ASSOCIATION OF OBESITY, DIABETES MELLITUS, AND HYPERTENSION WITH DENGUE SEVERITY

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ASSOCIATION OF OBESITY, DIABETES MELLITUS, AND HYPERTENSION WITH DENGUE SEVERITY

ABSTRACT

Dengue fever (DF) has unpredictable clinical progressions and outcomes. With the increasing prevalence of obesity, diabetes, and hypertension, these risk factors will likely play a more significant role in increasing the burden and mortality related to dengue infection. This study seeks to determine the association between obesity, diabetes, hypertension, and dengue severity (DS) in confirmed dengue cases among those aged 15 years and above in Malaysia's primary health care setting. A cohort study was conducted in the central region of Peninsular Malaysia from May 2016 to November 2017. We collected demographic, clinical history, physical examination, and laboratory examination information. The exposures of interest were obesity, diabetes, and hypertension. DS was defined as either dengue with warning signs or severe dengue. Participants underwent daily follow-up during which we recorded their vital signs, full blood count, and random blood sugar (RBS) results. The prevalence and incidence of DS were modelled using multivariable and random intercept logistic regression. The changes in platelet and hematocrit levels were modelled using random intercept linear regression. The final model of obesity was adjusted for age, gender, and ethnicity. Similarly, the final model of diabetes and hypertension was adjusted for age, gender, ethnicity, and Body Mass Index (BMI) categories. Out of 362 dengue patients, 48.9% were diagnosed with DS at enrolment. Based on the association between obesity and DS, we found significant associations between: (i) BMI (kg/m^2) and prevalence of DS (Adjusted Odds Ratio (aOR)=1.04; 95% Confidence Interval (CI):1.00,1.08; p=0.034); (ii) BMI (kg/m^2) and incidence of DS (aOR=1.15; 95% CI:1.03,1.28; p=0.016); (iii) BMI (kg/m^2) and hematocrit (%) (Adjusted Beta (aβ)=0.09; 95% CI:0.01,0.17; p=0.021); (iv) BMI (kg/m^2) and changes in hematocrit (%) (aβ=0.07; 95% CI:0.003,0.15; p=0.041); (v) obese and

hematocrit (%) (aβ=1.77; 95% CI:0.53,3.01; p=0.005); (vi) obese and changes in hematocrit (%) (aβ=1.39; 95% CI:0.25,2.53; p=0.017); (vii) waist circumference (cm) and changes in platelet ($x10^3/\mu L$) at phase 3 of DF (aβ=0.84; 95% CI:0.24,1.45; p=0.006); and (viii) abdominal obesity and changes in platelet ($x10^3/\mu L$) at phase 3 of DF (aβ=21.08; 95% CI:6.03,36.13; p=0.006). For the association between diabetes and DS, we found significant associations between: (i) RBS (mmol/L) among non-diabetes and incidence of DS (aOR=1.57; 95% CI:1.13,2.18; p=0.007); (ii) RBS (mmol/L) and platelet ($x10^3/\mu L$) (aβ=-2.49; 95% CI:-4.83,-0.16; p=0.037); (iii) RBS (mmol/L) and changes in platelet ($x10^3/\mu L$) at phase 2 of DF (aβ=-2.74; 95% CI:-4.93,-0.54; p=0.014); (iv) RBS (mmol/L) among non-diabetes and changes in platelet levels at phase 2 of DF (aβ=-4.53; 95% CI:-7.72,-1.34; p=0.005); (v) diabetes and incidence of DS at phase 1 of DF (aOR=20.89; 95% CI:1.08,403.96; p=0.044); and (vi) diabetes and changes in platelet ($x10^3/\mu L$) at phase 3 of DF (aβ=44.29; 95% CI:21.28,67.30; p<0.001). In the association between hypertension and DS, we only found significant associations between hypertension and changes in platelet ($x10^3/\mu L$) at phase 3 of DF (aβ=37.70; 95% CI:11.50,63.91; p=0.005). This is the first study that determines the associations between obesity, diabetes, hypertension and DS. These risk factors play an important role in risk stratifying dengue patients.

Keywords: dengue severity, obesity, BMI, diabetes, hypertension.

HUBUNGAN DIANTARA OBESITI, KENCING MANIS, DAN DARAH TINGGI DENGAN DENGGI TERUK

ABSTRAK

Jangkitan denggi yang parah mempunyai tahap perkembangan klinikal yang tidak dapat diramal. Dengan peningkatan insiden obesiti, diabetes, dan hipertensi, faktor risiko baharu ini mungkin memainkan peranan yang lebih penting dalam meningkatkan beban dan kadar kematian yang disebabkan oleh jangkitan denggi. Kajian kami bertujuan untuk menentukan hubungkait diantara obesiti, diabetes, dan hipertensi dengan jangkitan denggi parah yang telah disahkan dikalangan pesakit yang berumur 15 tahun dan ke atas, di klinik-klinik kesihatan yang terpilih di Malaysia. Kajian kohort pesakit denggi ini dilakukan di Semenanjung Malaysia dari bulan Mei 2016 sehingga November 2017. Maklumat yang dikumpul termasuk maklumat demografi, sejarah klinikal, pemeriksaan fizikal, dan keputusan pemeriksaan makmal pada setiap lawatan. Tahap keparahan denggi adalah berdasarkan diagnosa denggi dengan tanda amaran atau denggi parah. Maklumat mengenai rawatan susulan pada setiap hari seperti pemeriksaan fizikal, pemeriksaan darah penuh, dan keputusan gula dalam darah telah direkodkan. "*multivariable logistic regression*" dan "*random intercept logistic regression*" telah diaplikasi untuk menghasilkan model prevalen dan inseiden keparahan denggi. Manakala "*random intercept linear regression*" telah menghasilkan model bagi perubahan platelet dan hematokrit. Umur, jantina, dan etnik telah dikawal bagi model akhir obesiti. Manakala, untuk model akhir diabetes dan hipertensi, umur, jantina, etnik, dan indeks jisim tubuh (BMI) telah dikawal. Keputusan menunjukkan bahawa 48.9% daripada 362 pesakit telah disahkan sebagai denggi parah. Hubungkait diantara obesiti dan denggi parah adalah signifikan bagi: (i) BMI (kg/m^2) dan prevalen denggi parah (aOR=1.04; 95% CI:1.00,1.08; p=0.034); (ii) BMI (kg/m^2) dan insiden denggi parah (aOR=1.15; 95% CI:1.03,1.28; p=0.016); (iii) BMI (kg/m^2) dan hematokrit (%) (aβ=0.09; 95%

CI:0.01,0.17; p=0.021); (iv) BMI (kg/m^2) dan perubahan hematokrit (%) (aβ=0.07; 95% CI:0.003,0.15; p=0.041); (v) obesiti dan hematokrit (%) (aβ=1.77; 95% CI:0.53,3.01; p=0.005); (vi) obesiti dan perubahan hematokrit (%) (aβ=1.39; 95% CI:0.25,2.53; p=0.017); (vii) lilit pinggang (cm) dan perubahan platelet (x10^3/μL) pada tahap 3 demam denggi (aβ=0.84; 95% CI:0.24,1.45; p=0.006); dan (viii) obesiti sentral dan perubahan platelet (x10^3/μL) pada tahap 3 demam denggi (aβ=21.08; 95% CI:6.03,36.13; p=0.006). Mankala hubungkait diantara diabetes dan denggi parah adalah signifikan bagi: (i) RBS (mmol/L) dan insiden denggi parah (aOR=1.28; 95% CI:1.04,1.59; p=0.022); (ii) RBS (mmol/L) dan platelet (x10^3/μL) (aβ=-2.41; 95% CI:-4.52,-0.30; p=0.025); (iii) RBS (mmol/L) dan perubahan platelet (x10^3/μL) pada tahap 2 demam denggi (aβ=-2.48; 95% CI:-4.55,-0.41; p=0.019); (iv) RBS (mmol/L) pada bukan diabetes dan perubahan platelet (x10^3/μL) pada tahap 2 (aβ=--4.53; 95% CI:-7.72,-1.43; p=0.005); (v) diabetes dan insiden denggi parah pada tahap 1 demam denggi (aOR=20.89; 95% CI:1.08,403.96; p=0.044); dan (vi) diabetes dan perubahan platelet (x10^3/μL) pada tahap 3 demam denggi (aβ=44.29; 95% CI:21.28,67.30; p<0.001). Hubungkait diantara hipertensi dan denggi parah pula adalah signifikan bagi hipertensi dan perubahan platelet (x10^3/μL) pada tahap 3 demam denggi (aβ=37.70; 95% CI:11.50,63.91; p=0.005). Secara rumusan, ini adalah kajian prospektif pertama yang menentukan hubungkait diantara obesiti, diabetes, dan hipertensi dengan demam denggi. Faktor risiko yang baru dikenalpasti ini boleh digunakan bagi menjangka kadar risiko denggi parah.

Kata Kunci: denggi parah, obesiti, BMI, kencing manis, hipertensi.

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Dr Mohd Syis Bin Zulkipli, MD (UGM), MPH (Malaya).

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LIST OF ABBREVIATIONS

ALT	Alanine Transaminase
APC	Antigen-Presenting Cells
AST	Aspartate Transaminase
CPG	Clinical Practice Guidelines
CRP	C-Reactive Protein
DAGs	Directed Acyclic Graphs
DALYs	Disability-Adjusted Life Years
DBP	Diastolic Blood Pressure
DEN-1	Dengue Virus Type 1
DEN-2	Dengue Virus Type 2
DEN-3	Dengue Virus Type 3
DEN-4	Dengue Virus Type 4
DENV	Dengue Virus
DF	Dengue Fever
DHF	Dengue Hemorrhagic Fever
DIC	Disseminated Intravascular Coagulopathy
DSS	Dengue Shock Syndrome
DWS	Dengue with Warning Signs
DWWS	Dengue without Warning Signs
EDTA	Ethylene-diamine-tetra-acetic acid
FBC	Full Blood Count
FMS	Family Medicine Specialist
HbA1c	Hemoglobin A1c
HCT	Hematocrit
IASO	International Association for the Study of Obesity

IDF	International Diabetic Federation
IDI	International Diabetic Institute
IL-6	Interleukin 6
IL-8	Interleukin 8
IOTF	International Obesity Task Force
JNC	Joint National Committee
MA	Medical Assistant
MLT	Medical Lab Technician
NHMS	National Health and Morbidity Survey
NO	Nitric Oxide
PLT	Platelet
RBS	Random Blood Sugar
RNA	Ribonucleic Acid
SBP	Systolic Blood Pressure
SD	Severe Dengue
SN	Staff Nurse
TNF-α	Tumor Necrosis Factor Alpha
USD	United States Dollar
WAT	White Adipose Tissue
WBC	White Blood Cells
WHO	World Health Organisation
YLL	Years Life Lost

LIST OF APPENDIXES

CHAPTER 1: INTRODUCTION

1.1 Introduction

Chapter one introduces the conventional and risk factors for dengue infection. It consists of an evidence-based discussion on the epidemiology and possible associations between obesity, diabetes, hypertension, and dengue severity. It also explains what has been established in regard to this topic and the gaps that remain in our knowledge of the risk factors for dengue infection that led to the formulation of the research questions and study objectives.

1.2 Dengue Infection

Dengue is an acute systemic viral infection caused by the dengue virus. The majority of dengue infections patients are asymptomatic (Bhatt et al., 2013). Patients infected with the dengue virus may present symptoms such as fever, nausea, vomiting, diarrhea, joint pain, muscular pain, retro-orbital pain, and spontaneous bleeding (World Health Organization, 2012b). The most critical sign of severe dengue infection is a rapid reduction of platelet levels with increased hematocrit levels during the critical phase.

Dengue has become endemic in hundreds of countries that includes Africa, Western Pacific, America, Eastern Mediterranean, and South-East Asia regions. Globally, the incidence of dengue had increased from 8.3 to 58.4 million cases from 1990 to 2013 (Stanaway et al., 2016). Shepard et al. estimated that 58.4 million dengue patients were symptomatic with 13,586 fatal cases recorded during the same period (Shepard, Undurraga, Halasa, & Stanaway, 2016). In 2013, dengue accounted for 576,900 years life lost (YLL) and 1.14 million disability-adjusted life years (DALYs) (Stanaway et al., 2016). An estimate by Brady et al. on the prevalence of dengue showed that 3.9 billion people from 128 countries were at risk for dengue virus infection (Brady et al., 2012). The World Health Organisation (WHO) also reported that dengue has spread to new areas such as France, Europe, China, and Japan (World Health Organization, 2017a).

In Malaysia, dengue infection is endemic with multiple periods of hyperendemicity. The median trend of dengue infection continued to increase from 2010 to 2016 (Ministry of Health Malaysia, 2017). The incidence of dengue infection has continued to increase year by year since 2010. In 2015, Malaysia marked the highest number of dengue cases with 120,836 incidents reported (Ministry of Health Malaysia, 2016). 2016 recorded a 16% decrease in the incidence of dengue cases (Ministry of Health Malaysia, 2017) with a reduction in the case fatality rate from 0.28 to 0.23 (Remote Sensing Agency Malaysia, 2017).

Dengue infection has a broad spectrum of clinical presentations and outcomes. Each case can progress to a more severe form of infection known as severe dengue (SD) (Horstick et al., 2012). Additionally, the development of severe dengue is related to repeated dengue infections, the presence of co-morbidities, and extremes of age. Currently, no single sign can predict the development and progression of dengue infection to a more severe form (L. K. Lee et al., 2012; Rathakrishnan et al., 2014; Thein, Gan, Lye, Yung, & Leo, 2013).

Recently, there has been a shift in the mean age of patients with acute dengue infection. It was observed that dengue infection is more common among adults especially those aged 30 years and above (Pang et al., 2012). Individuals aged 30 to 39 and 40 to 49 years were also found to have 40% more risks of developing dengue hemorrhagic fever (Pang et al., 2012). A systematic review from Malaysia on the epidemiology of dengue in Malaysia concluded that there were changes in the age groups of dengue patients from children to adults during the 2000 to 2012 epidemics (Mohd-Zaki, Brett, Ismail, & L'Azou, 2014).

1.3 Conventional Risk Factor for Severe Dengue

The conventional risk factors for severe dengue refer to the commonly researched risk factors for dengue infection. Epidemiological studies have identified a number of risk factors for severe dengue, including secondary infection, the two extremes of age, pregnancy, under-nutrition, and over-nutrition (Guzman, Alvarez, & Halstead, 2013; Hammond et al., 2005; Kalayanarooj & Nimmannitya, 2005; M. S. Lee, Hwang, Chen, Lu, & Chen, 2006; Machado et al., 2013). Clinical signs and symptoms during acute illness have been associated with severe dengue such as gastrointestinal symptoms, hepatomegaly, bleeding manifestations, high serum urea, low serum protein and low lymphocyte proportion (Cao et al., 2002; V. J. Lee, Lye, Sun, & Leo, 2009; Phuong et al., 2004).

Currently, there is limited and inconclusive evidence on the other potential risk factors for severe dengue infection such as diabetes, hypertension, and obesity (C. Y. Chen et al., 2015; Figueiredo et al., 2010; Htun et al., 2015; Kalayanarooj & Nimmannitya, 2005; Pang et al., 2012; Teixeira et al., 2015). With increasing numbers of morbidity and mortality among dengue patients, there is an urgent need to investigate these risk factors for the development of severe dengue infection.

1.4 Risk Factors for Dengue Severity

1.4.1 Obesity

The combined global prevalence of overweight and obesity rose by 27.5% for adults between 1980 and 2013. There was also a marked increase in the number of overweight and obese individuals from 857 million in 1980 to 2.1 billion in 2013 (Ng et al., 2014). In 2016, the World Health Organisation (WHO) reported that the global prevalence of overweight and obesity was 1.9 billion and 650 million adults, respectively (World Health Organization, 2017g). The WHO also reported that at least 2.8 million people died due to being overweight or obese with an estimated 35.8 million or 2.3% of global Disability-Adjusted Life Years (DALYs) (World Health Organization, 2017c).

In 2016, the WHO reported that the prevalence of overweight and obesity were: (i) 21.9% and 4.7% in South-East Asia region; (ii) 49% and 20.8% in the Eastern Mediterranean region; (iii) 31.7% and 6.4% in the Western Pacific region; (iv) 31.1% and 10.6% in the African region; and (v) 62.5% and 28.6% in the America region, respectively (World Health Organization, 2017d, 2017e). There was a higher prevalence of obesity among upper-middle-income and high-income groups compared to low-income and lower-middle-income groups. Interestingly, all income groups showed an increasing trend in the prevalence of overweight and obesity from 1975 to 2016 (World Health Organization, 2017h, 2017i).

In Malaysia, the 2015 National Health and Morbidity Survey (NHMS) estimated that the national prevalence of overweight and obesity were 33.4% and 30.6%, respectively (National Institute of Health Malaysia, 2016). Comparing NHMS 2015 with NHMS 2011, the prevalence of overweight and obesity increased by 0.6% and 2.6%, respectively (National Institute of Health Malaysia, 2016).

Obesity may affect the dengue severity through inflammation pathways. The increased deposition of white adipose tissue (WAT) leads to increased production of Interleukin-six (IL-6), interleukin-eight (IL-8), and Tumor Necrosis Factor alpha (TNF-α) (Bosch et al., 2002; Calabro, Chang, Willerson, & Yeh, 2005; Juffrie et al., 2001). These are essential mediators of the inflammation pathway that increases capillary permeability and could underlie the process of continuous and severe plasma leakage. There is scarce and inconclusive evidence linking obesity with dengue severity (Maria Mahdalena Tri Widiyati, Ida Safitri Laksanawati, & Endy Paryanto Prawirohartono, 2013; Pichainarong, Mongkalangoon, Kalayanarooj, & Chaveepojnkamjorn, 2006). Together, the increased prevalence of overweight and obesity, spreading of dengue virus to a new area, and the hypothesised link between overweight and obesity and plasma leakage increases the risk of a more severe dengue infection and mortality.

1.4.2 Diabetes

Globally, 415 million adults have diabetes, with 46.5% of adults undiagnosed (International Diabetes Federation, 2015). The International Diabetes Federation (IDF) estimated that by the year 2040, there would be approximately 642 million people living with diabetes globally (International Diabetes Federation, 2015). Moreover, almost 12% (USD 673 billion) of the global health expenditure was spent on diabetes (International Diabetes Federation, 2015).

In Malaysia, the prevalence of diabetes is increasing. In the recent Malaysian National Health and Morbidity Survey (NHMS) in 2015, the prevalence of diabetes was estimated at 17.5% (National Institute of Health Malaysia, 2016). This figure was supported by the IDF report in 2015 where the prevalence of diabetes in Malaysia was 17.9%, with about 3.3 million people living with diabetes and 52% were undiagnosed with it (International Diabetes Federation, 2015).

A meta-analysis published in 2015 by Htun NS et al. found that diabetes was associated with 75% (95% CI=1.08,2.84; p=0.022) higher risk of severe clinical manifestations of dengue compared to non-diabetes. Moreover, a 2015 hospital-based retrospective case-control study by Chen et al. found that 56% of the diabetes patients developed dengue hemorrhagic fever (DHF) or Dengue Shock Syndrome (DSS) (C. Y. Chen et al., 2015). Furthermore, lower platelets counts were observed among diabetes patients during the first three days of infection (C. Y. Chen et al., 2015). As a summary, currently available evidence suggests an association between diabetes and severe dengue infections.

The pathophysiology on how dengue leads to severe clinical manifestation among diabetes patients is not well understood. Prior studies have shown endothelial dysfunction and increased production of cytokines in patients with type-2 diabetes (Geerlings & Hoepelman, 1999; Hsueh, Lyon, & Quinones, 2004; Kaye et al., 1986). Brausewetter et

al. in their 2001 study also found that capillary permeability was increased in both types of diabetes patients (Brausewetter et al., 2001). Similarly, in 2005 Cardier et al. concluded that in-vitro induction of apoptosis in endothelial cells was associated with a high level of tumor necrosis factor, TNF-α in dengue patients' serum (Cardier et al., 2005). In addition, Lee YR et al. recorded a high level of monocyte chemoattractant protein-1 (MCP-1) in severe dengue patients related to the in-vitro increase of the vascular endothelial cell permeability (Y. R. Lee et al., 2006).

In summary, cytokine increases the capillary permeability in people with diabetes compared to non-diabetics. The increased capillary permeability in people with diabetes dengue patient may predispose them to a higher risk of vascular leakage that results in a more rapid and severe third space fluid loss.

1.4.3 Hypertension

At a global level, the number of DALYs in 2013 attributed to hypertension was 208 million while the number of deaths was approximately 10.4 million (Britney Wong & Norman Campbell, 2015). The prevalence of hypertension in low, lower-middle and upper-middle income countries was 5% higher than in high-income countries (World Health Organization, 2013a). Hypertension was responsible for at least 45% of deaths due to heart disease and 51% of deaths due to stroke (World Health Organization, 2011). Moreover, the WHO estimated that the global crude and age-standardised prevalence of hypertension were at 22.3% and 20.5% respectively (World Health Organization, 2013a).

In Malaysia, hypertension is a significant public health problem (Rampal, Rampal, Azhar, & Rahman, 2008). According to the National Health and Morbidity Survey in 1996 and 2011, the prevalence of hypertension has increased from 32.9% in 1996 to 43.5% in 2011 in adults aged 30 years and above (Naing et al., 2016). In NHMS 2015, the overall prevalence of hypertension (known and undiagnosed) among adults of 18 years and above was 30.3% with 17.2% undiagnosed (National Institute of Health Malaysia, 2016).

Currently, the literature on hypertension and severe dengue is inconclusive. A case-control study in 2010 found no statistically significant association between hypertension and DHF (Figueiredo et al., 2010). However, in another study, the authors concluded that patients with hypertension had 2.1 times the risk of developing a severe clinical presentation of dengue compared to non-hypertension patients (Pang et al., 2012). In 2015, Teixeira et al. found that the progression of dengue infection to severe clinical presentation was associated with self-reported hypertension (Teixeira et al., 2015). Also, hypertension was proposed to have modified risk of the severe outcome in dengue patients with diabetes (Pang et al., 2012).

The mechanism by which hypertension might increase the risk of dengue infection progression to the severe clinical manifestation of dengue is not well understood. Current evidence suggests that hypertension leads to endothelial dysfunction and vascular damage, promoting inflammatory activation of the endothelium, changing the regulation of vascular tone and flow (Savoia et al., 2011). In addition, there were studies show that higher levels of circulating CRP are related to higher blood pressure (Bermudez, Rifai, Buring, Manson, & Ridker, 2002; Sesso et al., 2003).

The commonly elevated C-reactive protein (CRP) level in a patient with hypertension can promote detrimental effects on the vascular wall thereby inducing endothelial dysfunction and reducing the nitric oxide (NO) bioavailability (Hage, 2014; Pauletto & Rattazzi, 2006).

The effect of elevated CRP may lead to increased vascular permeability, coagulopathy, loss of fluid, and hypovolemic shock, which are the characteristics of dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS).

