13 (9.29)	39.45 (9.22)	0.669
PaO_2 (mm/Hg), mean (SD)	47.27 (56.47)	42.39 (32.56)	0.847
HCO_3 (mmol/L), mean (SD)	23.08 (3.89)	22.72 (4.10)	0.582
Lactate (mmol/L), mean (SD)	2.5 (4.0)	2.4 (2.0)	0.597
FBC			
Hb level day 1 (g/dL), mean (SD)	13.7 (1.9)	14.8 (2.5)	**0.001***
Hb level day 2 (g/dL), mean (SD)	13.6 (1.8)	14.5 (2.4)	**0.003***
Hb level day 3 (g/dL), mean (SD)	13.4 (2.1)	14.4 (2.3)	**0.011***
Hb level day 4 (g/dL), mean (SD)	12.9 (2.0)	14.1 (2.7)	0.134
HCT level day 1 (%), mean (SD)	41.1 (5.3)	44.7 (7.0)	**0.000***
HCT level day 2 (%), mean (SD)	40.4 (5.5)	43.3 (6.4)	**0.001***
HCT level day 3 (%), mean (SD)	39.9 (6.8)	43.0 (6.5)	0.051

HCT level day 4 (%), mean (SD)	38.4 (5.2)	42.2 (7.5)	0.080
Platelet count day 1 (x10⁹/L), mean (SD)	101 (72)	126 (76)	**0.023***
Platelet count day 2 (x10⁹/L), mean (SD)	77 (57)	97 (67)	0.064
Platelet count day 3 (x10⁹/L), mean (SD)	74 (55)	81 (64)	0.841
Platelet count day 4 (x10⁹/L), mean (SD)	77 (45)	82 (62)	0.853
WBC day 1 (x10⁹/L), mean (SD)	4.5 (3.8)	5.8 (3.4)	**0.000***
WBC day 2 (x10⁹/L), mean (SD)	4.4 (2.6)	5.1 (3.2)	0.077
WBC day 3 (x10⁹/L), mean (SD)	5.4 (3.1)	5.7 (3.6)	0.676
WBC day 4 (x10⁹/L), mean (SD)	6.4 (3.7)	5.8 (3.4)	0.520
Others			
CK (U/L), mean (SD)	457.22 (527.27)	3902.57 (19017.68)	**0.000***
PT (s), median (IQR)	13.6 (13.1 – 14.8)	14.0 (13.2 – 14.7)	0.890
aPTT (s), median (IQR)	50. 1 (44.7 – 61.6)	49.0 (40.8 – 56.2)	0.158
INR, median (IQR)	1.03 (0.98 – 1.15)	1.06 (0.99 – 1.13)	0.934
SBP, mean (SD)	118 (14.2)	120 (18.3)	0.529
DBP, mean (SD)	72 (10.5)	71 (12.6)	0.417
HR, mean (SD)	91 (21.0)	95 (19.0)	**0.043***
Days of fever, mean (SD)	4.83 (1.621)	4.37 (1.447)	**0.015***

***** Indicates variables with *P* value < 0.05 which were selected for binary logistic regression analysis

Investigation of laboratory parameters in relation to the groups of dengue patients was stated in Table 4.2. A number of parameters were found to have significant association with the groups of dengue patients. Dengue patients with bacteria co-infection seemed to have higher mean urea at 5.3 mmol/L (p = 0.006) than the control group without bacteria co-infection at 3.89 mmol/L. Besides, mean creatinine (p < 0.001) was also observed to be much higher in dengue patients with bacteria co-infection at 107.6 µmol/L, compared to dengue group without bacteria co-infection at 71.6 µmol/L. ALT which was also significant at p = 0.005, was higher in

dengue with bacteria co-infection group, 128.5 U/L in comparison to 73.9 U/L in dengue without bacteria co-infection group. On the other hand, mean albumin ($p = 0.045$) was found to be slightly lower in dengue with bacteria co-infection group, 34.7 g/L compared to 36.4 g/L in the other group. As for the full blood count blood test, mean Hb level day 1 ($p = 0.001$), mean Hb level day 2 ($p = 0.003$), mean Hb level day 3 ($p = 0.011$), mean HCT level day 1 ($p < 0.001$), mean HCT level day 2 ($p = 0.001$), mean platelet count day 1 ($p = 0.023$) and mean WBC day 1 ($p < 0.001$) were significant parameters. All the readings were higher marginally in the dengue with bacteria co-infection group. The mean CK level ($p < 0.001$) was strikingly higher in dengue patients with bacteria co-infection (3902.57 U/L) than dengue patients without bacteria co-infection (457.22 U/L). The mean heart rate ($p = 0.043$) and days of fever ($p = 0.015$) were also found to have significant association with the groups. The mean heart rate only higher by a slight extent in dengue with bacteria co-infection group at 95 beats per minute compared to 91 beats per minute in dengue without bacteria co-infection group. The average days of fever for both groups were closely similar, between four to five days.