1.5 What is Already Known?

1. The incidence of dengue infection is increasing globally and locally.
2. More adults are infected with dengue infection compared to children since the year 2000.
3. Clear evidence is available on the association between conventional risk factor and dengue infection from earlier research.
4. Scarce evidence is available on the association between risk factors (obesity, diabetes, and hypertension) and severity of dengue infection.
5. Dengue patients with co-morbidities such as diabetes, allergies, and hypertension were observed to have a more severe infection and poorer prognosis.
6. Patients with dengue infection were found to have glucose intolerance during the acute phase of infection.
7. Diabetes patient infected with dengue have more severe thrombocytopenia compared to non-diabetic patient.
8. Currently, the literature on hypertension and severe dengue is inconclusive due to their small sample sizes.
9. As a result of climate change, there were more geographical areas at high risk of dengue infection. Thus, the possibility that individuals with obesity, diabetes, and hypertension are infected with dengue virus increases, which in turn increases the risk of mortality and morbidity from dengue infection.

1.6 Justification of the Study

Dengue infection was previously known as a pediatric disease (Elling, Henneke, Hatz, & Hufnagel, 2013). Recently, the mean age among those infected has increased together with increased risk of mortality (Pang et al., 2012). The number of dengue infection among adults is higher compared to children. With the increase in cause-specific mortality, despite a well-organized dengue prevention program in Malaysia, there is a need to better risk stratify dengue patients seen in the community primary health care setting. Previous studies suggested that there might be an association between obesity, diabetes, hypertension, and severe dengue infection. A recent systematic review concluded that there is an association between diabetes and dengue severity, but the authors suggested that further prospective studies are needed to measure the impact of diabetes and hyperglycemia at time of dengue diagnosis on the severity of dengue (Htun et al., 2015). It has been discovered that uncontrolled diabetes can result in increased mortality and morbidity caused by an impaired immune system that resulted from impaired phagocytosis, intracellular killing, and chemotaxis of polymorphonuclear leukocytes (Alexiewicz, Kumar, Smogorzewski, Klin, & Massry, 1995; Delamaire et al., 1997). Furthermore, in a systematic review by Zulkipli et al., the authors found that obese children have 38% higher odds of developing severe dengue infection compared to non-obese children (Zulkipli et al., 2018).

There are many studies on dengue infection covering broad aspects that include epidemiology, diagnosis, treatment, and control. However, there is sparse evidence exploring risk factors for dengue severity especially obesity, diabetes, and hypertension. Obesity, diabetes, and hypertension are significant public health problems. The incidence of dengue is on the rise despite various prevention programs and improvements in management. Increasing incidence of dengue severity increases the economic burden in the management of dengue.

To our knowledge, this study is the first prospective study in Malaysia that focuses on the associations between obesity, diabetes, hypertension, and severe dengue infection among those aged 15 years and above. Previous studies to determine the association between diabetes and dengue were mostly retrospective with small sample sizes (Guzman et al., 2013; Hammond et al., 2005; Kalayanarooj & Nimmannitya, 2005; M. S. Lee et al., 2006; Machado et al., 2013). With the increasing prevalence of obesity, diabetes, and hypertension, coupled with the expanding geographical areas susceptible for dengue infection, there is an increased risk of individuals with obesity, diabetes, and hypertension to become infected with dengue. Thus, with the hypothesised link between obesity, diabetes, hypertension, and dengue severity, we might see an increased number of severe dengue infection cases.

This cohort study of dengue patients in the primary health clinics provides crucial evidence on the role of several risk factors (obesity, diabetes, and hypertension) with dengue severity and is set to improve the identification of high-risk dengue patients.

1.7 Research Questions

1. What is the risk of developing severe dengue infection among obese dengue patients compared to non-obese dengue patients?
2. What is the risk of developing severe dengue infection among diabetic dengue patients compared to non-diabetic dengue patients?
3. What is the risk of developing severe dengue infection among hypertensive dengue patients compared to non-hypertensive dengue patients?
4. Does daily monitoring of blood sugar enable effective and early risk stratification of dengue patients?

1.8 General Objective

The general objective of this study was to determine the association between risk factors (obesity, diabetes, hypertension) and dengue severity together with changes in platelet and hematocrit levels in confirmed dengue cases aged ≥ 15 years in the community primary health care setting.

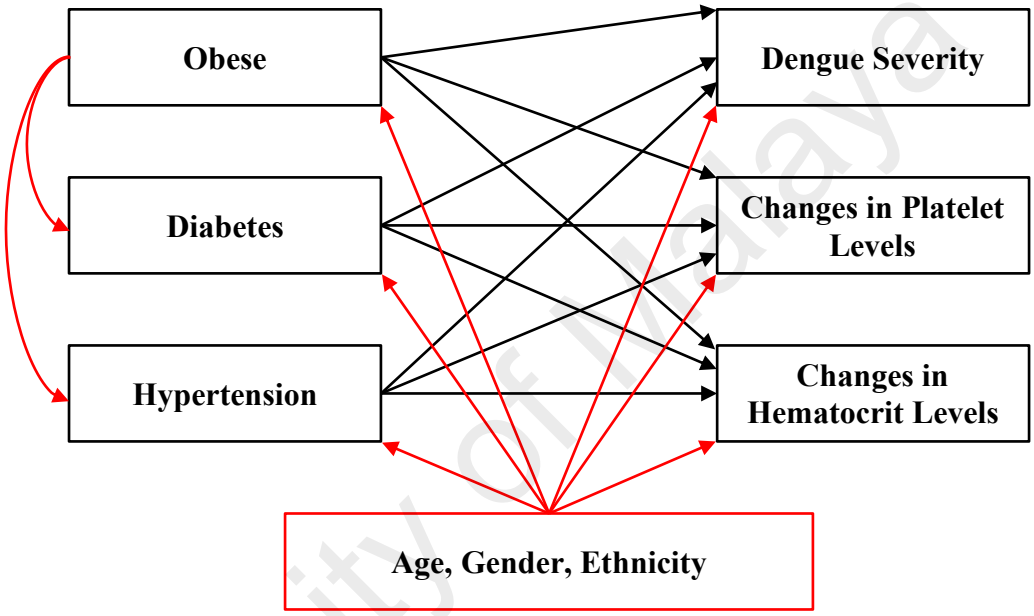

Figure 1.1: Causal diagram of the association between risk factors (obesity, diabetes, and hypertension) and severe dengue infection

1.9 Specific Objectives

1. Obesity and dengue severity
    a. To determine the cross-sectional and longitudinal association between obesity and dengue severity.
    b. To determine the cross-sectional and longitudinal association between obesity and platelet levels.
    c. To determine the cross-sectional and longitudinal association between obesity and hematocrit levels.

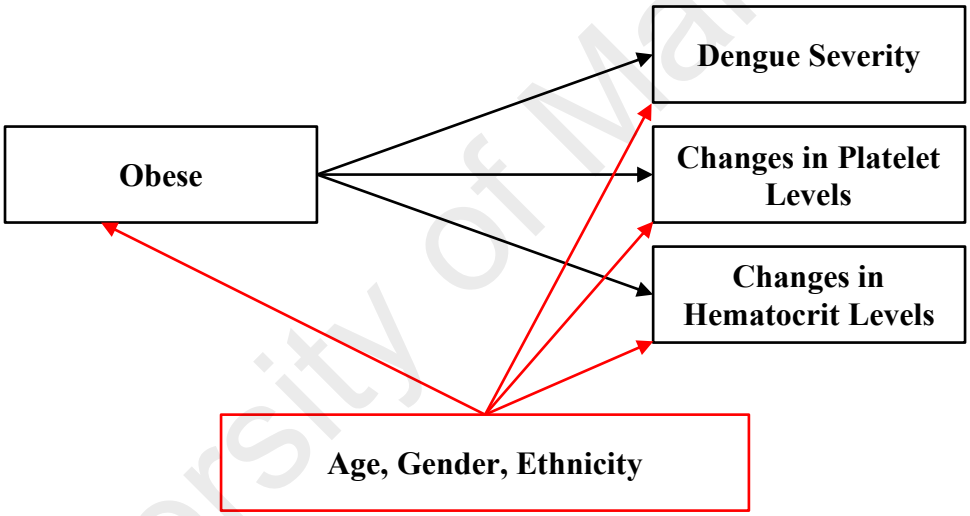

Figure 1.2: Causal diagram of the association between obesity and dengue severity

2. Diabetes and dengue severity

    a. To determine the cross-sectional and longitudinal association between diabetes and dengue severity.

    b. To determine the cross-sectional and longitudinal association between diabetes and platelet levels.

    c. To determine the cross-sectional and longitudinal association between diabetes and hematocrit levels.

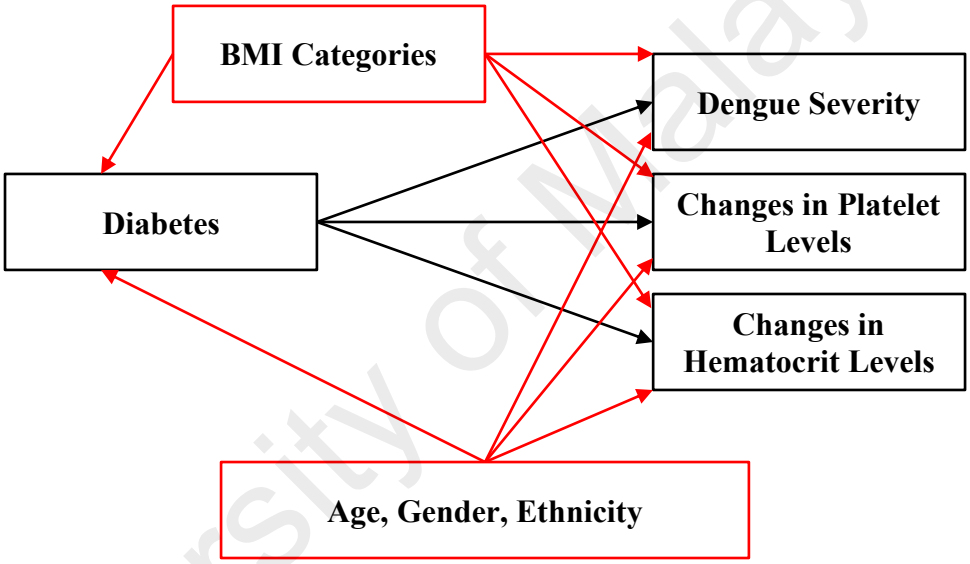

Figure 1.3: Causal diagram of the association between diabetes and dengue severity

3. Hypertension and dengue severity

    a. To determine the cross-sectional and longitudinal association between hypertension and dengue severity.

    b. To determine the cross-sectional and longitudinal association between hypertension and platelet levels.

    c. To determine the cross-sectional and longitudinal association between hypertension and hematocrit levels.

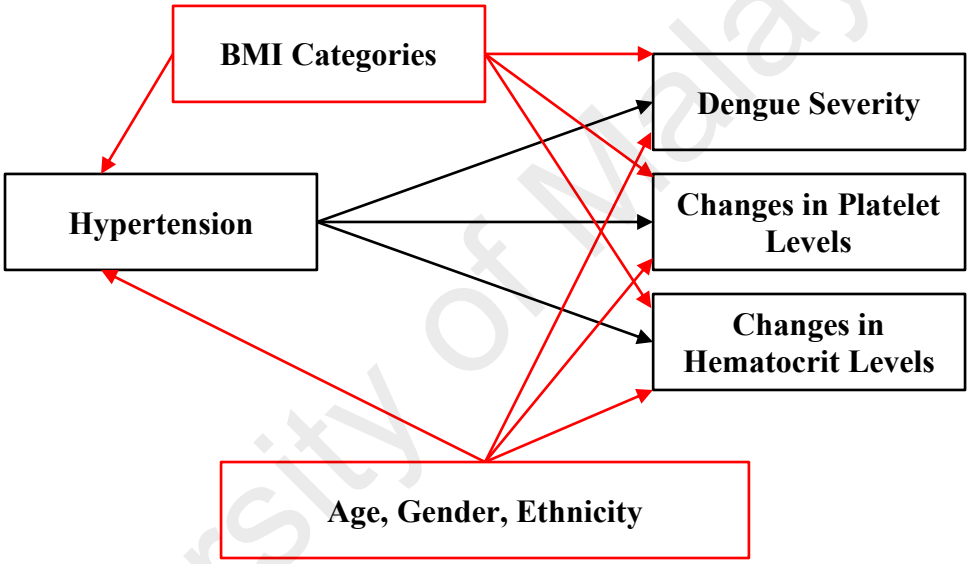

Figure 1.4: Causal diagram of the association between hypertension and dengue severity

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

This chapter provides an extensive literature review of the association between obesity, diabetes, hypertension, and dengue severity. We start the review by discussing the history of dengue infection, global and local epidemiology of dengue infection, virology, sign and symptoms, and diagnosis. We further review and extract relevant information on thrombocytopenia, hematocrits and the pathophysiology behind plasma leakage in dengue infection. Moreover, we performed an extensive review of obesity, diabetes, and hypertension that includes the definition, epidemiology, association with dengue, and its hypothesised link with the development of severe dengue infection. At the end of the literature review, we summarise the findings with a conceptual framework. The conceptual framework aims to guide our thinking on the hypotheses and study.

2.2 Dengue Infection

Dengue is an acute infectious disease caused by a flavivirus (R. Chen & Vasilakis, 2011). It is one of the most important arboviral infections in the world and is transmitted by Aedes mosquitoes, mainly *Aedes Aegypti* and also *Aedes Albopictus* (World Health Organization, 2017b). The earliest historical evidence of dengue-like illness is found in China around 992 A.D and described the disease as 'water poison' and associated with flying insects (Bleijs, 2017). Historically, in 1780, Benjamin Rush, a physician and a founding father of the United States of America, found the first account of a febrile disease resembling symptoms of dengue infection (Barnett, 2017). In 1905, Thomas Lane Bancroft found that the mosquito *Stegomyia Fasciata* was the carrier for the dengue fever pathogen (Australian National Herbarium, 2013). In 1907, Captain P. M. Ashburn and Lieutenant Charles F. Craig of the US Army Medical Corps reviewed all work previously done on the etiology as well as transmission of dengue fever and proved in their primary study in 1906 that a virus caused dengue infection (Ashburn & Craig, 1907).

Currently, there are four genetically related but antigenically distinct dengue virus (DENV) serotypes (DEN-1, DEN-2, DEN-3, and DEN-4) (R. Chen & Vasilakis, 2011). In 1943, the Japanese scientists Ren Kimura and Susumu Hotta, were first to discover the dengue virus type 1 (DEN-1) (Hotta, 1952). Subsequently, in 1944, dengue virus type 2 (DEN-2) was discovered by Albert Sabin (Sabin, 1952). Later on, the year 1994 and 1981 saw the introduction of DEN-3 and DEN-4 dengue serotypes, respectively (Gubler, 1998). The presentation of fever with a flu-like illness characterised dengue infection, which occasionally develops into a potentially fatal complication known as severe dengue (World Health Organization & Special Programme for Research and Training in Tropical Diseases, 2009).

Dengue symptoms usually begin four to six days after infection and includes: (i) high fever; (ii) severe headache; (iii) pain behind eyes (retro-orbital pain); (iv) joint pain (arthralgia); (v) muscle pain (myalgia); (vi) fatigue; (vii) nausea; (viii) vomiting; (ix) diarrhea; and (x) skin rash (World Health Organization, 2017a). Recovery from infection by one serotype provides lifelong immunity against that particular serotype (Soo, Khalid, Ching, & Chee, 2016). However, there could be partial and temporary cross-immunity to the other serotypes after recovery. Subsequent infections by other serotypes increase the risk of developing severe dengue (Soo et al., 2016).

Severe dengue (previously known as Dengue Hemorrhagic Fever and Dengue Shock Syndrome) was first recognised during the dengue epidemics in Thailand and Philippines in the 1950s (World Health Organization, 2017b). This severe form of dengue subsequently spread throughout Southeast Asia and expanded globally (World Health Organization, 2017b). Today, dengue is the leading cause of hospitalisation and mortality among children and adults across Africa, Western Pacific, America, Eastern Mediterranean, and South-East Asia regions (Bhatt et al., 2013).

In Malaysia, Skae reported the first known presentation of dengue on November 15, 1902 (Skae, 1902). He described a dengue outbreak in the northern state of Penang from December 1901 to March 1902 (Skae, 1902). Furthermore, in the 1960s, dengue cases began to spread into the urban areas of Penang and Kuala Lumpur. By the early 1970s, dengue infection spread to the whole Malaysia and has since become a significant health burden (Wallace et al., 1980).

2.2.1 Epidemiology of Dengue (Worldwide)

In 2012, the WHO classified dengue as the most important mosquito-borne viral disease in the world (World Health Organization, 2012a). Recently, there was an increased incidence of dengue infection at an alarming rate around the globe, especially in the tropical and sub-tropical regions (World Health Organization, 2017b). While the actual numbers of dengue cases might be underreported, an estimate of WHO in 2013 indicated that there were 390 million dengue infections occur in a year (World Health Organization, 2017b). Among that, about 96 million of dengue infection manifests clinically (Bhatt et al., 2013). Besides, earlier studies reported that around 50 to 200 million dengue infections, 500,000 severe dengue, and 20,000 dengue-associated deaths occur yearly (Gubler, 2002; Shepard, Coudeville, Halasa, Zambrano, & Dayan, 2011). In 2013, dengue infection cases occurred in Florida (United States of America) and the Yunnan province of China. It continues to affect several South American countries, notably Costa Rica, Honduras and Mexico (World Health Organization, 2017a). A study by Brady et al. in 2012 estimated that around 3.9 billion people from 128 countries were at risk of dengue infection (Brady et al., 2012).

In Asia, Singapore reported an increase in dengue infection cases after a lapse of several years (Hapuarachchi et al., 2016). Likewise, Laos reported an increasing number of dengue infection outbreaks (Soukaloun, 2014). In 2014, the People's Republic of China, the Cook Islands, Fiji, and Vanuatu recorded increasing trend of dengue infection cases (World Health Organization, 2017a). Moreover, Japan reported an autochthonous outbreak of dengue type 1 in Tokyo from August to October 2014 after a lapse of over 70 years (Seki et al., 2015). In 2015, an outbreak with over 15,000 dengue infection cases struck Delhi in India, while The Pacific island countries of Fiji, Tonga and French Polynesia continued to record increasing dengue infection cases (Travasso, 2015; World Health Organization, 2017a).

Worldwide, there were significant dengue infection outbreaks in 2016. The America region reported more than 2.38 million cases of dengue infection in 2016, where Brazil alone contributed less than 1.5 million dengue infection cases, approximately three times higher than in 2014 (World Health Organization, 2017a). The Solomon Islands in 2016 declared an outbreak of dengue infection with more than 7,000 suspected dengue cases (Pacific Island Report, 2016).

Up to the first 11 weeks of 2017, the region of America reported 50,172 cases of dengue fever; a reduction as compared with corresponding periods in previous years (World Health Organization, 2017a). An estimation of 500,000 people with severe dengue infection required hospitalisation each year and approximately 2.5% of those affected eventually died. Researchers believed that rapid urbanisation and climate change contributed to the increase in the incidence of dengue infection both locally and globally (Ebi & Nealon, 2016; Gubler, 2011). Similarly, a 37 years retrospective study in Singapore found that 86% of the increase in the incidence of dengue infection was due to population growth and 14% from the increase in temperature (Struchiner, Rocklov, Wilder-Smith, & Massad, 2015).

2.2.2 Epidemiology of Dengue (Malaysia)

In Malaysia, dengue is predominantly an urban disease due to the abundance of the principal vector *Aedes Aegypti,* which is at proximity to high densities of susceptible hosts (Mohd-Zaki et al., 2014). Dengue confined to the densely populated and urbanised areas of Peninsular Malaysia, which cater for 20 million of the country's 29 million inhabitants (Mohd-Zaki et al., 2014). Since the notification of dengue was made mandatory in Malaysia in the 1970s, dengue epidemic activity in Malaysia has been increasing in frequency and intensity over the past 40 years (Mohd-Zaki et al., 2014; Shekhar & Huat, 1992).

In 2000, the dengue incidence in Malaysia was 31.6 cases per 100,000 population (Mohd-Zaki et al., 2014). Also, the incidence rate of dengue infection rose approximately 500% from 31.6 to 159.7 cases per 100,000 population in 2009 compared to the year 2000 (Mohd-Zaki et al., 2014). In 2015, Malaysia reported the highest number of dengue cases with 120,836 incidents (Ministry of Health Malaysia, 2016). However, in 2016, the incidence of dengue cases in Malaysia decreased by 16%, with a reduction in case fatality rate from 0.28 to 0.23 (Ministry of Health Malaysia, 2017; Remote Sensing Agency Malaysia, 2016).

Economically, Malaysia holds an economic burden of dengue illness at approximately USD 3.72 per capita with the total burden of USD 102.25 million per year (Donald et al., 2013). Factors like: (i) population growth in urban areas; (ii) the indiscriminate disposal of waste coupled with the lack of efficient solid waste management; (iii) the increased and efficient movement of dengue viruses in infected humans through modern transportation, increasing temperature; (iv) rainfall; (v) humidity; and (vi) vast urbanisation have contributed to the marked increase in the occurrence of dengue (Hii, Zaki, Aghamohammadi, & Rocklov, 2016; Mohd-Zaki et al., 2014).

2.2.3 Virology

The dengue virus is an Arbovirus from the Flaviviridae family and Flavivirus genus (Paranjape & Harris, 2010). It is an enveloped virus, 40-60 nm in size, with an isometric nucleocapsid of 25-30 nm and linear, positive-sense RNA genome (Dimmock, Easton, & Leppard, 2007). It exists as four serotypes (DEN 1-4) and genetically related to other flaviviruses such as tick-borne encephalitis and yellow fever viruses (Dimmock et al., 2007; Paranjape & Harris, 2010). The dengue virus may undergo two different transmission cycles and amplifications, namely the sylvan and urban cycle (Burke & Monath, 2001).

It undergoes rounds of infection, amplification, and re-infection between non-human primates and arthropod vectors in the sylvan cycle (Burke & Monath, 2001). This infected arthropod vector was believed to have been migrating from jungle to urban environment and initiated the urban cycle. In the urban cycle, infection, amplification, and re-infection occur among human as the host and *Aedes Aegypti* as the common vector species (Burke & Monath, 2001).

Throughout the years, different dengue serotypes have been detected to predominate in Malaysia (Holmes, Tio, Perera, Muhi, & Cardosa, 2009; E. L. Pang & Loh, 2016). All four dengue serotypes can be isolated in Malaysia where the concurrent existence of all four serotypes shows that Malaysia is “hyperendemic” for dengue (R. Chen & Vasilakis, 2011).

2.2.4 Sign and Symptoms

Dengue infection can be inapparent or asymptomatic. It may instigate a broad spectrum of manifestation that ranges from classical fever to plasma leakage, spontaneous bleeding, shock and even death (Whitehorn, Van, & Simmons, 2014). People are infected through the bite of *Aedes* mosquitoes that usually breed in domestic water containers, drains, broken toilets, as well as clean stagnant water.

During the infection period, dengue viruses infect the liver parenchyma and cells of the reticuloendothelial system (Guzman et al., 2010). A sudden fever accompanied by anorexia, prodromal chills, headache, myalgia, retro-orbital pain, rashes, erythematous mottling of the skin, and facial flushing are common symptoms of classical dengue fever within the first three to four days of the febrile phase (Martina, Koraka, & Osterhaus, 2009).

As the infecting virus is circulating in the peripheral blood of patients, a mosquito's bite during febrile viremic stage would result in the disease being transmitted to another host after an extrinsic incubation period (Guzman et al., 2010). Severe dengue infection and dengue shock syndrome usually emerge during the time of defervescence, where increased propensity of capillary leakage was observed before hypovolemic shock (Whitehorn & Simmons, 2011).

2.2.5 Diagnosis

Previously, dengue infection was diagnosed based on the WHO 1997 classification. The WHO 1997 classified dengue infection into dengue fever, dengue hemorrhagic fever, and dengue shock syndrome (Khursheed et al., 2013). The usage of the WHO 1997 criteria has not been without criticism (Bandyopadhyay, Lum, & Kroeger, 2006; Deen et al., 2006). Among the criticism were that it underestimates patients who develop shock or severe dengue (Bandyopadhyay et al., 2006). In 2009, the WHO revised the dengue classification criteria as a result of the observation of a multi-centred study in Latin America and South-East Asia (World Health Organization, 2012b).

The new WHO 2009 classification classified dengue into probable dengue, dengue without warning signs, dengue with warning signs, and severe dengue (World Health Organization, 2012b). According to the WHO 2009 classification, probable dengue is defined when an individual who lives in or travels to dengue-endemic area is present with fever and any two of the following criteria; (i) nausea; (ii) vomiting; (iii) rash; (iv) aches and pains; (v) positive tourniquet test; and (vi) leukopenia (World Health Organization, 2012b).

A confirmed case of dengue without warning signs is made when an individual fulfils the criteria set in the probable dengue group together with laboratory confirmation of dengue (World Health Organization, 2012b). Dengue with warning signs can be determined based on the laboratory confirmation of dengue patients with any of the following warning signs: (i) abdominal pain and/or tenderness; (ii) persistent vomiting and/or diarrhea (more than three times over 24 hours); (iii) third space fluid accumulation such as ascites, pleural and pericardial effusion; (iv) spontaneous bleeding; (v) lethargy and/or restlessness and/or confusion; (vi) liver tenderness; and (vii) raised hematocrits with rapid drop in platelets (World Health Organization, 2012b).

Severe dengue can be determined based on a laboratory-confirmed dengue patient with the development of either one of the following conditions: (i) severe plasma leakage that leads dengue shock syndrome or fluid accumulation with respiratory distress; (ii) severe bleeding; and (iii) severe organ involvement that includes increase in liver profile, impaired consciousness, and organ failure (World Health Organization, 2012b).

The new WHO 2009 criteria has proven to be useful in detecting severe dengue cases which provides an advantage in the diagnosis for poor resources countries where laboratory investigation is not feasible (Horstick et al., 2014). Furthermore, the WHO 2009 classification allows precise detection of severe dengue patients. Studies have found that the WHO 2009 dengue classification into severity levels was 53% more sensitive in capturing severe dengue infection than the WHO 1997 dengue classification (Basuki et al., 2010; Narvaez et al., 2011). Additionally, a Singapore based study reviewed the performance of warning signs from the WHO 2009 classification in predicting severe dengue among adults and concluded that the absence of any warning signs could rule out the development of dengue severity and no predictor can accurately predict severe dengue (Leo et al., 2013).

Leucopoenia, neutropenia, lymphopenia, thrombocytopenia, increased liver enzymes and prolonged prothrombin time may appear as early as day three of dengue infection (Ministry of Health Malaysia, 2015a). Severe dengue patients with plasma leakage and gallbladder thickening exhibited marked lymphopenia and thrombocytopenia which appears three days after onset of fever (Binh, Matheus, Huong, Deparis, & Marechal, 2009). Furthermore, early signs of liver damage are significantly associated with the subsequent occurrence of plasma leakage, gallbladder thickening, and internal bleeding (Junia, Garna, & Setiabudi, 2007; M. S. Lee et al., 2006; Lovera et al., 2016; Rathakrishnan et al., 2014).

2.2.6 Thrombocytopenia

Thrombocytopenia has always been the most used criteria by WHO guidelines as an indicator for clinical dengue severity (World Health Organization, 2012b). The WHO 2009 guidelines described thrombocytopenia as a rapid decline in platelet count or platelet count less than 150,000 per microliter of blood (World Health Organization, 2012b). Lower platelet counts were observed more in a patient with severe dengue infection compared to non-severe dengue infection.

The mechanism involved in thrombocytopenia and bleeding during the dengue infection period is not fully understood. We hypothesised that thrombocytopenia occurs based on three main reasons such as: (i) impaired production; (ii) increased destruction or consumption; and (iii) splenic sequestration. A study in 1997 hypothesised that dengue infection could directly or indirectly affect the bone marrow progenitor cells (Murgue, Cassar, Guigon, & Chungue, 1997).

The dengue virus inhibits megakaryopoiesis and infects and induces apoptotic cell death in a subpopulation of early megakaryocytic progenitors which leads to thrombocytopenia in dengue infection (Basu, Jain, Gangodkar, Shetty, & Ghosh, 2008). In addition, there is evidence that the dengue virus can induce bone marrow hypoplasia, which inhibits the production of platelets. This supports the hypothesis of impaired thrombocyte production in dengue (Nakao, Lai, & Young, 1989).

Additionally, researchers found that dengue infection causes: (i) an increase in platelet consumption due to disseminated intravascular coagulation (DIC); (ii) increased destruction by increased apoptosis; and (iii) lysis results from the involvement of complement system and antiplatelet antibodies (Funahara, Sumarmo, & Wirawan, 1983; C. F. Lin, Wan, Cheng, Lei, & Lin, 2006).

In the early febrile phase, platelet counts are typically within the normal range (Bongsebandhu-Phubhakdi, Hemungkorn, Thisyakorn, & Thisyakorn, 2008; M. S. Lee et al., 2006; Ministry of Health Malaysia, 2015a). Subsequently, as the disease progresses to late febrile ort the defervescence stage, the platelet count decreases rapidly and may remain low in the first few days of the recovery period (Ministry of Health Malaysia, 2015a; World Health Organization, 2012b).

To date, there is no precise definition and cut-off values to determine the rapid decrease of platelets in dengue infection. Based on a meta-analysis in 2013, there were 33% increased odds of developing severe dengue for every 10,000 platelet cells decrement (Huy et al., 2013). Thus, further studies should be done to observe the trend and pattern of platelet counts as well as to define "rapid drop in platelet count" as the universal predictor for dengue severity (Huy et al., 2013).

2.2.7 Hematocrit

The increase in hematocrit is a marker of plasma leakage in dengue infection (Ministry of Health Malaysia, 2015a; World Health Organization, 2012b). The levels of hematocrit during dengue infection can be used to differentiate between severe and non-severe dengue infection. However, patients with early fluid therapy as well as those with concurrent bleeding can mask the values of hematocrit (Lum, Ng, & Khoo, 2014).

Increased hematocrit levels accompanied by a rapid decrease in platelet is suggestive of severe dengue (Lum et al., 2014). Due to the difficulties of obtaining baseline hematocrit in dengue patients, a cut-off of more than 40% in females and more than 46% in males is used as the baseline hematocrit (Lum et al., 2014). In severe dengue infection, the hematocrit level increases due to the development of plasma leakage and dehydration (Centers for Disease Control and Prevention, 2017; Lum et al., 2014). Based on a meta-analysis in 2013, with every 1% increase in the hematocrit value, the log odds ratio (logOR) of plasma leakage increases by 20.5% (Huy et al., 2013).

2.2.8 Plasma Leakage

Plasma leakage is a process in which the protein-rich, fluid component of the blood leaks from blood vessels into the surrounding tissue (Centers for Disease Control and Prevention, 2017; Srikiatkhachorn, 2009). In dengue infection, the presence of plasma leakage distinguishes severe infection from non-severe dengue infection. Some dengue patients develop severe dengue infection once their fever begins to disappear (Lum et al., 2014).

According to the pathophysiology of dengue virus infection, the immune response against the dengue virus begins when an infected adult mosquito releases viral particles by inserting its proboscis into the epidermal layer of human skin (Centers for Disease Control and Prevention, 2017). These viral particles are taken up by cells of the immune system known as antigen-presenting cells (APCs) in the human body (Centers for Disease Control and Prevention, 2017).

During the incubation period, dengue virus replicates and causes the APC to become mature (Centers for Disease Control and Prevention, 2017). The mature APC enters an afferent lymphatic vessel where it travels from the site of infection to the lymph node of the infected person (Martina et al., 2009). In the lymph node, the mature APC produces chemical messengers that attract and activate T-cells (Martina et al., 2009). These activated T-cells react and secrete pro-inflammatory molecules, such as TNF-alpha, IFN-gamma, IL-6, and IL-8 (Martina et al., 2009; Shi, Jiang, & Zeng, 2006).

In severe dengue infection, the production of the pro-inflammatory molecules is significantly higher than in non-severe dengue infection. When these molecules enter the bloodstream, they help eliminate the virus and might also play a role in plasma leakage (Martina et al., 2009; Shi et al., 2006). The endothelial surface glycocalyx and cell-to-cell junctions maintained the integrity of blood vessels (Reitsma, Slaaf, Vink, van Zandvoort, & oude Egbrink, 2007).

The glycocalyx is a network of proteoglycans and glycoproteins that project from the surface of endothelial cells and acts as the primary barrier against the leakage of proteins and fluid across the vascular wall (Reitsma et al., 2007). The mechanism causing increased vascular permeability during severe dengue was not well understood. However, available evidence suggests that reactive oxygen species, enzymes, and pro-inflammatory molecules start to break down the glycocalyx layer, allowing plasma to reach the underlying intercellular junctions and leak out into the tissues (Puerta-Guardo, Glasner, & Harris, 2016).

The increased vascular permeability causes an increase in hematocrit levels, also referred to as hemoconcentration. The term hemoconcentration is defined as a 20% increase in hematocrit levels and widely used as an indicator of plasma leakage (Srikiatkhachorn, 2009). Severe plasma leakage can result in hypovolemic shock, pleural effusions, and ascites, which can result in respiratory problems (Srikiatkhachorn, 2009).

2.3 Obesity and Dengue Severity

Obesity is defined as a condition of abnormal or excessive fat accumulation in adipose tissue to the extent that health is impaired (J. S. Garrow, 1988). The amount of excess fat in absolute terms, and its distribution in the body, either around the waist and trunk (abdominal, central or android obesity) or peripherally around the body (gynoid obesity) have significant health implications. Depending on age, different methods are available to measure a body's healthy weight according to age group.

Based on the World Health Organisation criteria, a BMI <18.5kg/m^2 is considered underweight, 18.5 to 24.9 kg/m^2 as ideal weight, and 25 to 29.9kg/m^2 as overweight or pre-obese (World Health Organization, 2017f). The obese category is sub-divided into obese class I (30 to 34.9kg/m^2), obese class II (35 to 39.9kg/m^2) and obese class III (≥ 40kg/m^2) (World Health Organization, 2017f).

Obesity is a major public health problem. Globally, the prevalence of overweight and obesity rose by 27.5% for adults between 1980 and 2013 (Ng et al., 2014). The number increased from 857 million in 1980 to 2.1 billion in 2013 (Ng et al., 2014). In 2016, the WHO reported that the prevalence of overweight and obesity were at: (i) 21.9% and 4.7% in South-East Asia region; (ii) 49% and 20.8% in Eastern Mediterranean region; (iii) 31.7% and 6.4% in Western Pacific region; (iv) 31.1% and 10.6% in African region; and (v) 62.5% and 28.6% in Americas region, respectively (World Health Organization, 2017d, 2017e). An estimate by Brady et al. on the prevalence of dengue showed that 3.9 billion people in 128 countries are at risk for dengue virus infection (Brady et al., 2012). The WHO also reported that dengue has spread to new areas such as France, Europe, China, and Japan (World Health Organization, 2017a).

In Malaysia, the 2015 national prevalence of overweight was 33.4% (95% CI=32.5,34.4) while the prevalence of obesity was 30.6% (95% CI=29.5, 31.6) (National Institute of Health Malaysia, 2016). Compared with the National Health and Morbidity Survey (NHMS) in 2011, the prevalence of obesity and overweight in 2016 increased by 2.6% and 0.6%, respectively (National Institute of Health Malaysia, 2011, 2016).

Hypothetically, obesity could affect the severity of dengue infection through the inflammation pathways. The increased deposition of white adipose tissue (WAT) in obese individuals leads to the increased production of Interleukin-six (IL-6), Interleukin-eight (IL-8), and Tumor Necrosis Factor Alpha (TNF-α) (Bosch et al., 2002; Calabro et al., 2005; Juffrie et al., 2001). IL-6, IL-8, and TNF-a were essential mediators of the inflammation pathway that increases capillary permeability (Puerta-Guardo et al., 2016). An increased capillary permeability in obese dengue patients could progressively underlie the process of severe plasma leakage.

In 2013, Huy et al. published a systematic review and meta-analysis of 198 studies up to September 2010 on factors associated with dengue shock syndrome. The authors found that obesity had no significant association between DSS and overweight or obesity (Huy et al., 2013). In 2016, Trang et al. published a systematic review and meta-analysis of 13 studies up to August 2013 on the association between nutritional status and dengue infection. The meta-analysis found that there was no significant association between overweight/obese and DSS. However, the authors failed to show substantial consistency regarding the relationship between studies and concluded that the effects of nutritional status on dengue outcomes were controversial (Trang et al., 2016). Similarly, both systematic reviews by Huy et al. and Trang et al. focused only on the association between nutritional status and dengue infection with studies up to August 2013 (Huy et al., 2013; Trang et al., 2016).

We performed a systematic review and meta-analysis to explore the association between obesity and dengue severity further. Based on the result of the systematic review, we found a significant association between obesity and dengue severity among children (Zulkipli et al., 2018). We found that there were 38% higher odds of developing severe dengue infection among obese dengue patients compared to non-obese patients (Zulkipli et al., 2018). The results of this review support our hypothesis that obese patients have more severe dengue infection compared to non-obese patients. However, we only managed to include studies with pediatric patients due to the unavailable studies involving adult dengue patients.

These findings represent crucial information on the association between obesity and dengue severity. With the increasing prevalence of obesity and increasing populations susceptible to dengue infection, the number of obese individuals susceptible to dengue will significantly increase. Thus, a more severe dengue infection can occur thereby increasing the burden of dengue infection.

2.4 Diabetes and Dengue Severity

Diabetes is a growing epidemic in South Asia. Malaysia observed an increased prevalence of diabetes (National Institute of Health Malaysia, 2011, 2016). The Malaysian National Health and Morbidity Survey (NHMS) in 2011 estimated that the prevalence of diabetes among individuals aged 18 years old and above was 15.2% or approximately 2.6 million (National Institute of Health Malaysia, 2011). In 2105, the prevalence of diabetes increased to 17.5%, 2.3% higher compared to NHMS 2011 (National Institute of Health Malaysia, 2016). Similarly, in 2015, the International Diabetic Federation (IDF) reported that the prevalence of diabetes in Malaysia was 17.9% with 3.3 million people living with diabetes and more than half (52%) were undiagnosed (International Diabetes Federation, 2015).

At this point, the confirmation of dengue infection was based solely on the WHO 2009 criteria (Ministry of Health Malaysia, 2015a; World Health Organization, 2012b). This criteria do not emphasise on the screening of co-morbidities among adults to predict the risk for developing severe dengue. Thus, finding new and straightforward predictors to predict dengue severity during the initial presentation using widely available markers such as blood glucose level to detect individuals with high risk especially among those living in dengue-endemic regions is essential.

The presence of diabetes requires a stricter observation for clinical progression due to its risk. However, the pathophysiology of diabetes leading to severe dengue is not well understood. Several studies have suggested that diabetes could result in immune and endothelial dysfunction (Geerlings & Hoepelman, 1999; Quinones, Nicholas, & Lyon, 2005). There is scarce evidence available especially in determining the association between obesity, diabetes, hypertension, and severity of dengue infection.

To date, there is only one systematic review on the association between diabetes and dengue severity published Htun et al. in 2015 summarising all research available until February 2014 (Htun et al., 2015). The authors found 10 papers by using the search terms "("dengue"[MeSH Terms] OR "dengue" [All Fields]) AND ("diabetes" [MeSH Terms] OR ("diabetes" [All Fields] AND "mellitus" [All Fields]) OR "diabetes" [All Fields] OR "diabetes" [All Fields]".

Of the ten papers reviewed, five were case-control studies and five were case-series studies. In the systematic review, the authors summarised that currently available evidence is limited and only suggestive in addressing the effects of diabetes on the clinical presentation of dengue (Htun et al., 2015). However, the authors suggested for more prospective studies to assess the effect of pre-existing diabetes as well as hyperglycemia at the time of dengue diagnosis and to obtain the appropriate sign and symptoms in identifying the risk factors of dengue severity and death (Htun et al., 2015).

Furthermore, Muhammad et al. found a significant association between dengue and diabetes. The authors also found that glucose intolerance was frequently associated with dengue fever during its early course (Muhammad et al., 2012). A previous study reported that there was a higher proportion of diabetes cases was found among dengue mortality cases (Leo et al., 2011). Figueiredo et al. found that among those with diabetes, there was twice the risk of developing severe dengue infection compared to non-diabetes (Figueiredo et al., 2010). In addition, Karunakaran et al. found a significant association between diabetes and higher dengue mortality (Karunakaran, Abbas, Sheen, Jose, & Nujum, 2014).

Chen et al. published a hospital-based retrospective study on the association between diabetes and thrombocytopenia among dengue patients (C. Y. Chen et al., 2015). In the study, the researchers observed the characteristics of dengue patients admitted to a university hospital during a dengue epidemic in Taiwan between June and December 2002. As a result, the authors concluded that dengue patients with diabetes tend to have lower platelet counts compared to non-diabetic dengue patients and they were more likely to develop severe dengue infection (C. Y. Chen et al., 2015).

The escalating global prevalence of both dengue and diabetes together with the limited evidence on its relationship as well as clinical progression justifies the need for additional study.

2.5 Hypertension and Dengue Severity

Hypertension is defined as: (i) a systolic blood pressure (SBP) of 140 mmHg or more; or (ii) a diastolic blood pressure (DBP) of 90 mmHg or more; or (iii) taking antihypertensive medication (Benjamin et al., 2017). Based on recommendations of the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) (United States Department of Health and Human Services, 2003), the classification of blood pressure for adults aged 18 years or older has been as follows:

(i) Normal: SBP <120 mmHg, DBP < 80 mmHg;

(ii) Pre-hypertension: SBP 120 to 139 mmHg, DBP 80 to 89 mmHg;

(iii) Stage 1- Hypertension: SBP 140 to 159 mmHg, DBP 90 to 99 mmHg;

(iv) Stage 2 –Hypertension: SBP ≥ 160 mmHg, DBP ≥ 100 mmHg.

Hypertension could be primary, which could develop as a result of environmental or genetic causes, or secondary, which has multiple etiologies, including renal, vascular, and endocrine causes (United States Department of Health and Human Services, 2003). Primary or essential hypertension accounts for 90 to 95% of adult cases, and secondary hypertension accounts for 2% to 10% of total cases. In 2010, it was estimated that hypertension accounted for 17.8% of the premature deaths (9.4 million deaths, 162 million years of life lost) and 7% of disability (173 million disability-adjusted life years [DALYs]) globally (Campbell et al., 2015; Lim et al., 2012; World Health Organization, 2008). Furthermore, the prevalence of hypertension in low, lower-middle and upper-middle income countries is 5% higher than in high-income countries (World Health Organization, 2013b).

Hypertension was responsible for at least 45% of deaths due to heart disease and 51% of deaths due to stroke (World Health Organization, 2008). In 2015, the WHO estimated that the global crude and age-standardised prevalence of hypertension at 22.3% and 20.5%, respectively (World Health Organization, 2015).

In Malaysia, hypertension is a significant public health problem (Rampal et al., 2008). According to the NHMS in 1996 and 2011, the prevalence of hypertension has increased from 32.9% in 1996 to 43.5% in 2011 among adults aged 30 years and above (Naing et al., 2016; National Institute of Health Malaysia, 2011). In the NHMS 2015, the overall prevalence of hypertension (known and undiagnosed) among adults aged 18 years and above was 30.3% (95% CI=29.3, 31.2) with 17.2% undiagnosed (95% CI=16.4, 18.0) (National Institute of Health Malaysia, 2016). The high prevalence of hypertension contributed to the increasing healthcare expenditure in Malaysia (Muna AS, Mohamed Azmi AH, & Mohamed Izham MI., 2010).

The literature on the association between hypertension and severe dengue infection is inconclusive. Maria et al. in a 2010 case-control study found no significant association between hypertension and DHF (Maria Aparecida A. Figueiredo, Rodviputal, & Barrets, 2010). In 2012, Pang et al. concluded that patients with hypertension had 2.1 times the risk of developing a severe clinical presentation of dengue compared to non-hypertension patients (Pang et al., 2012). Moreover, in 2015, Teixeira et al. found that the progression of dengue infection to a more severe clinical presentation was associated with self-reported hypertension among those with dengue infection (Teixeira et al., 2015). The mechanism by which hypertension might increase the risk of a more severe clinical manifestation of dengue is not well understood. Current evidence suggests that hypertension leads to the endothelial dysfunction and vascular damage, promoting inflammatory activation of the endothelium, and changing the regulation of vascular tone and flow (Savoia et al., 2011).

Furthermore, commonly elevated C-reactive protein (CRP) in hypertension can promote detrimental effects on the vascular wall such as inducing endothelial dysfunction and reducing the nitric oxide (NO) bioavailability (Hage, 2014; Pauletto & Rattazzi, 2006). The effect of CRP on the vascular wall could lead to increased vascular permeability, coagulopathy, loss of fluid, and hypovolemic shock, which are the characteristics of severe dengue infection.

2.6 Summary of Literature Review and Conceptual Framework

Dengue research focused on epidemiology of dengue, pathology, virology, vector, etiology, serotypes, symptoms, diagnosis, surveillance, monitoring, prevention, control, risk factor, and vaccination. (Cheah, Ng, Marzilawati, & Lum, 2014; Hii et al., 2016; Johansson, Hombach, & Cummings, 2011; Snow, Haaland, Ooi, & Gubler, 2014; Soo et al., 2016; Zhao et al., 2012). Limited studies have focused on the association between co-morbidities such as obesity, diabetes, and hypertension and dengue severity (Htun et al., 2015; Kalayanarooj & Nimmannitya, 2005). The conceptual framework in Figure 2.1 summarises the reviewed literature on the association of obesity, diabetes, and hypertension with the development of severe dengue infection.

Mainly, the development of severe dengue infection was due to plasma leakage and thrombocytopenia. The presence of plasma leakage leads to hemoconcentration and third space fluid accumulation, which further results in severe dengue infection. Thrombocytopenia in dengue infection leads to spontaneous bleeding. Obesity, diabetes, and hypertension increase the risk of severe dengue infection due to the predispose increased capillary permeability prior to dengue infection. Thus, combined with dengue infection, presence of obesity, diabetes, or hypertension, it further accelerates and worsens the progression of severe dengue infection.

Furthermore, there is a suggestive association between age, gender, ethnicity, high blood pressure, glucose intolerance, hypertriglyceridemia, thrombocytopenia, low serum albumin, blood types, dengue serotypes, and the development of a more severe dengue infection (C. Y. Chen et al., 2015; Hasanat, Ananna, Ahmed, & Alam, 2010; Lye, Lee, Sun, & Leo, 2010; Pang et al., 2012). Also, the presence of co-morbidities such as obesity, hypertension, diabetes, and allergy treated with steroids is related to severe dengue infection (Htun et al., 2015; Widiyati, Laksanawati, & Prawirohartono, 2013).