Table 4.3. Binary logistic regression analysis for predictors of dengue with bacteria co-infection

Clinical variables	Exp (B)	95% CI		p-value
		Lower	Upper	
Warning signs: Presence	0.479	0.264	0.868	**0.015***
Chills: Presence	0.627	0.379	1.039	0.070
Severe dengue: Presence	0.074	0.008	0.682	**0.022***
Severe leakage: Presence	0.153	0.031	0.749	**0.021***
Days of fever	0.787	0.669	0.925	**0.004***
Heart rate	1.009	0.999	1.020	0.086
Urea	0.911	0.705	1.178	0.479
Creatinine	1.016	0.995	1.037	0.143

ALT	1.002	0.999	1.006	0.243
Albumin	0.942	0.887	1.001	0.055
Hb day1	1.743	1.227	2.476	**0.002***
Hb day2	1.028	0.679	1.557	0.895
Hb day3	0.806	0.574	1.133	0.214
HCT day1	1.314	1.023	1.687	**0.033***
HCT day2	0.928	0.717	1.202	0.572
Platelet day1	1.017	0.989	1.045	0.239
WBC day1	1.476	0.945	2.305	0.087
CK	1.001	1.000	1.001	**0.000***

***** Indicates variables with *P* value < 0.05; Exp (B) = odds ratio; CI = 95% confidence interval for the odds ratio
Same comment for the asterisk *. What is CI? What is Exp (B)? All need to mention.

The clinical and laboratory parameters that were significant in the chi-square tests were further analyzed using binary logistic regression to determine if they can be significant predictors for dengue with bacteria co-infection model. The regression analysis showed that the presence of warning signs (p = 0.015), severe dengue (p = 0.022), severe leakage (p = 0.021), average days of fever (p = 0.004), mean Hb level day 1 (p = 0.002), mean Hb level day 2 (p = 0.033) and mean CK level (p < 0.001) were significant at their respective p-values.

Statistically, Exp (B) stands for odds ratio, that is the ratio between probabilities: the probability of an event favourable to an outcome of interest to the probability of an event against the same outcome (Sperandei, 2014). In logistic regression, the interpretations for continuous and categorical variables are different. For categorical variable, a value greater than 1 means that the event is more likely at the presence of the clinical variables. On the contrary, if the Exp (B) is less than 1, it indicates that the event is less likely at the presence of the variables (Anderson, Jin & Grunkemeier, 2003). The Exp (B) are normally presented with their 95% confidence interval (CI). The 95% CI means that we can be 95% confident that the range of values

contains the values of the odds ratio in a population. This helps us to assess the significance of the values practically.

From the results in table 4.3, the presence of warning signs, severe dengue and severe leakage are significant at their respective p-values. However, the odds that they are present in dengue with bacteria co-infection are less likely because their odds were less than 1 (OR < 1). For example, the presence of warning signs is only 0.479 times higher in dengue with bacteria co-infection group compared to dengue without bacteria co-infection. In other words, these parameters are less likely to be shown in dengue with bacteria co-infection. The 95% CI for the presence of warning signs were between 0.264 and 0.868. It means that we can be 95% certain that the odds ratio for presence of warning signs will lie between 0.264 and 0.868 in the population.

As for continuous variables, if the Exp (B) is greater than 1, it means that the event is more likely to happen as the predictor increases by a unit (Anderson, Jin & Grunkemeier, 2003). On the other hand, if the Exp (B) is less than 1, it shows that the event is less likely to occur when the predictor increases by a unit. The average number of days of fever is considered a poor predictor sign for the model because as the mean number of days of fever increases by one unit, the odds of associating with bacteria co-infection decreases by 21.3%. Conversely, the means of haemoglobin levels day 1 and 2 as well as CK levels can be good predictor signs for the model. For instance, as the CK levels increase by one unit, the odds of associating with bacteria co-infection increases by 0.1%. As for Hb levels on day 1 and day 2, their odds of associating with

bacteria co-infection increase by 74.3% and 2.8% respectively as their values increase by a unit.