In addition, age, gender, and ethnicity are related to the development of obesity, diabetes, hypertension, and dengue severity. Given this, we decided to investigate these co-morbidities in focus obesity, diabetes, and hypertension as risk factors for dengue severity.

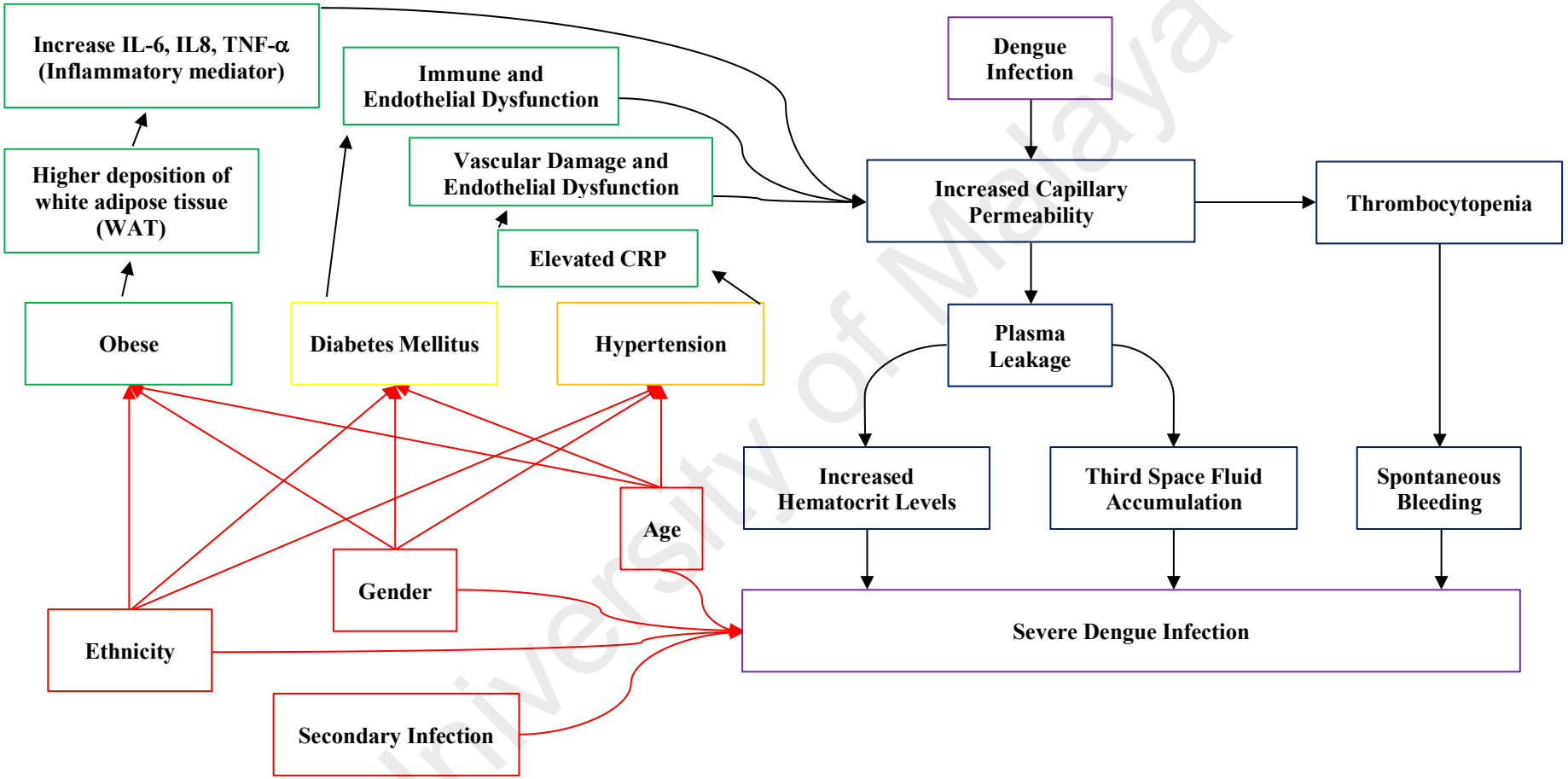

Figure 2.1: Conceptual framework

CHAPTER 3: METHODOLOGY

3.1 Introduction

This chapter details the research methodology. It includes explanations of the study design, study settings, study population, sampling frame, sampling strategy, inclusion and exclusion criteria, and sample size calculation. Furthermore, it explains the study flow and processes that include ethics approval, site initiation, study training, data collection process, and closing of study sites. We also describe the study variables used in this study which includes the exposure variables (obesity, diabetes, hypertension), outcome variables (dengue severity), confounding, and effect modification. Moreover, we describe the instrument used in this study that includes the computerised form, anthropometry measurement tools, vital sign measurement tools, and biomarkers. We further explain the data collection processes in detail, data extraction, data management, as well as analysis. Finally, we describe the ethics approval and all the grants secured at the end of this chapter.

3.2 Study Design

A multicentre, prospective, dynamic cohort design was used to investigate the association between risk factors (obesity, diabetes, and hypertension) and severity of dengue infection. In this study, the entry time of a participant into the cohort was the day of fever on the first visit by the participant. The exit time was the day of fever on the last follow up visit or at time of outcome ascertainment. Participants had variable number of study visits between them. As the time of presentation and follow-up time differs between participants, a dynamic cohort design is more suitable for this study. Thus, the longitudinal analysis accounted for this by modelling the days of fever rather than visit number. Data collection was conducted from May 2016 and November 2017 (18 months).

3.3 Study Settings

This study involved 24 primary health care clinics in six districts from the state of Melaka, Negeri Sembilan, and Selangor. All three states were located in the central region of Peninsular Malaysia. Figure 3.1 shows the location of selected states in the Peninsular Malaysia.

Figure 3.1: The Peninsular of Malaysia map

3.4 Study Population

The population for this study was dengue patients aged 15 years and above from Melaka, Negeri Sembilan, and Selangor. Based on previous studies, 80 percent of reported dengue cases were mainly 15 years old and above. (Cheah et al., 2014; Sam, Omar, Teoh, Abd-Jamil, & AbuBakar, 2013) Thus, we selected 15 years old as the cut-off point for the inclusion of our study participants. In practice, any patient who visits the clinic with high fever will undergo a full blood count (FBC) investigation. We diagnosed patients with probable dengue if they fulfil the diagnostic criteria of probable dengue infection. All probable dengue patients need to undertake a dengue confirmatory diagnostic test using the SD Bioline Dengue Duo NS1 Ag & IgG/IgM (SD Bioline, Korea) dengue rapid combo test (NS1, IgG, IgM). The SD Bioline Dengue Duo NS1 Ag & IgG/IgM rapid combo test is a simple, easy to perform, inexpensive, sensitive, and require no sophisticated protocols, and, therefore can be used in any location (Jang et al., 2019). It also showed no cross reactivity with Chikungunya virus infected serum (Jang et al., 2019).

3.5 Inclusion and Exclusion Criteria

We enrolled patients with dengue rapid combo test confirmed dengue infection as our participants. We confirmed dengue patient based on the dengue rapid combo test result of either: (i) NS1 positive; (ii) IgM positive; (iii) NS1 & IgM positive; (iv) NS1 & IgG positive; (v) IgG & IgM positive; or (vi) all NS1, IgG & IgM positive. We excluded patients with sepsis, malaria, pregnant, and breastfeeding as well as those on antiplatelet or anticoagulant treatment. We only accepted patients who fulfil the inclusion and exclusion criteria as research participants.

3.6 Sampling

In this study, we randomly selected 6 out of 18 districts from Melaka, Negeri Sembilan and Selangor. Next, from 6 districts, 24 primary health care clinics with Family Medicine Specialist (FMS) were selected using the list provided by each District Health Office. The selection of these study sites should not affect the external validity of this study as the association between the severity of dengue infection and its predictors in the study population was likely to be similar compared to other districts in Malaysia as well as urban and rural settings (Chew et al., 2016; Muhammad Azami, Salleh, Neoh, Syed Zakaria, & Jamal, 2011). Furthermore, we selected all patients from each primary health care clinics who fulfilled the inclusion and exclusion criteria to ensure selection transparency.

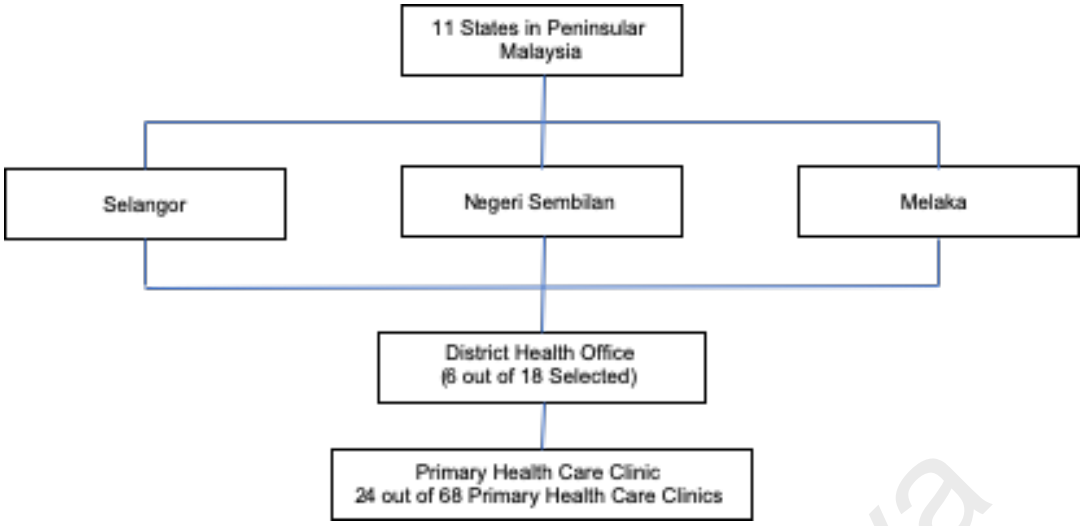

Figure 3.2: Sampling of Primary Health Care Clinics

Table 3.1: Selected primary health care clinics by districts

Districts	Primary Health Clinics
Melaka Tengah, Melaka	1. Klinik Kesihatan Ayer Keroh 2. Klinik Kesihatan Ayer Molek 3. Klinik Kesihatan Peringgit 4. Klinik Kesihatan Tengkera 5. Klinik Kesihatan Cheng 6. Klinik Kesihatan Ujong Pasir
Seremban, Negeri Sembilan	1. Klinik Kesihatan Ampangan 2. Klinik Kesihatan Seremban 3. Klinik Kesihatan Seremban 2
Gombak, Selangor	1. Klinik Kesihatan Taman Ehsan 2. Klinik Kesihatan AU 2
Petaling, Selangor	1. Klinik Kesihatan Seksyen 19 2. Klinik Kesihatan Seksyen 7 3. Klinik Kesihatan Seri Kembangan 4. Klinik Kesihatan Puchong 5. Klinik Kesihatan Kelana Jaya
Hulu Langat, Selangor	1. Klinik Kesihatan Kajang 2. Klinik Kesihatan Bangi 3. Klinik Kesihatan Batu 9 4. Klinik Kesihatan Bandar Sri Putra 5. Klinik Kesihatan Ampang
Klang, Selangor	1. Klinik Kesihatan Pandamaran 2. Klinik Kesihatan Meru 3. Klinik Kesihatan Bukit Kuda

3.7 Sample Size

The required sample size for this study was calculated using OpenEpi, Version 3, open source calculator - SSCohort (Sullivan, Dean, & Soe, 2009). We estimated the sample size based on the association between the exposure of interest (obesity, diabetes, and hypertension) and the incidence of severe dengue infection.

We used a composite outcome termed severe dengue infection that consists of either dengue with warning signs or severe dengue in the calculation of sample size for this study. We expected a low incidence of severe dengue infection due to the findings in (Mohd-Zaki et al., 2014). Thus, we combined dengue with warning signs and severe dengue as a composite term of dengue severity.

To calculate the sample size, we search for available references on the odds of dengue severity and prevalence of dengue severity in Malaysia. Based on the available literature, the odds of dengue severity between obese, diabetes, hypertension, and dengue severity range from 1.75 to 2.75 (Figueiredo et al., 2010; Htun et al., 2015; Teixeira et al., 2015; Widiyati et al., 2013). We also found that the prevalence of dengue severity among dengue fever ranging from 47 percent to 50 percent (Ahmad et al., 2018; Guzman et al., 2013; Liew et al., 2016; Soo et al., 2016). Thus, a sample size of 153 was calculated with 80% power at a two-sided α=0.05 to the odds of dengue severity between exposed and non-exposed participants; assuming the odds of 2.75 and 50% prevalence of dengue severity.

Despite the existence of various opinions and schools of thought, Mary S Fewtrell et al. suggested that the minimum follow-up rate in a longitudinal study should be at 80% (Mary S Fewtrell et al., 2008). Thus, the maximum attrition rate allowed to maintain the study power was 20%. By using 20% as the expected amount of attrition or loss to follow-up, the final minimal sample size needed will be 183 participants.

3.8 Study Flow Chart

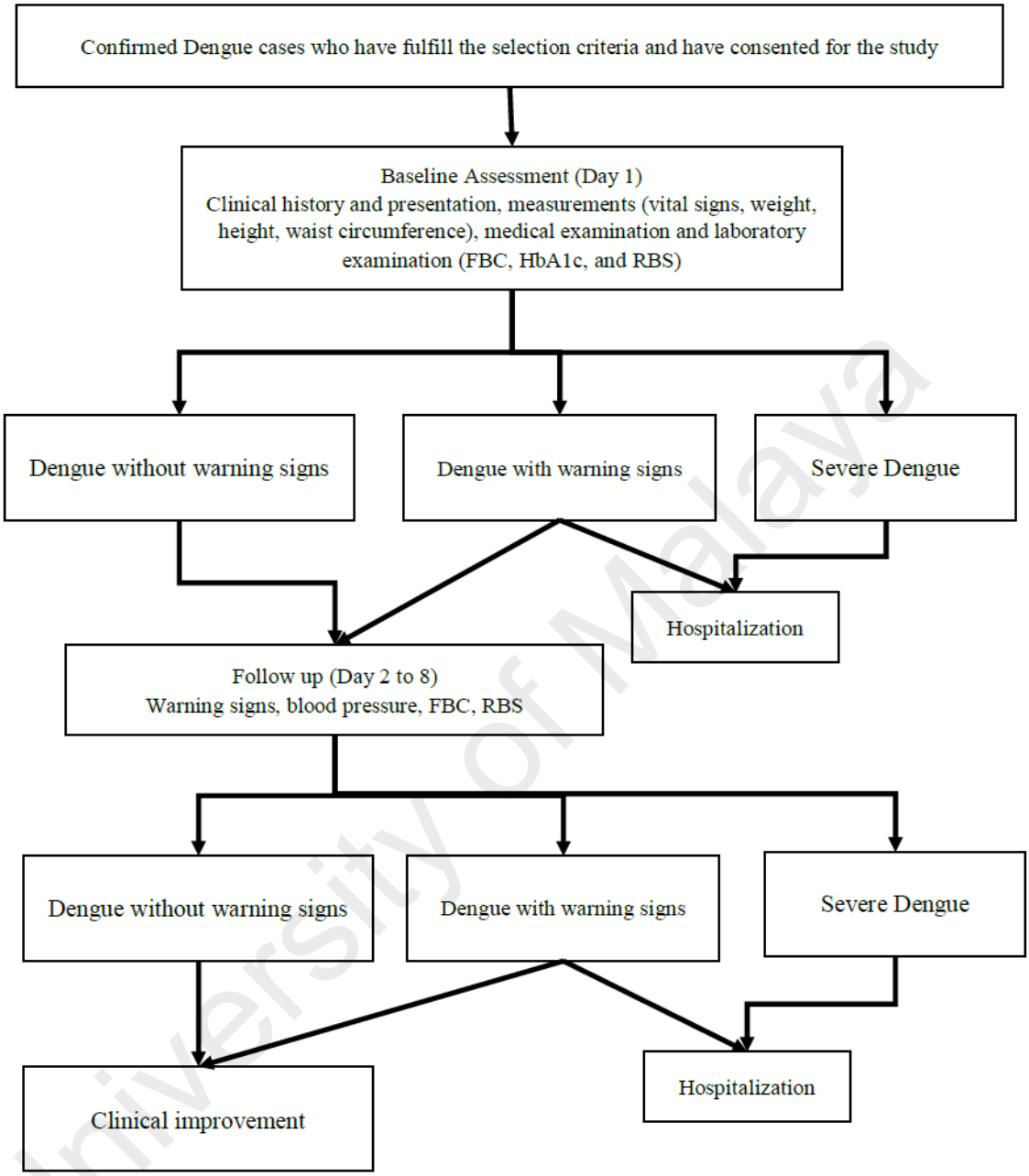

Figure 3.3: Study flow

To ensure a uniform and smooth enrolment process, we developed and followed a standard flow chart on the process of enrolment, baseline examination, and patient follow-up. The flow chart was an adaptation of the regular flow for dengue management in the primary health care clinics. It consists of three main steps: (i) selection and enrolment; (ii) baseline assessment; and (iii) follow-up.

In the first step, we screened all confirmed dengue patients for the eligibility criteria. We only enrolled patients with the consent and those who fulfilled the selection criteria. In step two, participants undergo baseline assessment that includes history taking, body composition measurements (weight, height, and waist circumference), medical examination (clinical examination, physical examination, and vital signs), and laboratory examination (full blood count, RBS, and HbA1c). Based on the baseline assessment, participants were diagnosed with either dengue without warning signs, dengue with warning signs, or severe dengue. Next, in step three, participants undergo follow-up. We schedule follow-up of participants daily starting from the first baseline visit. However, the time interval between successive visits may differ but are accounted for in the longitudinal analysis as the analysis uses a time metric based on day of fever. During follow-up, we monitored patients for warning signs, vital signs (temperature, blood pressure, and pulse rate), and laboratory values (full blood count and RBS). We stopped the follow-up once patients were hospitalised or discharged. We also provide participants with a dengue monitoring card, which record participants warning signs, BP, RBS, and FBC values on each follow-up. We recorded all required information in the paper-based data collection form.

3.9 Ethics

The National Medical Research Register, Ministry of Health Malaysia, reviewed and approved the research proposal (Registration number: NMRR-14-1777-20233 and Ref: (15)KKM/NIHSEC/P15-1089). The NMRR further reviewed and approved two amendments on inclusion of additional study sites (Ref: (9)KKM/NIHSEC/P15-1089 and Ref: (13)KKM/NIHSEC/P15-1089). In addition, the University Malaya Medical Centre Medical Ethics Committee reviewed and approved the research proposal. (Ref: MECID.NO:20143-68). All procedures involving human participants were performed in

accordance with the ethical standards of the Institutional and National Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

3.10 Study Initiation, Training, and Closing

3.10.1 Administrative Approval

We began our study site approval application after obtaining the ethics approval on 11th September 2015. The process started by meeting the Health Director or Deputy Health Director (Public Health Division) from each state for initial study approval. Once approved by the State Health Department, we conducted the second meeting at district level with the Health District Officer and two representatives from each selected Primary Health Care clinics. We presented and discussed the process and feasibility to conduct this study in their clinics. Final agreement and approval for study was then obtained from each clinic and district officer.

3.10.2 Preliminary Training

After receiving the final approval from the Health District Office, we conducted preliminary training sessions for each district. The training session involved four representatives from each clinic that includes Family Medicine Specialist (FMS), Medical Officer (MO), Staff Nurse (SN) or Medical Assistant (MA), and Medical Laboratory Technician (MLT). The selection of representative was according to the flow of the dengue management in each clinic. We ensured that all representatives had at least one-year working experience at the primary health care clinic and completed district level training on dengue clinical practice guidelines (CPG). We conducted a centralised training for all selected primary health care clinics from the selected districts to ensure

uniformity and standardisation thus reducing room for error in communicating and performing the procedure for this study.

During each training, we give each representative from the primary health care clinics an overview of the study protocol. We also explained the study flow and provided initial training for standardisation based on their given task for each representative. The medical officers and FMSs received training on the study flow as well as on how to use the paper-based data collection form for baseline assessment and follow-up. Staff nurses or medical assistants received standardised training on how to measure body weight, height, waist circumference, blood pressure, RBS level and procedures on taking laboratory blood samples. MLTs received training on the standardised procedure for RBS level determination and reporting of blood investigations (FBC and HbA1c).

3.10.3 Site Initiation

The initial study site started with six primary health care clinics from the Melaka Tengah district in May 2016. Subsequently, we initiated two primary health care clinics from Gombak, five primary health care clinics from Petaling, five primary health care clinics from Hulu Langat, and three primary health care clinics from Klang between July and September 2016. In January 2017, we initiated the final three primary health care clinics in Seremban district. The gradual initiation of study sites over seven months was due to the administrative issues that include administrative delay, funding, and lack of the human resource at study sites. We addressed all issues accordingly to ensure no further delay in study site initiation.

3.10.4 Study Update Visit

To ensure the continuity of the data collection process, we performed the first study update visit in November 2016. During the first study update visit, we managed to identify several issues regarding the enrolment of participants of which was found to be low. Among the issues were: (i) reducing number of dengue cases in all districts; (ii) medical officers claim that clinic was too busy and did not have time to enrol patient; (iii) new medical officers did not know about study; and (iv) low motivation and interest of medical officers in enrolling patients. After the first study update visit, we discussed the issues among investigators and came with several remedial measures.

First, we re-briefed all data collectors of the involved clinics on the study objectives and study flow to ensure that all medical officers and staff were updated and motivated. Secondly, we employed staff nurses in clinics in Gombak, Petaling, and Hulu Langat to assist medical officers in performing data collection process.

We performed the second study update visit in May 2017. During this round of update, we found similar issues where most of the medical officers were not motivated to enrol patients. We met with the medical officers from each clinic to solve the issue and resulted in a resolution to increase the honorarium for the medical officers.

3.10.5 Closing of Study Sites

In the last week of November 2017, we stopped the data collection and closed all study sites from enrolling new patients for data collection. In total, from May 2016 until November 2017, we managed to collect 362 study samples from all primary health care clinics.

3.11 Study Variables

3.11.1 Definition of Exposures

In this study, obesity, diabetes, and hypertension were the three exposures explored as risk factors. We determined the obesity status using the body mass index (BMI) calculated based on the measured weight (kg) and height (metre) during baseline visit. We defined obesity as either general obesity or central obesity. General obesity was defined as BMI ≥ 30 kg/m^2. The comparison groups for obesity were normal weight (BMI ≥ 18.50, < 25.00 kg/m^2). In addition, we defined: (i) overweight as BMI ≥ 25.00 kg/m^2 and < 30.00 kg/m^2; and (ii) underweight as BMI < 18.50 kg/m^2.

We defined the cut-off BMI scores based on the WHO classification of obesity (World Health Organization, 2017g). We defined abdominal obesity as WC ≥ 90 cm for men and WC ≥ 80 cm for women. The comparison group for abdominal obesity was individual with WC < 90 cm for men and WC < 80 cm for women. We defined abdominal obesity based on the WHO/IDI/IASO/IOTF 2000 classification of obesity by waist circumference (World Health Organization, International Diabetes Institute, International Association for the Study of Obesity, & International Obesity Task Force, 2000).

We diagnosed diabetes using the hemoglobin A1c (HbA1c) levels taken at the baseline visit and previous diagnosis of diabetes from the medical record. We recorded participant with a HbA1c value of ≥ 6.3% without any previous diagnosis of diabetes in the medical record as diabetes. Similarly, we recorded participants with any HbA1c value together with a previous diagnosis of diabetes in the medical record as diabetics. The comparison group for diabetes was non-diabetes with no recorded diagnosis of diabetes in the medical report and a HbA1c level of < 6.3% at baseline. The definition of diabetes is based on the latest 2015 Malaysian Clinical Practice Guidelines on Management of Type-2 Diabetes (Ministry of Health Malaysia, 2015b).

Hypertension was epidemiologically diagnosed using medical records and the average of two readings of the systolic and diastolic blood pressure at baseline. Among non-hypertension patients, we defined hypertension as either systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg. Similarly, we recorded those with a previous diagnosis of hypertension in the medical records as hypertension. The comparison group for hypertension was non-hypertension which includes individuals with SBP < 140 mmHg or DBP < 90 mmHg or those with no previous diagnosis of hypertension in the medical record.

We defined hypertension based on the seventh report of the Joint National Committee (JNC) on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure in 2003 and the Malaysian Clinical Practice Guidelines on Management of Hypertension in 2013 (Ministry of Health Malaysia, Malaysian Society of Hypertension, & Academy of Medicine of Malaysia, 2013; United States Department of Health and Human Services, 2003).

3.11.2 Definition of Outcomes

The outcomes of interest in this study were severe dengue infection which is defined collectively as dengue with warning signs or severe dengue. In this study, we used the WHO 2009 dengue classification to classify the participants. The WHO 2009 classifies dengue infection as: (i) dengue without warning signs; (ii) dengue with warning signs; and (iii) severe dengue (World Health Organization & Special Programme for Research and Training in Tropical Diseases, 2009).

We diagnosed dengue infection based on the reported diagnosis at the baseline and diagnosis at the end point of follow-ups. We diagnosed patient as dengue without warning signs if they presented with fever, at least two of the following criteria: (i) nausea, vomiting; (ii) rash; (iii) aches and pains; (iv) positive tourniquet test; or (v) leucopenia (WBC < 3,500 per microliter), and a confirmed laboratory test (Ministry of Health Malaysia, 2015a).

We diagnosed the patient as having dengue with warning signs if they fulfilled the criteria for dengue without warning signs with any of the warning signs that include: (i) diarrhea more than three times in 24 hours; (ii) vomiting more than three times in 24 hours; (iii) abdominal pain or tenderness; (iv) lethargy/restlessness/confusion; (v) liver tenderness; (vi) clinical fluid accumulation; (vii) spontaneous bleeding; and (viii) raised hematocrit with rapid drop in platelets (Ministry of Health Malaysia, 2015a). If there is no baseline hematocrit level to monitor raised haematocrit of more than 20%, a cut-off point of > 46% (for men age 60 years and below), >40% (for men above 60 years old), and > 40% for women with reduction of platelet (no cut-off point) shall be used to determine as positive raised haematocrit with rapid drop in platelet (Ministry of Health Malaysia, 2015a).

We diagnosed the patient with severe dengue if they fulfilled the criteria for either dengue without or with warning signs with any of the following conditions: (i) severe plasma leakage leading to shock or fluid accumulation with respiratory distress; (ii) severe bleeding; or (iii) severe organ involvement as evidenced by liver ALT or AST ≥ 1,000, impaired consciousness, and failure of the heart or other organs (Ministry of Health Malaysia, 2015a). Hospitalisation and discharged eligibility were determined based on the participant's clinical condition and progress as assessed by the attending medical officer.

We stratified days of fever into three phases namely: (i) phase 1 (days of fever ≤ 3 days); (ii) phase 2 (days of fever between 4-6 days); (iii) phase 3 (days of fever ≥ 7 days). The stratification for days of fever was based on the classical dengue progression namely: (i) febrile phase; (ii) critical phase; and (iii) defervescence phase based on the clinical practice guidelines. (Ministry of Health Malaysia, 2015a).

3.11.3 Adjustments and Interaction

(a) Age, gender, ethnicity

Confounders are variables that are both an ancestor of the exposure and an ancestor of the outcome (Mark Gilthorpe, 2018). Based on our review of the literature, we found that age, gender, and ethnicity might be related to the severity of dengue infection, where more men and older patients progress to a more severe dengue infection (Guzman et al., 2013; Hammond et al., 2005; Kalayanarooj & Nimmannitya, 2005; M. S. Lee et al., 2006; Machado et al., 2013). Similarly, we found that age, gender, and ethnicity were related to the development of obesity, diabetes, and hypertension (Choi, Liu, Palaniappan, Wang, & Wong, 2013; Jin et al., 2013; Zeigler-Johnson, Weber, Glanz, Spangler, & Rebbeck, 2013). Based on the definitions given, we considered age, gender, and ethnicity as the primary confounders in this study.

(b) Obesity

Babu et al. published a meta-analysis on the association of obesity with hypertension and diabetes (Babu et al., 2018). Based on the meta-analysis, the authors concluded that obesity showed a significant association with diabetes and hypertension (Babu et al., 2018). Similarly, previous studies found that the development of diabetes from obesity happens due to the progressive defect in insulin secretion coupled with a progressive rise in insulin resistance (Golay & Ybarra, 2005). Also, we found an association between obese and severity of dengue infection in pediatric patients (Zulkipli et al., 2018). Given the available evidence, we considered obesity as a confounder for the association of diabetes and hypertension with dengue severity.

(c) Secondary Infection

Based on our literature review, we found that secondary infection has been known to cause a more severe dengue infection compare to those with primary infection (Guzman et al., 2013; Soo et al., 2016). However, we couldn't find any relationship between secondary infection with obesity and diabetes. Based on the definition of confounder by Rothman, secondary infection was not considered confounder. However, given the possibility for secondary infection to have an interaction effect on the association between obesity, diabetes, and dengue severity, we consider secondary infection as possible interaction and will be tested in an additional analysis against the final model.

3.12 Study Instrument

3.12.1 Data Collection Form

We developed a paper-based data collection form using the Cardiff TeleForm System (currently known as OpenText TeleForm, Waterloo, Canada). The form is developed based on the standard dengue clerking form used in the primary health care clinic with additional information that includes measurement of waist circumference, measurement of weight, measurement of height, HbA1c level, RBS level and sections for daily follow-up. It consists of twelve pages that include: (i) one front page; (ii) three pages of baseline visit section; (iii) one page for visit two until visit eight; and (iv) one back page.

The front page consists of information on patient's name, patient's phone number, and doctor's name. Page two to page four consists of baseline characteristics and information that includes: (i) patient's general information; (ii) demographic information; (iii) clinical information; (iv) co-morbidities; (v) warning signs; (vi) vital signs; (vii) laboratory examination; (viii) diagnosis at baseline; and (ix) referral status.

Pages five to 11 consists of follow-up visit two to eight information that includes: (i) warning signs; (ii) vital signs; (iii) laboratory examination; (iv) diagnosis; and (v) referral status at respective follow-up. Page twelve contains the inclusion and exclusion criteria, instruction on how to fill up the electronic paper-based data collection form, and researcher contact details. To ensure readability and consistency of the paper-based data collection form with the required study variables, two experts reviewed the contents and performed the initial testing of the completed form before printing.

3.12.2 Anthropometry Measurement

We measured height using a calibrated vertical SECA 213 Portable Stadiometer (Hamburg, Germany), to the nearest centimetre. The staff nurse or medical assistant first informed the participant that they would now like to measure their standing height. The participant was told to stand barefoot with their back against the vertical scale, feet parallel to each other, toes pointing forward and soles flat on the floor.

Next, the staff nurse or medical assistant ensured that the participant stands unsupported, with legs straight and with buttocks and shoulder blades touching the vertical scale. Their shoulders should be relaxed, with arms by their sides, and they should not be slouching or leaning to one side. With permission, the participant's head was positioned gently so that they are looking straight forward, with their ear holes in the same horizontal plane as the lower border of their eye sockets.

The participant was told to stand as tall as possible, to take a deep breath in then out, and to relax their shoulders. While the participant was taking a deep breath in, the staff nurse or medical assistant applied gentle pressure upwards to the bony prominence just behind their ears and brought the horizontal measure down on top of the participant's head. The measurement was read on the vertical scale (as indicated by the red or black arrows/line) to the nearest centimetre and the value entered into the paper-based data collection form.

We measured weight using the SECA 813 Portable Digital Electronic Scale (Hamburg, Germany), to the nearest decimal fraction of a kilogram. The staff nurse or medical assistant first informed the participant that they would now like to measure their weight. The scale was placed on a hard and flat surface.

Next, the staff nurse and medical assistant pressed firmly on the centre of the scale to turn it on. Once the zeros appear, the participant was asked to stand in the centre of the scale without support, with their arms loosely by their sides, head facing forward and with their weight distributed evenly on both feet. A reading appeared in a few seconds. The numbers changed and then stop. Once the numbers have stopped, the reading was recorded to the nearest 0.1 kg.

We measured waist circumference using the SECA 201 Flexible Measuring Tape (Hamburg, Germany), to the nearest 0.1 centimetres. The cross-hand technique used for measuring waist circumference. The objective was to minimise the gaps between the tape and the body surface and to minimise indentations of the body surface wherever possible. The staff nurse or medical assistant first informed the participant that they would now like to measure their waist circumference.

The participant was told to stand upright in a relaxed manner, feet comfortably apart, weight evenly balanced on both feet, and with their arms hanging by their side. The staff nurse or medical assistant positioned the tape, hold the casing of the tape using their right hand. Next, the stub end of the tape was given to the participant with their left hand. The participant passed the tape around their back and gave it back to the staff nurse or medical assistant.

Then, the staff nurse or medical assistant took hold of the stub with their right hand and holds both the stub and the casing, leaving the left hand free to manipulate the tape at the correct level. Enough tension was used on the tape with the right hand to hold the position. The participant told to put the tape at their waist level, the narrowest point between the lower borders of the rib cage and the iliac crest. Using the left hand to take hold of the stub, the tape was pulled across to the left into the cross-hand position, keeping enough tension on the tape to prevent it from slipping out of position.

Next, the tape was moved sideways with both hands as needed to position the zero line nearer the participant's side, rather than the middle. When the tape was at place, the participant was told to breathe normally. Gentle pressure was applied to the tape and reading recorded to the nearest 0.1 centimetres at the end of normal expiration.

3.12.3 Vital Signs Measurement

We measured systolic blood pressure, diastolic blood pressure, and pulse rate using the Omron HEM-7120 Automatic Digital Blood Pressure Monitoring System (Kyoto, Japan), in mmHg. First, the staff nurse or medical assistant first informed the participant that they would now like to measure their blood pressure and pulse rate. The participant was asked to be seated quietly for at least 5 minutes in a chair, with feet on the floor, and arm supported at the heart level.

We used an appropriate-sized cuff (cuff bladder encircling at least 80% of the arm) to ensure accuracy. Next, the staff nurse or medical assistant ensured that the cuff was wrapped snugly around the upper arm of the participant with the centre of the cuff bladder positioned over the brachial artery and the lower border of the cuff approximately 2 cm above the elbow bend. The cuff placed at heart level by supporting the arm. The staff nurse or medical assistant then pressed the start button to begin the measurement. To ensure reliability and accuracy, we obtained two blood pressure measurements at five minutes apart for each participant.

3.12.4 Biomarker Measurement

The study participant underwent blood examination for FBC, RBS, and HbA1c during the baseline assessment and each follow-up. To avoid error in blood-taking procedures that might lead to disruption of blood samples, we assigned a staff nurse or medical assistant from each primary health care clinic to perform the blood-taking procedure. The staff nurse or medical assistant first explained to the participant the objective and procedure for blood-taking. Ten millilitres of venous blood were drawn by an open technique using the ten millilitres Terumo Single Use Non-Toxic Non-Pyrogenic Latex-Free Syringe (Kanagawa, Japan) and size 23 Gauge with a 1" Terumo Single Use Non-Toxic Non-Pyrogenic Latex-Free Needle (Kanagawa, Japan).

Four millilitres of blood transferred into two BD Hemogard$_{TM}$ EDTA vacuum container (New Jersey, United States) for full blood count and HbA1c analysis. Two millilitres of blood transferred into a Fluoride Oxalate vacuum container (New Jersey, United States) for RBS. Immediately, all three containers were inverted for eight to ten times to ensure proper mixture of the additives and to avoid micro clotting of the blood (Becton, 2010; Kocijancic, Cargonja, & Delic-Knezevic, 2014). The tubes labelled with participant name and identity card number for identification.

We analysed full blood counts using: (i) Sysmex Hematology Analyser (Kobe, Japan) for Klang, Melaka Tengah, Seremban districts; (ii) Mindray Three-part Hematology Analyser (Shenzen, China) for Hulu Langat and Gombak districts; and (iii) Nihon Kohden Hematology Analyser (Tokyo, Japan) for Petaling district. On daily basis, all hematology analyser will be calibrated using the standard procedure provided by the supplier. Based on available literature, all three analyser showed now statistically difference in blood values and have good concordance (Longair, Briggs, & Machin, 2011; Sun et al., 2019; Wang, Zhao, Su, & Liu, 2019). Moreover, all three analysers comply with the International Council for Standardisation in Hematology (ICSH) standard. Thus,

we are confident that there will be no difference in the values. We measured the HbA1c level using Bio-Rad D-10 HbA1c Program (California, United States) for all primary health care clinics. We measured RBS value using the portable Abbot Freestyle Glucometer (Illinois, United States).

3.13 Data Collection

We conducted data collection from May 2016 until November 2017 for a total period of 18 months. On participant first visit, the attending medical officer recorded participant baseline characteristics such as age, gender, ethnicity, day of fever, dengue infection, sign and symptoms, warning signs, physical examination findings (including waist circumference, weight, and height), laboratory values (including hbA1c and RBS levels), diagnosis, and referral status.

Next, during each follow-up, the attending medical officer recorded the presence of warning signs, blood pressure, and laboratory values (includes RBS). The medical officer was responsible for completing the paper-based data collection form for each patient. Participants undergo follow-up daily until discharged or hospitalised. We appointed one member of the primary research team to monitor the data collection processes in each primary health care clinic. Also, we collected the paper-based data collection form for verification from each clinic every two weeks.

3.14 Data Extraction System

After completing the data collection process, we verified the paper-based data collection forms. We assessed the completeness and readability of the forms before beginning the scanning process. We verified any missing data with the respective medical officer and study participant. Next, we scanned all completed and verified forms into the TeleForm computer database system using the Kodak i280 Document Scanner (Eastman Kodak Company, Document Imaging, Rochester, NY). After completing the scanning process, we verified all scanned data with the paper-based data collection form for any error. At the end of the data extraction process, we exported all verified data to the STATA database format for further data management and analysis.

3.15 Data Management

The data management process was performed after the completion of the data extraction process. Using STATA (Texas, United States) version 14, we performed series of steps on the primary research data to verify, organise, transform, integrate and extract the recorded data in an appropriate output form for subsequent future use. We documented all method of processing to ensure the utility and integrity of the data. We produced a ".do" file for each process and recorded all outputs in an individual ".log" file. All primary materials, research data, and records for this study were stored in Dropbox (California, United States), a cloud-based file hosting service that offers cloud storage and file synchronisation. Additionally, we kept an updated backup of all material, research data, and records in a designated USB drive.

3.16 Statistical Analysis

Summary statistics consisted of the frequency (n) and percentage (%) of responses in each category for discrete variables and the mean and standard deviation (SD) for continuous variables. We also presented the summary for the continuous variable using quartiles. We rounded all continuous values to one decimal place higher than the measured value. Histograms, stem and leave, and Q-Q plots were used to examine the distribution of data. Kolmogorov-Smirnov test was used to test for normality.

Differences between proportions was tested using a Chi-square test. Fisher's exact tests were used when any assumptions of Chi-square were violated. The differences between two and three or more means was tested using independent sample t-test and ANOVA, respectively. The differences between two and three or more medians was tested using the Wilcoxon-Mann-Whitney test or Kruskal-Wallis test.

To ensure no missing data, we performed the verification and initial validation of raw data in the paper-based data collection form before and after the data extraction process. Before the data extraction process, we manually verified each item in the paper-based data collection form with the medical record of study participant. We also called up participants for confirmation, reappointment, and reassessment if there was no available data for the missing item in the medical record. After the data extraction process, we rechecked the imported data with the paper-based data collection form to ensure no missing or erroneous data.

The odds of dengue severity were modelled using logistic regression. Changes in hematocrit and platelet levels were modelled using linear regression. We used multivariable logistic and multivariable linear regression models to differentially adjust for plausible confounding based on the postulated causal relationships.

This cohort study followed up dengue fever participants from baseline visit until discharge or development of dengue severity. The data structure included repeated measures of the random blood sugar, blood pressure, haematocrit, platelets, and dengue warning signs. We used a random intercept regression models to account for the longitudinal nature of the data. We modelled the time metric as days of fever. The day of fever ranges from Day 1 to Day 14. Day of fever was initially modelled as a continuous covariate but was later categorized into 3 phases: Dengue Fever Phase 1 (Day 0-3), Dengue Fever Phase 2 (Day 4-6), Dengue Fever Phase 3 (Day ≥ 7). The categorization of days of fever was performed acknowledging the classical dengue progression into: (i) febrile phase; (ii) critical phase; and (iii) defervescence phase; based on the Malaysian clinical practice guidelines.

The longitudinal analysis of the association between exposures (e.g. BMI) and outcomes (e.g. Dengue Severity) included determining the cross-sectional association and the longitudinal association. The cross-sectional association is an association between BMI and Dengue severity that exist at baseline and is constant through time. An example of the model specification between BMI and dengue severity is given below to help in the understanding of the cross-sectional association in the longitudinal analysis.

Model 1

$$\text{Logit (P(DS))} = (\beta_0 + \beta_{0i}) + \beta_1 BMI_i + \beta_2 P2_i + \beta_3 P3_i + \varepsilon_i$$

Where;

- BMI = measured as kg/m^2 and following a distribution centered at 25 kg/m^2
- Dengue fever phase categorized into phase 1 (day 0-3), phase 2 (day 4-6), and phase 3 (day ≥ 7)
- β_1: Estimates the cross-sectional association between BMI and odds of dengue severity that exist at baseline and is constant through time
- β_2: Estimates the association of dengue fever days and odds of dengue severity comparing phase 2 to phase 1 controlling for BMI
- β_3: Estimates the association of dengue fever days and odds of dengue severity comparing phase 2 to phase 1 controlling for BMI

The longitudinal association is the association between BMI and Dengue severity that varies by time. We modelled this longitudinal effect by adding a time interaction. An example of the model specification between BMI and dengue severity is given below to help in the understanding of the longitudinal association in the longitudinal analysis.

Model 2

$$\text{Logit (P(DS))} = (\beta_0 + \beta_{0i}) + \beta_1 BMI_i + \beta_2 P2_i + \beta_3 P3_i + \beta_4 BMI_i * P2_i + \beta_5 BMI_i * P3_i + \varepsilon_i$$

- β_1: Estimates the association between BMI and odds of dengue severity at phase 1
- β_2: Estimates the association of dengue fever days and odds of dengue severity by comparing phase 2 to phase 1 controlling for BMI
- β_3: estimates the association of dengue fever days and odds of dengue severity by comparing phase 2 to phase 1 controlling for BMI
- B_4: Estimates the association between BMI and odds of dengue severity at phase 2
- B_5: Estimates the association between BMI and odds of dengue severity at phase 3

The significance of the longitudinal association was tested using a Likelihood Ratio test comparing the model 1 and model 2.

We performed two additional exploratory analysis: (i) mediation analysis; and (ii) interaction analysis on the association between exposures and outcomes. In the mediation analysis, determination of possible mediator was done initially using Directed Acyclic Graph to determine the causal pathway. Furthermore, we used Generalized Structural Equation Model (GSEM) analysis to further computes the indirect effect and total effect of each tested mediator variable tested. In the interaction analysis, we tested the interaction effect of secondary dengue infection in the association between exposures and outcomes. Only significant main association was tested for interaction using likelihood-ratio test (lrtest) to compare model without interaction term and model with interaction term.

We used a two-sided significance level of $\alpha = 0.05$ for all statistical tests performed in this study. The confidence intervals presented has 95% degree of confidence. We rounded all p-values to three decimal places. We presented all p-values that round to 0.000 as < 0.001 and all p-values that round to 1.000 as > 0.999. P-values ≤ 0.05 are considered statistically significant. For all statistical analysis, we used the STATA statistical software version 14.2 to produce all summaries, listings, statistical analyses, and graph.

3.17 Funding

The University Malaya Research Grant Program - HTM (Wellness) (Grant No: RP034B-15HTM) and the University Malaya Postgraduate Research Grant - Research (Grant No: PG163-2015B) funded all the related expenses to run this study.

CHAPTER 4: RESULTS

4.1 Introduction

This chapter presents the research findings according to the study objectives. The first section describes the general characteristics of participants at baseline, general characteristics of participants by dengue severity at baseline, general characteristics of cohort participants at baseline, and general characteristics of cohort participants by study visits. In the second section, we describe the general and clinical characteristics of participants by BMI categories. Furthermore, we describe the characteristics of exposures based on non-severe and severe dengue infection groups, platelet quartiles, and hematocrit quartiles. Moreover, we describe the prevalence and incidence, crude and adjusted association of BMI, BMI categories, WC, and abdominal obesity with dengue severity as well as platelet and hematocrit levels. Similarly, in the third section, we describe the characteristics and associations of RBS and diabetes with dengue severity as well platelet and hematocrit level. The last section describes the characteristics and association between hypertension and dengue severity as well as platelet and hematocrit levels.

4.2 Characteristics of Participants

4.2.1 Baseline Characteristics of Participants

Participants for this study were dengue confirmed patients aged 15 years and above who had sought treatment from May 2016 to November 2017. We enrolled dengue patients fulfilling the inclusion and exclusion criteria from the 24 Primary Health Care Clinics in Melaka Tengah, Seremban, Hulu Langat, Petaling, Gombak, and Klang districts into this study. A total of 362 patients participated in the period.

Table 4.1 shows the baseline characteristics of dengue patients. The mean age was 35.8 ± 14.10 years. The majority of participants were males with a total number of 186 patients (51.4%) compared to 176 female patients (48.6%). According to the distribution by ethnic group, 244 participants (67.9%) were Malay, followed by 66 Chinese participants (18.2%), 42 Indians (11.6%), and 10 participants (2.8%) of other ethnic groups. A total of 78 participants (21.6%) have previous dengue infections based on their dengue IgG test, and 284 participants (78.5%) had no previous dengue infection. Among those with previous dengue infection, 69 (88.5%) were unaware of previous dengue infection. Only nine participants (11.5%) were aware of their previous dengue infection.

The mean days of fever at first visit was 3.9 ± 1.63 days. The mean BMI at baseline was 25.2 ± 5.69 kg/m^2. Based on the classification of BMI, 158 participants (43.7%) were of normal weight, followed by 112 overweight participants (30.9%), 63 obese participants (17.4%), and 29 underweight participants (8.0%). Based on the abdominal obesity status, 184 participants (50.8%) presented with abdominal obesity compared to 178 participants (49.2%) with no abdominal obesity. Among those with abdominal obesity, 45.1% were overweight, 34.2% obese, and 20.7% of normal BMI. Among those with no abdominal obesity, 67.4% have normal BMI, 16.3% overweight, and 16.3% underweight.