Table 4.4. Clinical presentations in relation to leptospiral co-infection

Clinical presentations	Types of bacteria co-infection		P-value
	Non-leptospiral (n = 45)	Leptospiral (n = 26)	
	% (n)	% (n)	
Lethargy	26.7 (12)	15.4 (4)	0.273
Persistent vomiting (3 times or more/day)	35.6 (16)	53.8 (14)	0.133
With warning signs	77.8 (35)	80.8 (21)	0.766
Abdominal pain	44.4 (20)	30.8 (8)	0.256
Mucosal/gum bleeding	6.7 (3)	7.7 (2)	0.609
Persistent diarrhea (3 times or more/day)	31.1 (14)	34.6 (9)	0.761
Headache	48.9 (22)	50.0 (13)	0.928
Retro-orbital pain	15.6 (7)	19.2 (5)	0.464
Chills	44.4 (20)	26.7 (7)	0.143
Rigor	35.6 (16)	23.1 (6)	0.273
Cough	15.6 (7)	11.5 (3)	0.464
Sore throat	6.7 (3)	7.7 (2)	0.609
Rhinorrhea	6.7 (3)	0.0 (0)	0.248
Myalgia	62.2 (28)	57.7 (15)	0.707
Arthralgia	62.2 (28)	61.5 (16)	0.954
Severe dengue	55.6 (25)	42.3 (11)	0.282
Severe leakage	2.2 (1)	7.7 (2)	0.301
Rash	6.7 (3)	0.0 (0)	0.248
Organ failure	2.2 (1)	0.0 (0)	0.634
Pleural effusion	0.0 (0)	3.8 (1)	0.366
Dengue infection phase			
Febrile	65.1 (28)	56.5 (13)	0.141
Defervescence	34.9 (15)	34.8 (8)	
Critical	0.0 (0)	8.7 (2)	

***** Indicates variables with *P* value < 0.05 which were selected for binary logistic regression analysis

Table 4.4 showed the investigation of clinical presentations and laboratory parameters in relation to leptospiral co-infections. None of the parameters showed significant association with leptospiral co-infections.

Table 4.5. Laboratory parameters in relation to leptospiral co-infection

Laboratory parameters	Types of bacteria co-infection		P-value
	Non-leptospiral (n = 44)	Leptospiral (n = 26)	
Renal profile			
Urea (mmol/L), mean (SD)	5.30 (4.81)	5.19 (3.65)	0.923
K (mmol/L), mean (SD)	3.7 (0.5)	3.7 (0.7)	0.992
Na (mmol/L), mean (SD)	131.9 (4.0)	133.7 (4.8)	0.114
Creatinine (µmol/L). mean (SD)	106.4 (81.8)	113.3 (69.3)	0.429
Liver function tests			
AST (U/L), mean (SD)	171.9 (200.3)	354.6 (838.2)	0.446
ALT (U/L), mean (SD)	106.3 (137.6)	166.2 (308.9)	0.505
Albumin (g/L), mean (SD)	33.93 (5.6)	35.70 (6.8)	0.442
ABG/VBG			
pH, median (IQR)	7.383 (7.358 – 7.415)	7.379 (7.350 – 7.404)	0.189
$PaCO_2$ (mm/Hg), mean (SD)	38.23 (7.72)	41.55 (11.25)	0.279
PaO_2 (mm/Hg), mean (SD)	45.06 (36.26)	37.79 (25.03)	0.582
HCO_3 (mmol/L), mean (SD)	22.60 (3.96)	22.90 (4.40)	0.874
O_2 saturation (%), mean (SD)	60.20 (26.35)	52.63 (21.65)	0.439
Lactate (mmol/L), mean (SD)	2.25 (1.12)	2.54 (2.93)	0.652
FBC			
Hb level day 1 (g/dL), mean (SD)	14.6 (2.5)	14.9 (2.4)	0.623
Hb level day 2 (g/dL), mean (SD)	14.0 (2.4)	15.1 (1.7)	0.062
Hb level day 3 (g/dL), mean (SD)	14.1 (2.6)	14.8 (1.7)	0.155
Hb level day 4 (g/dL), mean (SD)	13.5 (3.0)	14.9 (1.6)	0.068
HCT level day 1 (%), mean (SD)	43.9 (6.9)	45.8 (7.2)	0.274
HCT level day 2 (%), mean (SD)	42.2 (6.7)	44.6 (4.6)	0.055
HCT level day 3 (%), mean (SD)	42.2 (7.3)	43.8 (4.8)	0.241
HCT level day 4 (%), mean (SD)	40.5 (8.5)	44.5 (4.7)	0.052