According to the presence of diabetes, 60 participants (16.6%) were found to have diabetes, and 302 participants (83.4%) were non-diabetics. For the hypertension status, a total number of 43 participants (11.9%) were hypertensive, and 319 participants (88.1%) were non-hypertensive. Based on the diagnosis of participants at first visit, there were 185 participants (51.1%) diagnosed as dengue without warning signs (DWWS), 174 participants (48.1%) diagnosed as dengue with warning signs (DWS), and 3 were participants (0.8%) diagnosed as severe dengue (SD).

Table 4.1: Baseline characteristics of participants

General Characteristics	Baseline (N = 362) n (%) / mean ± SD
Age, years	35.8 ± 14.10
Gender	
Male	186 (51.4%)
Female	176 (48.6%)
Ethnicity	
Malay	244 (67.4%)
Chinese	66 (18.2%)
Indian	42 (11.6%)
Others	10 (2.8%)
Previous Dengue Infection	
No	284 (78.5%)
Yes	78 (21.5%)
Unaware	69 (88.5%)
Aware	9 (11.5%)
Days of Fever, days	3.9 ± 1.63
BMI, kg/m^2	25.2 ± 5.69
BMI Category	
Underweight	29 (8.0%)
Normal	158 (43.7%)
Overweight	112 (30.9%)
Obese	63 (17.4%)
Abdominal Obesity	
No	178 (49.2%)
Underweight	29 (16.3%)
Normal	120 (67.4%)
Overweight	29 (16.3%)
Obese	0 (0.0%)
Yes	184 (50.8%)
Underweight	0 (0.0%)
Normal	38 (20.7%)
Overweight	83 (45.1%)
Obese	63 (34.2%)
Diabetes	
Yes	60 (16.6%)
Hypertension	
Yes	43 (11.9%)
Diagnosis	
DWWS[a]	185 (51.1%)
DWS[b]	174 (48.1%)
SD[c]	3 (0.8%)

[a] DWWS: Dengue without Warning Signs; [b] DWS: Dengue with Warning Signs; [c] SD: Severe Dengue.

4.2.2 Baseline Characteristics of Participants by Dengue Severity

We defined the non-severe group as cases diagnosed with dengue without warning signs and the severe group as cases diagnosed with either dengue with warning signs or severe dengue. There were 185 participants (51.1%) in the non-severe group and 177 participants (48.9%) in the severe group. Table 4.2 describes the characteristics of participants based on dengue severity groups. Mean age among those in the non-severe group was 36.0 ± 14.28 years compared to 35.6 ± 13.94 years in the severe group. According to gender, there were 97 males (52.4%) and 88 females (47.6%) in the non-severe group. In the severe group, 89 participants (50.3%) were male, and 88 participants (49.7%) were female. Based on ethnicity, there were 122 (66.0%) Malay participants in the non-severe group, followed by 31 (16.8%) Chinese, 25 (13.5%) Indians, and 7 (3.8%) of other ethnic groups. In the severe dengue group, 122 participants (68.1%) were Malay, 35 participants (19.8%) were Chinese, 17 participants (9.6%) were Indians, and 3 participants (1.7%) were from other ethnic groups.

According to the status of previous dengue infection, 38 participants (20.5%) with had a dengue infection and 145 participants (79.5%) were without a previous dengue infection in the non-severe group. In the severe dengue group, 40 participants (22.6%) had a previous dengue infection while the other 137 participants (77.4%) had no previous dengue infection. Among participants with a previous dengue infection, 35 (92.1%) are in the non-severe group and 34 participants (85.0%) in the severe group were unaware. According to days of fever, the non-severe group had a mean of 3.9 ± 1.66 days and the severe groups a mean of 3.9 ± 1.60 days. Comparing the baseline characteristics of participants by dengue severity groups, there was no significant difference ($p>0.05$) between the non-severe and severe groups in all five characteristics at baseline.

Table 4.2: Baseline characteristics of participants by dengue severity group

General Characteristics	Non-Severe[a] (N = 185)	Severe[b] (N = 177)	P-value
	n (%) / mean ± SD	n (%) / mean ± SD	
Age, years	36.0 ± 14.28	35.6 ± 13.94	0.762
Gender			0.682
Male	97 (52.4%)	89 (50.3%)	
Female	88 (47.6%)	88 (49.7%)	
Ethnicity			0.363
Malay	122 (66.0%)	122 (68.1%)	
Chinese	31 (16.8%)	35 (19.8%)	
Indian	25 (13.5%)	17 (9.6%)	
Others	7 (3.8%)	3 (1.7%)	
Previous Dengue Infection			0.634
No	147 (79.5%)	137 (77.4%)	
Yes	38 (20.5%)	40 (22.6%)	
Unaware	35 (92.1%)	34 (85.0%)	0.482
Aware	3 (7.9%)	6 (15.0%)	
Days of Fever, days	3.9 ± 1.66	3.9 ± 1.60	0.706

[a] Non-severe group defined as patients with dengue without warning signs (DWWS)
[b] Severe group defined as patients with dengue with warning signs (DWS) and severe dengue (SD)

4.2.3 Baseline Characteristics of Cohort Participants

Participants for the cohort study were dengue confirmed patients diagnosed as dengue without warning signs, who had sought treatment from May 2016 to November 2017 at the 24 primary health care clinics in Melaka Tengah, Seremban, Hulu Langat, Petaling, Gombak, and Klang districts. Table 4.3 described the general characteristics of all 185 cohort participants based on their age, gender, ethnicity, previous dengue infection, awareness of previous dengue infection, and days of fever. The mean age of the cohort participants was 36.0 ± 14.28 years. Based on gender, there were 97 male participants (52.4%), and 88 female participants (47.6%). According to the distribution by ethnic group, 122 participants (66.0%) were of Malay ethnicity, followed by 31 participants (16.8%) of Chinese ethnicity, 25 participants (13.5%) of Indians ethnicity, and 7 participants (3.8%) of others ethnicity. A total of 38 participants (20.5%) had previous dengue infection based on their dengue IgG test, and 147 participants (79.5%) had no previous dengue infection. From those with previous dengue infection, 35 participants (92.1%) were unaware of previous dengue infection, and only 3 participants (7.9%) were aware of their previous dengue infection. The mean days of fever at first visit was 3.9 ± 1.66 days.

Table 4.3: Baseline characteristics of cohort participants

General Characteristics	Baseline (N = 185) n (%) / mean ± SD
Age, years	36.0 ± 14.28
Gender	
Male	97 (52.4%)
Female	88 (47.6%)
Ethnicity	
Malay	122 (66.0%)
Chinese	31 (16.8%)
Indian	25 (13.5%)
Others	7 (3.8%)
Previous Dengue Infection	
No	147 (79.5%)
Yes	38 (20.5%)
Unaware	35 (92.1%)
Aware	3 (7.9%)
Days of Fever, days	3.9 ± 1.66

4.2.4 Characteristics of Cohort Participants by Study Visits

Table 4.4 describes and compares the characteristics of the cohort participants according to visits. The comparison includes the general characteristics as well as platelet counts and hematocrits levels. The mean age ranges from 34.2 ± 12.57 years to 38.0 ± 15.52 years. We found that there was no significant difference (p=0.989) in the mean age of participants according to study visits.

According to gender, there was a higher percentage of females compared to males in each visit. No significant difference (p=0.948) was found in the distribution if gender between visits. According to the ethnic groups, there was no significant difference (p=0.999) in the distribution of ethnicity between study visits. The percentage of Malay participants was higher compared to other ethnicities in all groups. There was no significant difference in the status of previous dengue infection (p=0.631). The majority of the participants with a previous dengue infection were unaware of their infection status despite the non-significant differences (p=0.896).

Based on the mean days of fever by visits, we found that there was a significant difference (p<0.001) between visits. The mean number of days increases from 3.9 ± 1.66 days to 9.7 ± 1.15 days. Similarly, there is a significant difference (p=0.007) in the mean platelet between visits. The mean platelets decreased from 145.0 ± 50.62 $x10^3/\mu L$ in the first visit to 128.9 ± 53.19 $x10^3/\mu L$ in the second visit and increased to 175.5 ± 73.35 $x10^3/\mu L$ in visit 5. The mean platelet decreased in visit 6 to 158.0 ± 61.50 $x10^3/\mu L$ and increased to 201.3 ± 42.57 $x10^3/\mu L$ in visit 7. There was no significant difference (p=0.652) in hematocrit levels between visits.

Table 4.4: Characteristics of cohort participants according to study visits

General Characteristics	Visit 1 (N = 185)	Visit 2 (N = 135)	Visit 3 (N = 78)	Visit 4 (N = 46)	Visit 5 (N = 18)	Visit 6 (N = 9)	Visit 7 (N = 3)	P-value
	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	
Age, years	36.0 ± 14.28	35.5 ± 13.65	35.5 ± 13.01	34.2 ± 12.57	35.2 ± 13.09	37.1 ± 16.74	38.0 ± 15.52	0.989
Gender								0.948
Male	97 (52.4%)	74 (54.8%)	41 (52.6%)	24 (52.2%)	11 (61.1%)	6 (66.7%)	1 (33.3%)	
Female	88 (47.6%)	61 (45.2%)	37 (47.4%)	22 (47.8%)	7 (38.9%)	3 (33.3%)	2 (66.7%)	
Ethnicity								0.999
Malay	122 (66.0%)	93 (68.9%)	53 (68.0%)	32 (69.6%)	13 (72.2%)	6 (66.7%)	3 (100.0%)	
Chinese	31 (16.8%)	22 (16.3%)	13 (16.7%)	6 (13.0%)	3 (16.7%)	1 (11.1%)	0 (0.0%)	
Indian	25 (13.5%)	14 (10.4%)	9 (11.5%)	6 (13.0%)	1 (5.6%)	1 (11.1%)	0 (0.0%)	
Others	7 (3.8%)	6 (4.4%)	3 (3.9%)	2 (4.4%)	1 (5.6%)	1 (11.1%)	0 (0.0%)	
Previous Dengue Infection								0.631
No	147 (79.5%)	109 (80.7%)	63 (80.8%)	38 (82.6%)	12 (66.7%)	6 (66.7%)	2 (66.7%)	
Yes	38 (20.5%)	26 (19.3%)	15 (19.2%)	8 (17.4%)	6 (33.3%)	3 (33.3%)	1 (33.3%)	
Unaware	35 (92.1%)	24 (92.3%)	15 (100.0%)	8 (100.0%)	6 (100.0%)	3 (100.0%)	1 (100.0%)	0.896
Aware	3 (7.9%)	2 (7.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Days of Fever, days	3.9 ± 1.66	5.0 ± 1.49	5.9 ± 1.43	6.7 ± 1.36	7.9 ± 1.53	8.2 ± 1.56	9.7 ± 1.15	<0.001
Platelets, x10^3/µL	145.0 ± 50.62	128.9 ± 53.19	138.1 ± 63.81	145.8 ± 66.50	175.5 ± 73.35	158.0 ± 61.50	201.3 ± 42.57	0.007
Hematocrits, %	41.0 ± 5.04	41.9 ± 4.57	41.3 ± 4.51	41.0 ± 4.60	40.9 ± 4.59	41.7 ± 4.97	38.7 ± 1.15	0.652

4.3 Obesity and Dengue Severity

4.3.1 Baseline Characteristics of Participants by BMI Categories

This study categorised participants into normal weight, underweight, overweight, and obese. The total number of obese participants among the confirmed dengue cases were 63 out of 362 cases (17.4%). Overweight, normal weight, and underweight accounts for 112 (30.9%), 158 (43.6%), and 29 (8.0%) participants, respectively. Table 4.5 describes the general characteristics of participants by BMI categories at baseline. We found a higher mean age of 38.4 ± 13.36 years among those in the obese group and 39.0 ± 12.59 years for those in the overweight group. Also, we found a lower mean age of 29.6 ± 17.32 years among the underweight group and 33.7 ± 14.13 years for those with normal weight. On statistical analysis, we found a significant (p=0.001) difference in the mean age between different categories of BMI.

According to gender, those in the normal weight (54.4%) and overweight (52.7%) groups had more male participants compared to those in the underweight (48.3%) and obese (42.9%) groups. We found no significant difference between BMI categories in our statistical analysis (p=0.456). There was a higher percentage of Malay participants in all different categories of BMI, followed by Chinese, Indian, and other ethnic groups. Comparing the ethnic distribution of participants by BMI categories, there was no significant difference between BMI category (p=0.244). Based on the status of previous dengue infection, 5 (17.2%) underweight participants had a previous dengue infection and 80% were unaware. In the normal weight group, 27 participants had previous dengue infection and 88.9% were unaware. The underweight and obese groups have 26 (23.2%) and 20 (31.8%) participants with previous dengue infection, respectively.

Among those in the overweight group, 84.6% were unaware of the previous dengue infection. Moreover, 95% of participants with a previous dengue infection in the obese group were unaware of it. There was no statistical significance for the differences in previous dengue infection status (p=0.101) and awareness of previous dengue infection (p=0.481). Similarly, no significant difference was found between BMI categories (p=0680).

Table 4.5: Baseline characteristics of participants by BMI categories

General Characteristics	Underweight (n = 29)	Normal (n = 158)	Overweight (n = 112)	Obese (n = 63)	P-value
	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	
Age, years	29.6 ± 17.32	33.7 ± 14.13	39.0 ± 12.59	38.4 ± 13.36	0.001
Gender					0.456
Male	14 (48.3%)	86 (54.4%)	59 (52.7%)	27 (42.9%)	
Female	15 (51.7%)	72 (45.6%)	53 (47.3%)	36 (57.1%)	
Ethnicity					0.244
Malay	22 (75.9%)	106 (67.1%)	69 (61.6%)	47 (74.6%)	
Chinese	6 (20.7%)	30 (19.0%)	19 (17.0%)	11 (17.5%)	
Indian	0 (0.0%)	18 (11.4%)	19 (17.0%)	5 (7.9%)	
Others	1 (3.5%)	4 (2.5%)	5 (4.5%)	0 (0.0%)	
Previous Dengue Infection					0.101
No	24 (82.8%)	131 (82.9%)	86 (76.8%)	43 (68.3%)	
Yes	5 (17.2%)	27 (17.1%)	26 (23.2%)	20 (31.8%)	
Unaware	4 (80.0%)	24 (88.9%)	22 (84.6%)	19 (95.0%)	0.481
Aware	1 (20.0%)	3 (11.1%)	4 (15.4%)	1 (5.0%)	
Days of Fever, days	4.1 ± 1.81	3.8 ± 1.76	4.0 ± 1.50	3.9 ± 1.43	0.680

4.3.2 Baseline Clinical Characteristics of Participants by BMI Categories

Table 4.6 compares the clinical characteristics of participants by BMI categories. According to warning signs, we found no significant differences between underweight, normal weight, overweight, and obese participants. However, there was a higher percentage of diarrhea (22.2%), vomiting (12.7%), abdominal pain (14.3%), and raised hematocrit with rapid decrease in platelets (30.2%) among those with obesity compared to underweight, normal weight, and overweight. According to the laboratory values, there were no significant differences between BMI categories in white blood cells level (p=0.131), platelets level (p=0.179), and hematocrits level (p=0.566).

Table 4.6: Baseline clinical characteristics of participants by BMI categories

Clinical Characteristics	Underweight (n = 29)	Normal (n = 158)	Overweight (n = 112)	Obese (n = 63)	P-value
	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	
Warning Signs					
Diarrhea					0.306
No	24 (82.8%)	138 (87.3%)	97 (86.6%)	49 (77.8%)	
Yes	5 (17.2%)	20 (12.7%)	15 (13.4%)	14 (22.2%)	
Vomiting					0.769
No	26 (89.7%)	144 (91.1%)	103 (92.0%)	55 (87.3%)	
Yes	3 (10.3%)	14 (8.9%)	9 (8.0%)	8 (12.7%)	
Abdominal Pain					0.171
No	28 (96.6%)	147 (93.0%)	105 (93.8%)	54 (85.7%)	
Yes	1 (3.5%)	11 (7.0%)	7 (6.3%)	9 (14.3%)	
Lethargy					0.211
No	28 (96.6%)	128 (81.0%)	90 (80.4%)	52 (82.5%)	
Yes	1 (3.5%)	30 (19.0%)	22 (19.6%)	11 (17.5%)	
Liver Tenderness					>0.999
No	29 (100.0%)	156 (98.7%)	111 (99.1%)	63 (100.0%)	
Yes	0 (0.0%)	2 (1.3%)	1 (0.9%)	0 (0.0%)	
Third Space Fluid Accumulation					-
No	29 (100.0%)	158 (100.0%)	112 (100.0%)	63 (100.0%)	
Yes	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Spontaneous Bleeding					0.876
No	29 (100.0%)	156 (98.7%)	110 (98.2%)	63 (100.0%)	
Yes	0 (0.0%)	2 (1.3%)	2 (1.8%)	0 (0.0%)	
Raised HCT[a] with drop in PLT[b]					0.809
No	22 (75.9%)	114 (72.2%)	85 (75.9%)	44 (69.8%)	
Yes	7 (24.1%)	44 (27.9%)	27 (24.1%)	19 (30.2%)	
Laboratory Values					
WBC[c], x10^3/µL	3.4 ± 1.36	3.7 ± 1.71	3.8 ± 1.72	4.2 ± 1.64	0.131
PLT[b], x10^3/µL	138.6 ± 51.14	133.0 ± 49.75	140.3 ± 61.52	122.0 ± 47.28	0.179
HCT[a], %	42.2 ± 3.86	41.7 ± 5.15	41.7 ± 4.93	42.6 ± 4.59	0.566

[a] HCT: Hematocrits; [b] PLT: Platelets; [c] WBC: White Blood Cells.

4.3.3 Associations Between BMI Categories, Abdominal Obesity, and Dengue Severity at Baseline

Table 4.7 describes the associations of BMI, BMI categories, waist circumference, abdominal obesity, and dengue severity at baseline. According to BMI, there was an association between the mean BMI and severe dengue infection (p=0.044). The mean BMI in the severe dengue infection group was higher with 25.8 ± 6.42 kg/m^2 compared to the mean of 24.6 ± 4.83 kg/m^2 for the non-severe dengue infection group. There was no significant association between different BMI categories and the prevalence of dengue severity (p=0.528). Furthermore, we observed a higher prevalence of participants with normal weight (44.3%) in the non-severe dengue infection compared to the severe dengue infection group (42.9%). There was a higher prevalence of underweight (8.7%) and overweight (32.4%) participants among the non-severe dengue infection group compared to the underweight (7.3%) and overweight (29.4%) participants in the severe dengue infection group. In contrast, there was a higher percentage of obese participants among the severe dengue infection group (20.3%) compared to non-severe dengue infection group (14.6%).

According to waist circumference, there was no significant (p=0.904) association between the mean waist circumference of participants and the prevalence of dengue severity. The mean waist circumference of participants in the non-severe dengue infection group was 85.3 ± 11.31 cm. Similarly, the mean waist circumference of participants in the severe dengue infection group was 85.1 ± 14.29 cm. In abdominal obesity, there were 93 participants (52.5%) without abdominal obesity in the severe dengue infection group compared to 84 participants (46.0%) in the non-severe group. In contrast, there were 100 participants (54.1%) with abdominal obesity in the non-severe group compared to 84 participants (47.5%) in the severe group. There was no significant difference (p=0.210)

in the association between the status of abdominal obesity and the prevalence of dengue severity.

Table 4.7: Associations between BMI categories and abdominal obesity and dengue severity at baseline

Exposures	Non-Severe (n = 185)	Severe (n = 177)	P-value
	n (%) / mean ± SD	n (%) / mean ± SD	
BMI, kg/m^2	24.6 ± 4.83	25.8 ± 6.42	0.044
BMI Categories			0.528
Underweight	16 (8.7%)	13 (7.3%)	
Normal	82 (44.3%)	76 (42.9%)	
Overweight	60 (32.4%)	52 (29.4%)	
Obese	27 (14.6%)	36 (20.3%)	
Waist Circumference, cm	85.3 ± 11.31	85.1 ± 14.29	0.904
Abdominal Obesity			0.210
No	84 (46.0%)	93 (52.5%)	
Yes	100 (54.1%)	84 (47.5%)	

Furthermore, a multivariable logistic regression analysis was performed to explore the crude and adjusted associations between BMI, BMI categories waist circumference, abdominal obesity, and dengue severity at baseline. Table 4.8 describes the crude and adjusted cross-sectional association of BMI, BMI categories, waist circumference, and abdominal obesity with dengue severity at baseline. According to BMI in kg/m^2, we found a significant association in the crude (p=0.047) and adjusted (p=0.034) association with dengue severity at baseline. The crude association concluded that with every 1 kg/m^2 increase in BMI, the odds dengue severity increases by 4% (OR=1.04; 95% CI:1.00,1.08). Similarly, the adjusted association concluded that with every 1 kg/m^2 increase in BMI, the odds of dengue severity increases by 4% with regard to age, gender, and ethnicity remain constant (aOR=1.04; 95% CI:1.00,1.08).

According to the BMI categories, we found no significant association in the crude association of underweight (p=0.746), overweight (p=0.786), and obesity (p=0.226) with dengue severity. Similarly, our adjusted association found no significant association of underweight (p=0.643), overweight (p=0.960), and obesity (p=0.263) with dengue severity. Using p-trend for BMI categories, we found no significant ordered relationship between BMI categories and dengue severity in the crude (p=0.243) and adjusted

(p=0.226) association. However, an increasing odds ratio of dengue severity with higher BMI categories in the crude and adjusted association were observed.

According to waist circumference, we found no significant association between waist circumference and the prevalence of dengue severity in the crude (p=0.903) and adjusted association (p=0.915). Similarly, we found no significant association in the crude (p=0.210) and adjusted association (p=0.205) between abdominal obesity and the prevalence of dengue severity.

Table 4.8: Crude and adjusted associations between BMI categories and abdominal obesity and the prevalence of dengue severity at baseline

Exposures	Prevalence of Dengue Severity			
	Crude	P-value	Adjusted*	P-value
	OR (95% CI)		aOR (95% CI)	
BMI, kg/m^2	1.04 (1.00,1.08)	0.047	1.04 (1.00,1.08)	0.034
BMI Categories				
Underweight	0.88 (0.40,1.94)	0.746	0.83 (0.37,1.85)	0.643
Normal	1.00	Ref	1.00	Ref
Overweight	0.94 (0.58,1.52)	0.786	0.99 (0.60,1.62)	0.960
Obesity	1.44 (0.80,2.59)	0.226	1.41 (0.77,2.56)	0.263
P-trend	-	0.243	-	0.226
Waist Circumference, cm	1.00 (0.98,1.02)	0.903	1.00 (0.98,1.02)	0.915
Abdominal Obesity	0.77 (0.51,1.16)	0.210	0.75 (0.48,1.17)	0.205

* Adjusted for age, sex, and ethnicity.

4.3.4 Associations Between BMI Categories, Abdominal Obesity, and Incidence of Dengue Severity

Table 4.9 describes the characteristics of BMI, BMI categories, waist circumference, abdominal obesity, and dengue severity between study visits. According to BMI, there was no significant difference found between study visits. The mean BMI of each visit ranges from 22.8 ± 2.77 kg/m^2 to 24.7 ± 4.71 kg/m^2. We also found no significant difference in the distribution of BMI categories between study visits. The majority of participants have normal body weight followed by overweight, obesity, and underweight. Similarly, no significant difference between study visits found for the mean waist circumference and abdominal obesity.