Platelet count day 1 (x10⁹/L), mean (SD)	131 (73)	118 (81)	0.308
Platelet count day 2 (x10⁹/L), mean (SD)	99 (65)	94 (71)	0.771
Platelet count day 3 (x10⁹/L), mean (SD)	80 (68)	84 (58)	0.455
Platelet count day 4 (x10⁹/L), mean (SD)	80 (59)	87 (70)	0.717
WBC day 1 (x10⁹/L), mean (SD)	6.0 (3.5)	5.4 (3.4)	0.455
WBC day 2 (x10⁹/L), mean (SD)	5.0 (2.8)	5.3 (3.8)	0.717
WBC day 3 (x10⁹/L), mean (SD)	5.6 (3.4)	5.8 (4.1)	0.510
WBC day 4 (x10⁹/L), mean (SD)	6.1 (4.1)	5.1 (2.1)	0.887
ESR, median (IQR)	15.5 (12.5 – 55.0)	17.0 (3.5 – 31.5)	0.584
Others			
CRP (mg/L), median (IQR)	80.76 (40.9 – 173.8)	3.6 (2.61 – 6.26)	**0.000***
CK (U/L), median (IQR)	713.00 (1779.5)	1352 (3296)	**0.048***
PT (s), mean (SD)	13.94 (1.16)	16.28 (8.59)	0.285
aPTT (s), mean (SD)	50.48 (13.58)	59.57 (33.12)	0.249
INR, mean (SD)	1.08 (0.18)	1.32 (0.93)	0.347
SBP, mean (SD)	120.75 (16.78)	119.62 (17.12)	0.725
DBP, mean (SD)	71.36 (10.95)	70.73 (14.83)	0.558
HR, mean (SD)	95.02 (15.96)	93.68 (22.62)	0.636
Days of fever, mean (SD)	4.18 (1.419)	4.72 (1.487)	0.243
Duration of stay in hospital, mean (SD)	9.69 (14.05)	7.4 (13.28)	0.263

***** Indicates variables with *P* value < 0.05 which were selected for binary logistic regression analysis

Table 4.5 demonstrated the data on laboratory parameters in relation to leptospiral co-infection. The examples of the laboratory parameters that were significant included CRP ($p < 0.001$) and CK ($p = 0.048$) levels. Both of the parameters were remarkably higher in the non-leptospiral co-infection group than leptospiral co-infection. The CRP and CK levels were about 22 and 14 times higher respectively in non-leptospiral co-infection than leptospiral co-infection group. Both of the

parameters were further analyzed using binary logistic regression to determine if they have significant relationship with the dengue leptospiral co-infection model.

Table 4.6. Binary logistic regression analysis for predictors of dengue with leptospiral co-infection

Clinical variables	Exp (B)	95% CI		p-value
		Lower	Upper	
CRP (mg/L)	0.892	0.805	0.989	**0.030***
CK (U/L)	1.000	0.999	1.001	0.896

***** Indicates variables with *P* value < 0.05; Exp (B) = odds ratio; CI = 95% confidence interval for the odds ratio

Same comment for asterisk *. What is CI? What is Exp (B)? All need to mention.

Based on the regression analysis, CRP level was found to have significant effect (p = 0.030) on the model. However, for every unit increase in the CRP level, the odds of leptospiral co-infection decreases by 10.8%. This means that higher CRP level is less likely to have association with leptospiral co-infection. Its 95% CI indicates that we can be 95% confident that for every unit increase in the CRP level, the odds of associating with leptospiral co-infection will decrease (between 1.1% to 19.5%) in a population.

CHAPTER 5

DISCUSSION

5.1 Overview

Our results showed a small but significant percentage of dengue patients with bacteria co-infection. In our study, 72 (4.2%) of all confirmed dengue patients had bacteria co-infection, which is closely in agreement with 4.1% among 268 dengue cases in another study who were co-infected with leptospirosis (Suppiah et al., 2017). The present study focused on determining clinical and laboratory parameters that can serve as predictor signs for dengue with bacteria co-infection as well as dengue with leptospiral co-infection.