According to the outcomes of dengue severity, 47 participants (25.4%) who came at first visit developed severe dengue infection compared to 138 participants (74.6%) with non-severe dengue infection. For those who came for the second visit, 26 participants (19.3%) developed severe dengue infection compared to 109 participants (61.5%) with non-severe infection. Among 78 participants who came for the third visit, 18 participants (23.1%) have severe dengue infection, and 60 participants (76.9%) have non-severe dengue infection. For the 46 participants who came for the fourth visit, 10 participants (21.7%) developed severe dengue infection while 36 participants (78.3%) were non-severe. Visit five, six, and seven have 4 (22.2%), 1 (11.1%), and 3 (100.0%) participants with severe dengue infection compared to 14 (77.8%). 8 (88.9%), and 0 (0.0%) participants with non-severe dengue infection.

A crude and adjusted random intercept multivariable logistic regression analysis was performed to explore the association between BMI, BMI categories, waist circumference, abdominal obesity, and the incidence of dengue severity. We stratified days of fever into three phases namely: (i) phase 1 (days of fever ≤ 3 days); (ii) phase 2 (days of fever between 4-6 days); (iii) phase 3 (days of fever ≥ 7 days).

Table 4.9: Characteristics of BMI categories, abdominal obesity, and dengue severity by study visits

Variables	Visit 1 (N = 185)	Visit 2 (N = 135)	Visit 3 (N = 78)	Visit 4 (N = 46)	Visit 5 (N = 18)	Visit 6 (N = 9)	Visit 7 (N = 3)
	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD
BMI, kg/m^2	24.6 ± 4.83	24.7 ± 4.71	24.6 ± 4.26	24.4 ± 4.26	24.2 ± 3.40	22.8 ± 2.77	24.3 ± 3.01
BMI Categories							
Underweight	16 (8.7%)	11 (8.2%)	5 (6.4%)	3 (6.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Normal	82 (44.3%)	61 (45.2%)	35 (44.9%)	21 (45.7%)	10 (55.6%)	7 (77.8%)	2 (66.7%)
Overweight	60 (32.4%)	44 (32.6%)	30 (38.5%)	17 (37.0%)	7 (38.9%)	2 (22.2%)	1 (33.3%)
Obesity	27 (14.6%)	19 (14.1%)	8 (10.3%)	5 (10.9%)	1 (5.6%)	0 (0.0%)	0 (0.0%)
Waist Circumference, cm	85.3 ± 11.31	85.8 ± 10.97	84.9 ± 10.05	84.4 ± 10.50	84.1 ± 11.26	83.2 ± 9.61	85.7 ± 14.57
Abdominal Obesity							
No	85 (46.0%)	63 (46.7%)	39 (50.0%)	23 (50.0%)	11 (61.1%)	6 (66.7%)	1 (33.3%)
Yes	100 (54.1%)	72 (53.3%)	39 (50.0%)	23 (50.0%)	7 (38.9%)	3 (33.3%)	2 (66.7%)
Dengue Severity							
Non-severe	138 (74.6%)	109 (61.5%)	60 (76.9%)	36 (78.3%)	14 (77.8%)	8 (88.9%)	0 (0.0%)
Severe	47 (25.4%)	26 (19.3%)	18 (23.1%)	10 (21.7%)	4 (22.2%)	1 (11.1%)	3 (100.0%)

Table 4.10 describes the crude and adjusted association between BMI, BMI categories, waist circumference, abdominal obesity, and the incidence of dengue severity. According to BMI, we found a significant crude association between BMI and the incidence of dengue severity controlled for dengue fever phase (p=0.026). With every 1 kg/m^2 increase in BMI, the odds of developing severe dengue infection increases by 13% with regard to dengue fever phase remains constant (OR=1.13; 95% CI:1.01,1.26). When stratified according to dengue fever phases, we found a significant association between BMI and the incidence of dengue severity at phase 1 of dengue fever (p=0.003). With every 1 kg/m^2 increase in BMI at phase 1, the odds of developing severe dengue infection increases by 27% (OR=1.27; 95% CI:1.08,1.50). No significant association was found in phase 2 (p=0.187) and phase 3 (p=0.902) of dengue fever.

In the adjusted association, there was a significant association between BMI and the incidence of dengue severity (p=0.016). With every 1 kg/m^2 increase in BMI, the odds of developing severe dengue infection increases by 15% with regard to age, gender, ethnicity, and dengue fever phase remains constant (aOR=1.15; 95% CI:1.03,1.28). There was also a significant association in the stratified analysis where with every 1 kg/m^2 increase in BMI at phase 1 of dengue fever, the odds of developing severe dengue infection increases by 29% with regard to age, gender, and ethnicity remain constant (aOR=1.29; 95% CI:1.10,1.53; p=0.002). No significant association was found in phase 2 (p=0.127) and phase 3 (p=0.757) of dengue fever.

Based on the P-interaction test, the effect of BMI on the incidence of dengue severity by dengue fever phase in the crude (p=0.127) and adjusted association (p=0.110) were not important. Thus, we selected the simple model without interaction for interpretation.

According to BMI categories, there was no significant crude association between underweight (p=0.243), overweight (p=0.322), obesity (p=0.239), and the incidence dengue severity compared to normal weight controlled for dengue fever phase. Similarly, we found no significant adjusted association between underweight (p=0.179), overweight (p=0.689), obesity (p=0.262), and the incidence dengue severity compared to normal weight adjusted for age, gender, ethnicity, and dengue fever phase. We also did not find any significant trend in the crude (p=0.418) and adjusted (p=0.349) association between different categories of BMI and dengue severity.

In the stratified analysis by dengue fever phases, we found a significant crude association between obesity and the incidence of dengue severity at phase 1 (p=0.011). The odds of dengue severity among those with obesity were 18.3 times higher compared to normal weight patients at phase 1 of dengue fever (OR=19.29; 95% CI:1.99,186.63). Similarly, there was a significant adjusted association between obesity and the incidence of dengue severity at phase 1 (p=0.012), where those with obesity have 17.7 times higher odds of developing dengue severity compared to normal weight patients at phase 1 of dengue fever with regards to age, gender, and ethnicity remain constant (aOR=18.74; 95% CI:1.92,182.65). Furthermore, no significant trend between BMI categories and the incidence of dengue severity in the crude (p=0.060) and adjusted association (p=0.057) in phase 1 was found. We also found no significant crude and adjusted association between different BMI categories and the incidence of dengue severity at phase 2 and phase 3. Similarly, no significant trend was found between BMI categories and the incidence of dengue severity in the crude and adjusted association at phase 2 and phase 3. Based on the P-interaction test, the effect of BMI categories on the incidence of dengue severity by dengue fever phase in the crude (p=0.255) and adjusted association (p=0.250) were not important. Thus, we selected the simple model without interaction for interpretation.

According to waist circumference, there was no significant crude (p=0.559) and adjusted (p=0.940) association between waist circumference and the incidence of dengue severity. Similarly, there was no significant crude and adjusted association between waist circumference and the incidence of dengue severity stratified by dengue fever phase. Based on the P-interaction test, the effect of waist circumference on the incidence of dengue severity by dengue fever phase in the crude (p=0.137) and adjusted association (p=0.111) were not important. Thus, we selected the simple model without interaction for interpretation.

According to abdominal obesity, there was a significant crude association between abdominal obesity and the incidence of dengue severity (p=0.031), where among those with abdominal obesity, the odds of dengue severity were 72% lower compared to those without abdominal obesity with regard to dengue fever phase remains constant (OR=0.28; 95% CI:0.09,0.89). In contrast, we found no significant adjusted association between abdominal obesity and the incidence of dengue severity (p=0.057). When stratified according to dengue fever phases, we found a significant crude association between abdominal obesity and the incidence of dengue severity at phase 2 (p=0.034) and phase 3 (p=0.012). Among those with abdominal obesity at phase 2, the odds of dengue severity were 73% lower compared to those without abdominal obesity with regard to dengue fever phase remains constant (OR=0.27; 95% CI:0.08,0.91). Among those with abdominal obesity at phase 3, the odds of dengue severity were 90% lower compared to those without abdominal obesity with regard to dengue fever phase remains constant (OR=0.10; 95% CI:0.02,0.60). However, when adjusted for age, gender, and ethnicity, no significant association was found in all three phases. Based on the P-interaction test, the effect of abdominal obesity on the incidence of dengue severity by dengue fever phase in the crude (p=0.246) and adjusted association (p=0.259) were not important. Thus, we selected the simple model without interaction for interpretation.

Table 4.10: Crude and adjusted associations between BMI categories and abdominal obesity and the incidence of dengue severity

Exposures	Dengue Severity			
	Crude	P-value	Adjusted*	P-value
	OR (95% CI)		aOR (95% CI)	
BMI, Centred at 25 kg/m²				
BMI at All Phases	1.13 (1.01,1.26)	0.026	1.15 (1.03,1.28)	0.016
BMI at Phase 1	1.27 (1.08,1.50)	0.003	1.29 (1.10,1.53)	0.002
BMI at Phase 2	1.08 (0.96,1.20)	0.187	1.09 (0.98,1.22)	0.127
BMI at Phase 3	1.01 (0.88,1.16)	0.902	1.02 (0.89,1.17)	0.757
P-Interaction[2]	-	0.127	-	0.110
BMI Categories				
All Phases				
Underweight	0.27 (0.03,2.40)	0.243	0.24 (0.03,1.94)	0.179
Normal	1.00	Ref	1.00	Ref
Overweight	0.53 (0.15,1.85)	0.322	0.77 (0.22,2.70)	0.689
Obesity	2.61 (0.53,12.91)	0.239	2.53 (0.50,12.74)	0.262
P-Trend	-	0.418	-	0.349
Phase 1				
Underweight	0.49 (0.02,12.99)	0.672	0.38 (0.02,9.36)	0.551
Normal	1.00	Ref	1.00	Ref
Overweight	0.81 (0.11,5.69)	0.831	1.11 (0.16,7.67)	0.914
Obesity	19.29 (1.99,186.63)	0.011	18.74 (1.92,182.65)	0.012
P-Trend	-	0.060	-	0.057
Phase 2				
Underweight	0.28 (0.02,3.24)	0.309	0.25 (0.02,2.81)	0.262
Normal	1.00	Ref	1.00	Ref
Overweight	0.65 (0.17,2.54)	0.539	0.92 (0.23,3.60)	0.902
Obesity	0.99 (0.18,5.41)	0.991	0.98 (0.18,5.43)	0.981
P-Trend	-	0.546	-	0.546
Phase 3				
Underweight	0.15 (0.005,4.80)	0.282	0.14 (0.005,4.03)	0.249
Normal	1.00	Ref	1.00	Ref
Overweight	0.13 (0.02,0.99)	0.049	0.19 (0.03,1.46)	0.112
Obesity	0.29 (0.02,3.64)	0.340	0.28 (0.02,3.41)	0.317
P-Trend	-	0.861	-	0.849
P-Interaction[2]	-	0.255	-	0.250
WC, Centred at 80 cm				
WC at All Phase	0.99 (0.95,1.03)	0.559	1.00 (0.96,1.05)	0.940
WC at Phase 1	1.04 (0.98,1.11)	0.224	1.06 (0.99,1.13)	0.102
WC at Phase 2	0.97 (0.93,1.02)	0.198	0.98 (0.94,1.03)	0.451
WC at Phase 3	0.95 (0.89,1.02)	0.173	0.97 (0.90,1.04)	0.326
P-Interaction[2]	-	0.137	-	0.111
Abdominal Obesity				
All phases				
No	1.00	Ref	1.00	Ref
Yes	0.28 (0.09,0.89)	0.031	0.32 (0.10,1.03)	0.057
Phase 1				
No	1.00	Ref	1.00	Ref
Yes	0.70 (0.13,3.63)	0.667	0.78 (0.15,4.17)	0.774
Phase 2				
No	1.00	Ref	1.00	Ref
Yes	0.27 (0.08,0.91)	0.034	0.30 (0.09,1.06)	0.061
Phase 3				
No	1.00	Ref	1.00	Ref
Yes	0.10 (0.02,0.60)	0.012	0.11 (0.02,0.69)	0.018
P-Interaction[2]	-	0.246	-	0.259

* Adjusted for age, gender, and ethnicity.

[1] P-Interaction tests between the model without and with interaction by dengue fever phase.

[2] Dengue fever was categorised into Phase 1 (Fever day 0-3), Phase 2 (Fever day 4-6), and Phase 3 (Fever day ≥7).

We use a spline graph to highlight the association between BMI and dengue severity. Figure 4.1 describes the association between BMI and dengue severity by dengue fever phase. At phase 1 of dengue fever, we observed that the odds of dengue severity reduce slightly but increases as the BMI increase above 27 kg/m^2. At phase 2 and phase 3 of dengue fever, the odds of dengue severity reduce below odds ratio of 1.0 with increasing BMI and increases as BMI increase above 29 kg/m^2. The effect of BMI on dengue severity was more at phase 1 compared to phase 2 and phase 3 of dengue fever.

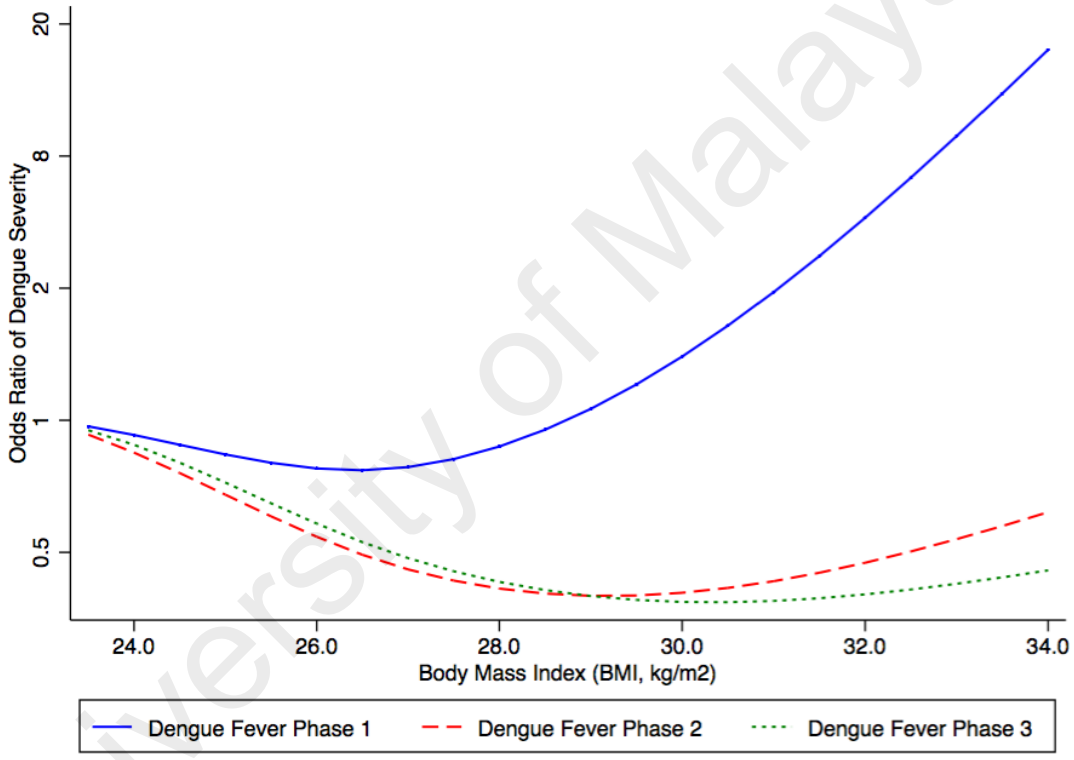

Figure 4.1: Association between BMI and dengue severity by dengue fever phase

4.3.5 Associations Between BMI Categories, Abdominal Obesity, and Platelet

Table 4.11 describes the associations between BMI, BMI categories, waist circumference, abdominal obesity, and platelet quartiles at baseline. Overall, only waist circumference showed a significant association between the mean waist circumference and different quartiles of platelets (p=0.043). There was a higher waist circumference in the second quartile (87.3 ± 12.67 cm) compared to first (86.4 ± 15.53 cm), third (84.8 ± 11.16 cm) and fourth (82.1 ± 11.50 cm) quartiles. No other significant association was found between BMI (p=0.295), BMI categories (p=0.176), abdominal obesity status (p=0.866), and platelet levels in quartiles.

Table 4.11: Association between BMI categories and abdominal obesity and platelet quartiles at baseline

Exposures	Platelet Quartiles				P-value
	Q1 (n = 83)	Q2 (n = 95)	Q3 (n = 105)	Q4 (n = 79)	
	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	
BMI, kg/m^2	25.9 ± 7.27	25.7 ± 5.39	24.6 ± 4.75	24.6 ± 5.27	0.295
BMI Categories					0.176
Underweight	8 (9.9%)	5 (5.3%)	6 (5.7%)	10 (12.7%)	
Normal	34 (41.0%)	42 (44.2%)	48 (45.7%)	34 (43.0%)	
Overweight	22 (26.5%)	26 (27.4%)	37 (35.2%)	27 (34.2%)	
Obese	19 (22.9%)	22 (23.2%)	14 (13.3%)	8 (10.1%)	
Waist Circumference, cm	86.4 ± 15.53	87.3 ± 12.67	84.8 ± 11.16	82.1 ± 11.50	0.043
Abdominal Obesity					0.866
No	41 (49.4%)	45 (47.4%)	50 (47.6%)	42 (53.2%)	
Yes	42 (50.6%)	50 (52.6%)	55 (52.4%)	37 (46.8%)	

Table 4.12 describes the crude and adjusted multivariable linear regression analysis between BMI, BMI categories, waist circumference, abdominal obesity, and platelet levels at baseline. The crude association tested the association between BMI, BMI categories, waist circumference, abdominal obesity, and platelet levels at baseline. The adjusted association tested the association between BMI, BMI categories, waist circumference, abdominal obesity, and platelet levels at baseline controlled for age, gender, and ethnicity.

According to BMI, no significant association was found in the crude (p=0.138) and adjusted (p=0.133) association. Based on BMI categories, we found no significant crude association between underweight (p=0.406), overweight (p=0.708), obesity (p=0.101), and platelet levels compared to normal weight at baseline. Similarly, we found no significant adjusted association between underweight (p=0.438), overweight (p=0.638), obesity (p=0.108), and platelet levels compared to normal weight at baseline adjusted for age, gender, and ethnicity. Furthermore, we observed a lower platelet levels among those with higher BMI categories. However, the trend was not statistically significant both in the crude (p=0.075) and adjusted (p=0.089) association.

According to waist circumference, we found a significant crude association between waist circumference and platelet levels at baseline (p=0.034). With every 1 cm increase in waist circumference, the platelet level decreases by 0.4 $x10^3/\mu L$ (β=-0.40; 95% CI:-0.77,-0.03). In contrast, the association was not significant when adjusted for age, gender, and ethnicity (p=0.116). Similarly, we found no significant crude and adjusted association between abdominal obesity and platelet levels in the crude (p=0.742) and adjusted (p=0.648) association. Furthermore, we performed the crude and adjusted random intercept multivariable linear regression analysis to determine the association of BMI, BMI categories, waist circumference, and abdominal obesity with the changes in platelet levels.

Table 4.12: Crude and adjusted associations between BMI categories and abdominal obesity and platelet levels at baseline

Exposures	Platelet, $x10^3/\mu L$			
	Crude	P-value	Adjusted*	P-value
	β (95% CI)		aβ (95% CI)	
BMI, kg/m^2	-0.64 (-1.48, 0.20)	0.138	-0.66 (-1.52, 0.20)	0.133
BMI Categories				
Underweight	7.69 (-10.49, 25.86)	0.406	7.17 (-11.02, 25.36)	0.438
Normal	1.00	Ref	1.00	Ref
Overweight	2.14 (-9.11, 13.40)	0.708	2.71 (-8.61, 14.04)	0.638
Obesity	-11.28 (-24.78, 2.21)	0.101	-11.15 (-24.74, 2.45)	0.108
P-trend	-	0.075	-	0.089
Waist Circumference, cm	-0.40 (-0.77, -0.03)	0.034	-0.32 (-0.71, 0.08)	0.116
Abdominal Obesity	-1.60 (-11.18, 7.97)	0.742	-2.35 (-12.48, 7.78)	0.648

* Adjusted for age, sex, and ethnicity.

Table 4.13 describes the crude and adjusted association between BMI, BMI categories, waist circumference, abdominal obesity and changes in platelet levels. According to BMI, we found no significant crude (p=0.756) and adjusted (p=0.690) association between BMI and changes in platelet levels controlled for dengue fever phase. When stratified according to dengue fever phases, we found no significant crude association between BMI and changes in platelet levels at phase 1 (p=0.236), phase 2 (p=0.594), and phase 3 (p=0.061) of dengue fever. Similarly, no significant adjusted association between BMI and changes in platelet levels at phase 1 (p=0.243), phase 2 (p=0.535), and phase 3 (p=0.096) of dengue fever. Based on the P-interaction test, the effect of BMI on platelet levels by dengue fever phase in the crude (p=0.032) and adjusted association (p=0.048) were important. Thus, we selected the complex model with interaction by dengue fever phase for interpretation.

According to BMI categories, we found no significant crude association between underweight (p=0.261), overweight (p=0.462), obesity (p=0.423), and changes in platelet levels compared to normal weight controlled for dengue fever phase. Similarly, we found no significant adjusted association between underweight (p=0.258), overweight (p=0.500), obesity (p=0.434), and changes in platelet levels compared to normal weight controlled for dengue fever phase. Lower platelet levels were observed with higher categories of BMI. However, we found no significant trend between BMI categories and changes in platelet levels in the crude (p=0.426) and adjusted association (p=0.448).

When stratified according to dengue fever phase, we found no significant crude and adjusted association between BMI categories and changes in platelet levels at all three phases of dengue fever. Similarly, we found no significant trend between BMI categories and changes in platelet levels in the crude and adjusted association at phase 1, phase 2, and phase 3.

Based on the P-interaction test, the effect of BMI categories on platelet levels by dengue fever phase in the crude (p=0.083) and adjusted association (p=0.107) were not important. Thus, we selected the simple model without interaction for interpretation.

According to waist circumference, we found no significant crude (p=0.146) and adjusted (p=0.302) association between abdominal obesity with the changes in platelet levels controlled for dengue fever phases. However, when stratified by dengue fever phases, significant crude associations were found between waist circumference and changes in platelet levels at phase 2 (p=0.021) and phase 3 (p=0.007) of dengue fever. Among those at phase 2 of dengue fever, every 1cm increase in waist circumference, the platelet level decreases by 0.47 $x10^3/\mu L$ (β=-0.47; 95% CI:-0.87,-0.07). For those at phase 3 of dengue fever, every 1cm increase in waist circumference, the platelet level increases by 0.83 $x10^3/\mu L$ (β=0.83; 95% CI:0.23,1.43).

In the adjusted stratified association, we only found significant association between waist circumference and changes in platelet levels at phase 3 (p=0.006). With every 1cm increase in waist circumference at phase 3, the platelet level increases by 0.84 $x10^3/\mu L$ with regard to age, gender, and ethnicity remain constant ($a\beta$=0.84; 95% CI:0.24,1.45). Based on the P-interaction test, the effect of waist circumference on platelet levels by dengue fever phase in the crude (p=0.001) and adjusted association (p<0.001) were important. Thus, we selected the complex model with interaction by dengue for interpretation.