5.1.1 Dengue with bacteria co-infection

Our study showed that the presence of warning signs (odds ratio [OR] = 0.479), severe dengue (OR = 0.074) and severe leakage (OR = 0.153) are less likely to have bacteria co-infection because their odds ratios were less than 1 (OR < 1). However, these findings contradict with some other related studies. For example, a study by Suppiah et al in 2017 demonstrated that the symptom shock was significantly associated with dengue-leptospirosis co-infection. Shock can be caused by severe plasma leakage in dengue patients. Another study also showed that dengue patients with concurrent bacteraemia were more likely to present with severe dengue shock syndrome and warning sign of haemoconcentration (Thein et al., 2015). Thien and others also highlighted that patients with bacteria co-infection would have more serious illness and poorer prognosis. Bacteria co-infection has been associated with severe dengue and higher mortality (Panda et al., 2018). The reason behind this is probably due to the more severe dengue infection caused by bacteria co-infection (Nagassar, Bridgelal-Nagassar, McMorris & Roye-Green, 2012; Trunfio et al., 2016).

The bacterial toxins, especially gram-negative bacteria seem to be correlated with severe dengue fever, causing exacerbated immune responses or increase viral load. The reason why our results might contradict could be due to different subject populations and sample size. For instance, our study involved a more generalized population while some only included adult patients, severe dengue patients while others excluded those with underlying medical conditions. Risk factors like age, gender, comorbidities and severity of dengue infection have been shown to have effect on bacteria co-infection in dengue patients.

Next, our results show that the average days of fever is also less likely to be associated with bacteria co-infection (OR = 0.787). This is in contrast to some of the of the findings as well. A study that involved adult patients, prolonged fever (>5 days) is suggestive of very high risk for concurrent bacteraemia, particularly when they have severe dengue fever. Prolonged fever was about 26 times higher more likely in co-infection than with severe dengue only (Lee et al., 2005). In a case report of a child with dengue fever, blood culture test revealed positive for *Salmonella typhi* co-infection when fever persisted despite other recovery signs (Jagadishkumar et al., 2016; Srinivasaraghavan et al., 2015). Other case reports were also tested positive for *Salmonella* co-infection after fever persisted for more than seven days (>7 days) (Bansal et al., 2015; Siddiqui et al., 2019). The reason why our results might differ is probably due to different sample size and study designs. In comparison, our study had a smaller number of dengue patients with bacteria co-infection. There are also limited studies on dengue with bacteria co-infection where most of them were presented in the form of case reports or case series which might not be indicative of a whole

population.You should provide possible explanation on your result which was different fr

As for haemoglobin and haematocrit levels on day 1, our results showed that they are more likely (OR >1) to be associated with bacteria co-infection. This agrees with a study that showed that haemoconcentration (haematocrit change >= 20%) was more likely to be observed in adult patients with concurrent bacteraemia than without concurrent bacteraemia (Thein et al., 2017). From our study findings, one fatal case was observed to have leptospiral co-infection. The clinical and laboratory parameters revealed that the deceased was in the critical phase of dengue fever, had warning sign of haemoconcentration (33%) with concurrent thrombocytopenia. His presentation was consistent with the clinical course of dengue infection (MaHTAS, 2015). The interpretation of haemoglobin and haematocrit levels can also be difficult as they can be confounded by factors such as bleeding or excessive fluid replacement. Although both haemoglobin and haematocrit levels have significant effect on the dengue bacteria co-infection model, they ought to be examined cautiously.

Another variable that is of great interest is the CK levels. Our analysis suggested that CK level is more likely to be associated with bacteria co-infection in dengue patients (OR = 1.001). CK is a biomarker which is normally released when there is injury to the muscle tissues. Myositis in dengue fever is rare and it is usually acute and mild (Paliwal et al., 2011). Study by Paliwal and others demonstrated that dengue myositis could have been caused by invasion of the virus directly into the muscles. In an *in vitro* study using human satellite cells, they are highly susceptible to dengue virus infection. Replication and infection of dengue virus are shown to be

highly efficient in the muscle cells. Another probable pathogenesis dengue myositis is due to the release of myotoxic cytokines. The cytokines were produced in response to the viral infection, leading to immune mediated damage of the muscle fibres. In another study by Ehelepola et al, *Staphylococcus aureus* has also shown to be capable of causing myositis (Ehelepola, Rajapaksha, Dhanapala, Thennekoon & Ponnamperuma, 2018). However, myositis and high serum CK level is not commonly seen in dengue complications (Aggarwal, Jain, Pawar, Jain & Mittal, 2014). Therefore, the authors highlighted possible co-infection and prompt examination when dengue patients show signs and symptoms of myositis with high CK level.

5.1.2 Dengue with leptospiral co-infection

Our regression analysis showed that CRP level is less likely (OR = 0.892) to be associated with dengue leptospiral co-infection. In other words, CRP is more likely to be associated with other bacterial co-infection. This agrees with a study by Chen et al in 2016. Chen and others also established that higher CRP level can be a sign of concurrent bacteraemia in severe dengue patients (Chen et al., 2016). However, another study marked CRP as a potential marker in dengue with leptospiral co-infection. CRP is a biomarker of inflammation in the human body (Donovan & Watson, 2019). Study by Le Turnier et al deduced that an increased in CRP level should raise suspicion of dengue leptospiral co-infection. They also demonstrated that a CRP value of higher than 50 mg/L is able to differentiate between leptospirosis and dengue fever (Le Turnier et al., 2019). Thus, physicians need to look for possible co-infection in dengue patients with elevated CRP level.

Although not significant, AST was observed to be significantly higher in dengue bacterial co-infected group, about two times when compared to no bacterial co-infection. Similarly, this could be observed in the leptospiral-coinfected dengue group. This might suggest a link between AST and dengue/leptospiral co-infection. AST and ALT are normally high in levels when there is damage to the liver. One possible cause can be due to acute bacteria-induced hepatitis. A study by Treeprasertsuk and Wilairatana deduced co-infection as one of the factors that can cause acute liver failure in dengue patients (Treeprasertsuk & Wilairatana, 2017). Other organs and cells involvement such as brain, red blood cells, kidney, cardiac and skeletal muscles can also lead to the release of AST (Schaefer & John, 2020; Trung et al.,2010). Acute kidney injury (AKI) is also very likely to happen in dengue patients with bacterial co-infection, which can cause increased AST. Rhabdomyolysis which is one of the atypical manifestations possibly shown in dengue patients with bacteria co-infection is known to be the cause of AKI. It is established that muscle injury leads to loss of fluid and subsequent blood flow to the kidneys. Eventually, a series of immune responses cause intrarenal vasoconstriction and AKI. Other than that, myoglobin and myoglobinuria can damage the renal tubules when produced tremendously from the breakdown of muscles (Pothapregada, Kamalakannan & Thulasingam, 2016; Puapatanakul, Lumlertgul & Srisawat, 2017). On top of that, rhabdomyolysis also correlates with high CK level (Lee et al., 2016). The mean CK level, although not significant in our regression model for dengue leptospiral co-infection, its value was seen to be notably higher by about two times than non-leptospiral co-infection (Table 4.5). This biomarker is also of significant interest in a study by Suppiah et al (Suppiah et al., 2017). Their findings demonstrated that CK level was almost three times higher in the co-infection group, although not statistically

significant in their findings probably due to small number of co-infected patients. They believed that extreme myalgia caused by bacteria co-infection in dengue patients raised the CK level to a considerably higher level.

CHAPTER 6

CONCLUSION

6.1 Summary/Conclusion

In conclusion, elevated CK level, haemoglobin and haematocrit levels could serve as potential predictors for dengue with bacteria co-infection. Nevertheless, other confounding factors need to be taken into consideration especially when examining the haemoglobin and haematocrit levels. Even though the presence of warning signs, severe dengue and severe leakage were not significant predictors for bacteria co-infection in our study, they have shown to be related to bacteria co-infection in some studies. Besides, the average number of days of fever was also found to be not a significant predictor for dengue bacteria co-infection in our analysis, but a number of findings have shown otherwise. Therefore, we believe that prolonged fever should raise suspicion of possible co-infection despite other recovery signs of dengue fever. The CRP level has always been a biomarker of interest in identifying dengue/leptospirosis co-infection. Although it is not a significant predictor in our study, this should not rule out the likelihood of dengue/leptospirosis co-infection.

6.2 Study Strengths & Limitations

Our study has several strengths and weaknesses. Current study serves as a preliminary study to further investigate the significant clinical and laboratory parameters associated with dengue with bacteria co-infection. Moreover, our findings may call for more studies to establish stronger evidence for the significant parameters in our study, focusing on a bigger population and locations. Besides, all the diagnoses of dengue fever were confirmed by laboratory tests. The constant effect of odds ratio in logistic regression allows us to understand how each change in the predictor variables uniquely affect the outcome.

However, our study was conducted in one centre only. The data available from the hospital were only of retrospective type. Thus, the conclusion made cannot be applied to other diseases not present in the setting. Furthermore, retrospective data are very much dependent on the completeness of the medical record in the hospital database. Sometimes, incomplete or missing data are inevitable. Therefore, some of our data set which were incomplete were not included in the analysis, and this reduces the sample size. Selection bias might also have occurred which can affect the analysis due to the retrospective nature of the study.

6.3 Recommendation

Based on the limitations encountered in our study, we recommend acquiring a more complete data set for more accurate analysis. Our initial intention was to collect the data for three-year period from 2014 to 2016, however, due to the Movement Control Order (MCO) upon the COVID-19 pandemic, we only managed to complete for one year. Therefore, we suggest to continue on the year 2014 and 2015. This might be able to compensate for the data unavailable in 2016. Other than that, we also propose that future studies involve a larger study area in order to deduce based on stronger evidence of the significant predictors. We also put forward the use of receiver operating curve (ROC) as the next step to test for the predictors' sensitivity and specificity as well as acquiring a cut-off value that could diagnose possible bacteria co-infection.

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APPENDICES

Supplementary Figure 1

JAWATANKUASA ETIKA & PENYELIDIKAN PERUBATAN
(Medical Research & Ethics Committee)
KEMENTERIAN KESIHATAN MALAYSIA
d/a Kompleks Institut Kesihatan Negara
Blok A, No 1, Jalan Setia Murni U13/52,
Seksyen U13, Bandar Setia Alam,
40170 Shah Alam, Selangor.

Tel: 03-3362 8888/8205

Ref : KKM/NIHSEC/ P19-2760 (7)
Date: 21- Jan -2020

Dr. Zulkefley Bin Othman
UNIVERSITY PUTRA MALAYSIA (UPM)

Dear Sir / Mdm,

ETHICS INITIAL APPROVAL: NMRR-19-3445-51176 (IIR)
A retrospective study of dengue and bacteria co-infection in patients admitted to hospital

This letter is made in reference to the above matter.

2. The Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia (MOH) has provided ethical approval for this study. Please take note that all records and data are to be kept strictly **CONFIDENTIAL** and can only be used for the purpose of this study. All precautions are to be taken to maintain data confidentiality. Permission from the District Health Officer / Hospital Administrator / Hospital Director and all relevant heads of departments / units where the study will be carried out must be obtained prior to the study. You are required to follow and comply with their decision and all other relevant regulations, including the Access to Biological and Benefit Sharing Act 2017.

3. The investigators and study sites involved in this study are:

HOSPITAL SERDANG
Associate Professor Dr. Chee Hui Yee
Associate Professor Tham Chau Ling
Dr Siti Zulaikha Binti Zakariah
Dr Wan Zul Haikal Hafiz Bin Wan Zukiman
Dr. Zulkefley Bin Othman (Penyelidik Utama)

4. The following study documents have been received and reviewed with reference to the above study:

Documents received and reviewed with reference to the above study:

1. Study Protocol with attached Data Collection Tables_Version 4, dated 13-Jan-2020
2. Investigator's documents : Declaration of Conflict of Interest (COI), IA-HOD-IA, and CV:
   a) Associate Professor Dr. Chee Hui Yee
   b) Associate Professor Tham Chau Ling
   c) Dr Siti Zulaikha Binti Zakariah
   d) Dr Wan Zul Haikal Hafiz Bin Wan Zukiman
   e) Dr. Zulkefley Bin Othman (Penyelidik Utama)

5. Please note that ethical approval is valid until **20- Jan-2021**. The following are to be reported upon receiving ethical approval. Required forms can be obtained from the Medical Research Ethics Committee (MREC) website (http://www.nih.gov.my/mrec).

i. **Continuing Review Form** has to be submitted to MREC within 2 month (60 days) prior to the expiry of ethical approval.
ii. **Study Final Report** upon study completion to the MREC.
iii. Ethical approval is required in the case of **amendments / changes** to the **study documents/ study sites/ study team**. MREC reserves the right to withdraw ethical approval if changes to study documents are not completely declared.