According to abdominal obesity, we found no significant crude (p=0.597) and adjusted (p=0.808) association between abdominal obesity and changes in platelet levels controlled by dengue fever phase. Similarly, we did not find any significant crude association at phase 1 (p=0.330) and phase 2 (p=0.835) as well as the adjusted association at phase 1 (p=0.288) and phase 2 (p=0.946) of the stratified analysis.

However, at phase 3 of dengue fever, we found significant crude (p=0.004) and adjusted (p=0.006) association between abdominal obesity and changes in platelet levels. In the crude association, those with abdominal obesity have 22.05 $x10^3/\mu L$ higher platelet levels compared to those without abdominal obesity at phase 3 (β=22.05; 95% CI:7.13,36.98). In the adjusted association, those with abdominal obesity have 21.08 $x10^3/\mu L$ higher platelet levels compared to those without abdominal obesity at phase 3 with regard to age, gender, and ethnicity remain constant (aβ=21.08; 95% CI:6.03,36.13).

Based on the P-interaction test, the effect of abdominal obesity on platelet levels by dengue fever phase in the crude (p=0.006) and adjusted association (p<0.001) were important. Thus, we selected the complex model with interaction by dengue for interpretation.

Table 4.13: Crude and adjusted association BMI categories and abdominal obesity and changes in platelet levels

Exposures	Platelet, x10^3/µL			
	Crude β (95% CI)	P-value	Adjusted* aβ (95% CI)	P-value
BMI, Centred at 25 kg/m^2				
BMI at All Phases	-0.12 (-0.89,0.65)	0.756	-0.16 (-0.94,0.62)	0.690
BMI at Phase 1	-0.72 (-1.90,0.47)	0.236	-0.71 (-1.90,0.48)	0.243
BMI at Phase 2	-0.25 (-1.17,0.67)	0.594	-0.29 (-1.23,0.64)	0.535
BMI at Phase 3	1.18 (-0.06,2.42)	0.061	1.06 (-0.19,2.30)	0.096
P-Interaction[2]	-	0.032	-	0.048
BMI Categories				
All Phases				
Underweight	9.71 (-7.22,26.65)	0.261	9.73 (-7.14,26.60)	0.258
Normal	Ref	-	Ref	-
Overweight	3.83 (-6.38,14.03)	0.462	3.53 (-6.72,13.77)	0.500
Obesity	-5.12 (-17.64,7.40)	0.423	-5.00 (-17.55,7.54)	0.434
P-Trend	-	0.426	-	0.448
Phase 1				
Underweight	3.94 (-21.81,29.69)	0.764	3.68 (-21.98,29.34)	0.779
Normal	Ref	-	Ref	-
Overweight	2.07 (-13.73,17.87)	0.797	1.68 (-14.11,17.46)	0.835
Obesity	-13.22 (-31.69,5.24)	0.160	-13.01 (-31.45,5.43)	0.167
P-Trend	-	0.504	-	0.481
Phase 2				
Underweight	19.94 (-0.81,40.70)	0.060	20.08 (-0.67,40.83)	0.058
Normal	Ref	-	Ref	-
Overweight	1.39 (-10.13,12.90)	0.814	1.03 (-10.53,12.58)	0.862
Obesity	-5.44 (-20.70,9.81)	0.484	-5.50 (-20.76,9.77)	0.481
P-Trend	-	0.112	-	0.106
Phase 3				
Underweight	-8.02 (-39.25,23.21)	0.615	-6.95 (-37.98,24.08)	0.661
Normal	Ref	-	Ref	-
Overweight	15.52 (-1.26,32.30)	0.070	14.63 (-2.15,31.40)	0.087
Obesity	14.48 (-8.05,37.73)	0.204	13.79 (-9.04,36.61)	0.236
P-Trend	-	0.482	-	0.334
P-Interaction[2]	-	0.083	-	0.107
WC, Centred at 80 cm				
WC at All Phase	-0.26 (-0.60,0.09)	0.146	-0.20 (-0.57,0.18)	0.302
WC at Phase 1	-0.41 (-0.93,0.12)	0.130	-0.37 (-0.91,0.17)	0.183
WC at Phase 2	-0.47 (-0.87,-0.07)	0.021	-0.43 (-0.84,-0.01)	0.046
WC at Phase 3	0.83 (0.23,1.43)	0.007	0.84 (0.24,1.45)	0.006
P-Interaction[2]	-	0.001	-	<0.001
Abdominal Obesity				
All phases				
No	Ref	-	Ref	-
Yes	2.36 (-6.40,11.11)	0.597	1.16 (-8.23,10.56)	0.808
Phase 1				
No	Ref	-	Ref	-
Yes	-6.57 (-19.81,6.66)	0.330	-7.38 (-21.01,6.24)	0.288
Phase 2				
No	Ref	-	Ref	-
Yes	1.07 (-9.04,11.19)	0.835	0.36 (-10.16,10.89)	0.946
Phase 3				
No	Ref	-	Ref	-
Yes	22.05 (7.13,36.98)	0.004	21.08 (6.03,36.13)	0.006
P-Interaction[2]	-	0.006	-	<0.001

[*] Adjusted for age, gender, and ethnicity.
[1] P-Interaction tests between the model without and with interaction by dengue fever phase.
[2] Dengue fever was categorised into Phase 1 (Fever day 0-3), Phase 2 (Fever day 4-6), and Phase 3 (Fever day ≥7).

Figure 4.2 describes the association between BMI and changes in platelet levels by dengue fever phase. At phase 1 of dengue fever, we observed a lower platelet levels with higher BMI. At phase 2 of dengue fever, the platelet level showed a more constant trend with slight variation with increasing BMI. In contrast to phase 1, there was an increasing trend of platelet levels with increasing BMI.

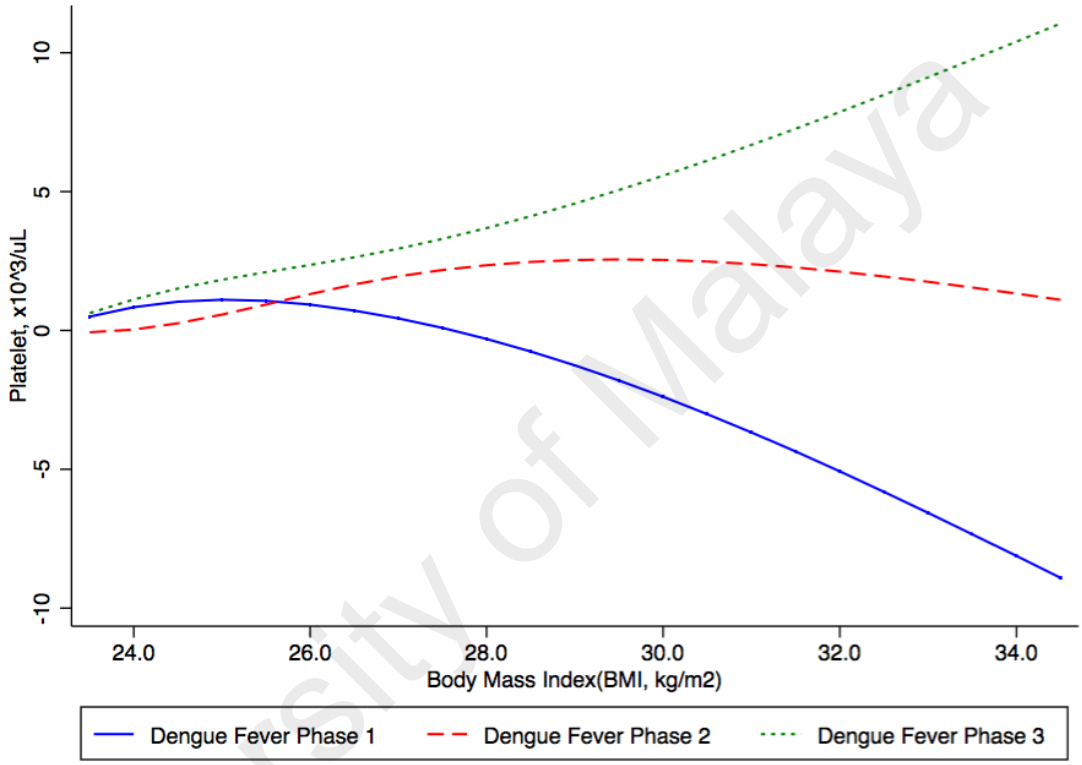

Figure 4.2: Association between BMI and changes in platelet levels by dengue fever phase

4.3.6 Associations Between BMI Categories, Abdominal Obesity, and Hematocrit

Table 4.14 describes the association of BMI, BMI categories, waist circumference, and abdominal obesity with hematocrit levels in quartiles at baseline. In the associations, only abdominal obesity showed a significant association between the status of abdominal obesity and different quartiles of hematocrit level (p=0.031). There were 68 participants (62.4%) with abdominal obesity compared with 41 participants (37.6%) with no abdominal obesity in the first quartile. In the second, third and fourth quartiles, 37 (48.7%), 40 (43.0%), and 39 (46.4%) participants had abdominal obesity compared to 39 (51.3%), 53 (57.0%), and 45 (53.6%) participants with no abdominal obesity, respectively. No significant association was found for BMI (p=0.816), BMI category (p=0.994), and waist circumference (p=0.157) with different quartiles of hematocrit levels.

Table 4.14: Association between BMI categories, abdominal obesity, and hematocrit levels in quartile at baseline

Exposures	Hematocrit Quartiles				P-value
	Q1 (n = 109)	Q2 (n = 76)	Q3 (n = 93)	Q4 (n = 84)	
	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	
BMI, kg/m^2	25.1 ± 6.01	25.6 ± 6.32	24.8 ± 5.12	25.4 ± 5.33	0.816
BMI Categories					0.994
Underweight	8 (7.3%)	7 (9.2%)	8 (8.6%)	6 (7.1%)	
Normal	49 (45.0%)	31 (40.8%)	40 (43.0%)	38 (45.2%)	
Overweight	33 (30.3%)	26 (34.2%)	30 (32.3%)	23 (27.4%)	
Obese	19 (17.4%)	12 (15.8%)	15 (16.1%)	17 (20.2%)	
Waist Circumference, cm	83.5 ± 13.66	84.7 ± 12.39	85.4 ± 12.80	87.7 ± 11.98	0.157
Abdominal Obesity					0.031
No	41 (37.6%)	39 (51.3%)	53 (57.0%)	45 (53.6%)	
Yes	68 (62.4%)	37 (48.7%)	40 (43.0%)	39 (46.4%)	

We performed the multivariable linear regression analysis to determine the association of BMI, BMI categories, waist circumference, and abdominal obesity with the changes in hematocrit levels at baseline. Table 4.15 describes the crude and adjusted cross-sectional association based on the multivariable linear regression analysis.

The crude association tested the linear association between BMI, BMI category, waist circumference, abdominal obesity, and hematocrit levels. The adjusted association tested the association between exposure variables and hematocrit levels with adjustment for age, gender, ethnicity, and diabetes status. According to the crude association, we found no significant association for BMI (p=0.496), underweight (p=0.569), overweight (p=0.939), and obesity (p=0.190). We also observed higher hematocrit levels among those with obesity, but no significant trend was found (p=0.375).

In contrast, we found significant associations between waist circumference (p=0.027), status of abdominal obesity (p=0.031), and hematocrit levels. For waist circumference, with every 1 cm increase in waist circumference, the hematocrit level increases by 0.04% (β=0.04; 95% CI:0.01,0.08). Among those with abdominal obesity, the hematocrit level decreases by 1.11% (β=-1.11; 95% CI:-2.11,-0.10).

When adjusted for age, gender, ethnicity, and diabetes status, there were significant associations between BMI (p=0.019), obesity (p=0.005), and hematocrit levels. With every 1 kg/m^2 increase in BMI, the hematocrit level increases by 0.09% with regard to age, gender, ethnicity, and diabetes status remain constant (aβ=0.09; 95% CI:0.02,0.17). According to the BMI categories, obese participants have a 1.77% higher hematocrit level compared to normal weight participants with regard to age, gender, ethnicity, and diabetes status remain constant (aβ=1.77; 95% CI:0.53,3.01). There was also a significant trend in hematocrit levels among BMI categories (p=0.022). In contrast, we found no significant adjusted association of underweight (p=0.469), overweight (p=0.474), waist circumference (p=0.077), and abdominal obesity (p=0.642) with hematocrit levels.

Table 4.15: Crude and adjusted association between BMI categories, abdominal obesity, and hematocrit levels at baseline

Exposures	Hematocrit, %			
	Crude	P-value	Adjusted*	P-value
	β (95% CI)		β (95% CI)	
BMI, kg/m^2	0.03 (-0.06,0.12)	0.496	0.09 (0.02,0.17)	0.019
BMI Categories				
Underweight	0.56 (-1.38,2.51)	0.569	0.61 (-1.05,2.28)	0.469
Normal	1.00	Ref	1.00	Ref
Overweight	0.05 (-1.14,1.24)	0.939	0.37 (-0.65,1.40)	0.474
Obesity	0.96 (-0.48,2.39)	0.190	1.77 (0.53,3.01)	0.005
P-trend	-	0.375	-	0.022
Waist Circumference, cm	0.04 (0.01,0.08)	0.027	0.03 (-0.004,0.07)	0.077
Abdominal Obesity	-1.11 (-2.11,-0.10)	0.031	0.22 (-0.71,1.15)	0.642

* Adjusted for age, gender, ethnicity, and diabetes status.

Table 4.16 described the crude and adjusted association of BMI, BMI categories, waist circumference, and abdominal obesity with the changes in hematocrit levels. According to BMI, we found no significant crude association of BMI with the changes in hematocrit levels controlled for dengue fever phase (p=0.739). In contrast, we found a significant adjusted association between BMI and changes in hematocrit levels where with every 1 kg/m^2 increase in BMI, the hematocrit level increases by 0.07% with regard to age, gender, ethnicity, and dengue fever phase remains constant (aβ=0.07; 95% CI:0.003,0.15; p=0.041). In the stratified association by dengue fever phase, no significant crude association was found between BMI and changes in hematocrit levels at phase 1 (p=0.586), phase 2 (p=0.499), and phase 3 (p=0.251) of dengue fever. Similarly, no significant adjusted association was found between BMI and changes in hematocrit levels at phase 1 (p=0.070) and phase 3 (p=0.927) of dengue fever. However, we found a significant adjusted association between BMI and the incidence changes in hematocrit levels at phase 2 where with every 1 kg/m^2 increase in BMI at phase 2, the hematocrit level increases by 0.09% with regard to age, gender, ethnicity, and diabetes status remain constant (aβ=0.09; 95% CI:0.02,0.17; p=0.017).

Based on the P-interaction test, the effect of BMI on hematocrit levels by dengue fever phase in the crude association was important (p=0.027). However, the effect of BMI on hematocrit levels by dengue fever phase in the adjusted association was not important (p=0.076). Thus, we selected the complex model with interaction by dengue fever phase for interpretation of the crude model and simple model for interpretation of the adjusted model.

According to BMI categories, there was no significant crude association between underweight (p=0.849), overweight (p=0.699), and obesity (p=0.395) with the changes in hematocrit levels compared to normal weight controlled for dengue fever phase. We observed a higher hematocrit levels among obese and underweight patients compared to normal weight. In contrast, lower hematocrit levels were found among overweight patients. Based on the p-trend, there was no significant trend between different BMI categories (p=0.569).

When adjusted for age, gender, ethnicity, diabetes status, and dengue fever phase, we found a significant association between obesity with the changes in hematocrit levels compared to normal weight (p=0.017). Among obese patients, the hematocrit level was 1.39% higher compared to normal weight patients with regard to age, gender, ethnicity, diabetes status, and dengue fever phase remains constant (aβ=1.39; 95% CI:0.25,2.53). No significant adjusted association was found between underweight (p=0.759), overweight (p=0.819), and changes in hematocrit levels adjusted for age, gender, ethnicity, diabetes status, and dengue fever phase. There was also a significant trend between BMI categories and the incidence changes in hematocrit levels in the adjusted association (p=0.044).

In the stratified association by dengue fever phase, there was no significant crude association between BMI categories and changes in hematocrit levels at phase 1, phase 2, and phase 3 of dengue fever. Similarly, no significant trend was found between BMI categories at all three phases. When adjusted by age, gender, ethnicity, and diabetes status, only obesity at phase 2 (p=0.012) was significantly associated with the changes in hematocrit levels. At phase 2 of dengue fever, those with obesity have 1.62% higher hematocrit levels compared to normal weight patients with regard to age, gender, ethnicity, and diabetes status remain constant (aβ=1.62; 95% CI:0.35,2.90). We also found a significant trend between BMI categories and changes in hematocrit levels in the adjusted association at phase 2 of dengue fever (p=0.031).

Based on the P-interaction test, the effect between BMI categories and changes in hematocrit levels by dengue fever phase in the crude (p=0.314) and adjusted association (p=0.319) were not important. Thus, we selected the simple model without interaction by dengue fever phase for interpretation.

According to waist circumference, there was a significant crude association between waist circumference and changes of hematocrit levels controlled for dengue fever phase (p=0.021). With every 1 cm increase in waist circumference, there was 0.04% increase in the hematocrit levels (β=0.04; 95% CI:0.01,0.08) with regard to dengue fever phase remains constant. However, the association was not significant when adjusted for age, gender, ethnicity, diabetes status, and dengue fever phase (p=0.062). In the stratified analysis by dengue fever phase, we found significant crude association between waist circumference and incidence changes in hematocrit levels at phase 2 (p=0.013). With every 1 cm increase in waist circumference at phase 2, there was 0.05% increase in the hematocrit levels (β=0.05; 95% CI:0.01,0.09).

Similarly, there was a significant adjusted association between waist circumference and changes in hematocrit levels at phase 2 (p=0.035). With every 1 cm increase in waist circumference at phase 2, there was 0.04% increase in the hematocrit levels with regard to age, gender, ethnicity, and diabetes status remain constant (aβ=0.04; 95% CI:0.002,0.07). Based on the P-interaction test, the effect of waist circumference on the incidence changes in hematocrit levels by dengue fever phase in the crude (p=0.347) and adjusted association (p=0.346) were not important. Thus, we selected the simple model without interaction by dengue fever phase for interpretation.

According to abdominal obesity, we found a significant crude association between abdominal obesity and changes in hematocrit levels controlled by dengue fever phase (p=0.016). Among those with abdominal obesity, the hematocrit levels were lower by 1.17% compared to non-abdominal obesity with regard to dengue fever phase remains constant (β=-1.17; 95% CI:-2.12,-0.22). In contrast, the association was not significant when adjusted for age, gender, ethnicity, diabetes status, and dengue fever phase (p=0.701). In the stratified analysis by dengue fever phase, we found significant crude association between abdominal obesity and changes in hematocrit levels at phase 1 (p=0.017) and phase 2 (p=0.040). Among those with abdominal obesity at phase 1, the hematocrit levels were 1.39% lower compared to those without non-abdominal obesity (β=-1.39; 95% CI:-2.53,-0.25). At phase 2, those with abdominal obesity have 1.05% lower hematocrit levels compared to those without non-abdominal obesity (β=-1.05; 95% CI:-2.05,-0.05). When adjusted for age, gender, ethnicity, and diabetes status, no significant association was found at phase 1 (p=0.970), phase 2 (p=0.563), and phase 3 (p=0.563) of dengue fever. Based on the P-interaction test, the effect between waist circumference and changes in hematocrit levels by dengue fever phase in the crude (p=0.755) and adjusted association (p=0.822) were not important. Thus, we selected the simple model without interaction by dengue fever phase for interpretation.

Table 4.16: Crude and adjusted association between BMI categories, abdominal obesity, and changes in hematocrit levels

Exposures	Hematocrit, %			
	Crude	P-value	Adjusted*	P-value
	β (95% CI)		aβ (95% CI)	
BMI, Centred at 25 kg/m²				
BMI at All Phases	0.01 (-0.07,0.10)	0.739	0.07 (0.003,0.15)	0.041
BMI at Phase 1	0.03 (-0.07,0.13)	0.586	0.08 (-0.01,0.17)	0.070
BMI at Phase 2	0.03 (-0.06,0.12)	0.499	0.09 (0.02,0.17)	0.017
BMI at Phase 3	-0.06 (-0.17,0.04)	0.251	0.003 (-0.09,0.10)	0.927
P-Interaction[2]	-	0.027	-	0.076
BMI Categories				
All Phases				
Underweight	0.18 (-1.67,2.03)	0.849	0.24 (-1.29,1.76)	0.759
Normal	Ref	-	Ref	-
Overweight	-0.22 (-1.34,0.90)	0.699	0.11 (-0.82,1.04)	0.819
Obesity	0.59 (-0.77,1.95)	0.395	1.39 (0.25,2.53)	0.017
P-Trend	-	0.569	-	0.044
Phase 1				
Underweight	-0.02 (-2.25,2.21)	0.986	0.28 (-1.66,2.23)	0.774
Normal	Ref	-	Ref	-
Overweight	-0.12 (-1.45,1.22)	0.866	0.17 (-1.01,1.34)	0.781
Obesity	0.69 (-0.95,2.33)	0.409	1.45 (0.01,2.89)	0.049
P-Trend	-	0.864	-	0.421
Phase 2				
Underweight	0.07 (-1.92,2.06)	0.943	-0.01 (-1.70,1.67)	0.990
Normal	Ref	-	Ref	-
Overweight	-0.04 (-1.21,1.13)	0.946	0.31 (-0.68,1.30)	0.538
Obesity	0.80 (-0.69,2.29)	0.295	1.62 (0.35,2.90)	0.012
P-Trend	-	0.287	-	0.031
Phase 3				
Underweight	0.91 (-1.64,3.45)	0.485	0.85 (-1.42,3.12)	0.463
Normal	Ref	-	Ref	-
Overweight	-0.96 (-2.33,0.41)	0.168	-0.60 (-1.81,0.61)	0.330
Obesity	-0.37 (-2.19,1.44)	0.688	0.53 (-1.09,2.51)	0.520
P-Trend	-	0.262	-	0.978
P-Interaction[2]	-	0.314	-	0.319
WC, Centred at 80 cm				
WC at All Phase	0.04 (0.01,0.08)	0.021	0.03 (-0.002,0.06)	0.062
WC at Phase 1	0.04 (-0.005,0.09)	0.079	0.03 (-0.01,0.07)	0.191
WC at Phase 2	0.05 (0.01,0.09)	0.013	0.04 (0.002,0.07)	0.035
WC at Phase 3	0.02 (-0.02,0.07)	0.284	0.01 (-0.03,0.06)	0.510
P-Interaction[2]	-	0.347	-	0.346
Abdominal Obesity				
All phases				
No	Ref	-	Ref	-
Yes	-1.17 (-2.12,-0.22)	0.016	0.17 (-0.68,1.01)	0.701
Phase 1				
No	Ref	-	Ref	-
Yes	-1.39 (-2.53,-0.25)	0.017	0.02 (-1.03,1.07)	0.970
Phase 2				
No	Ref	-	Ref	-
Yes	-1.05 (-2.05,-0.05)	0.040	0.27 (-0.64,1.17)	0.563
Phase 3				
No	Ref	-	Ref	-
Yes	-1.16 (-2.36,0.04)	0.057	0.11 (-0.99,1.20)	0.851
P-Interaction[2]	-	0.755	-	0.822

* Adjusted for age, gender, ethnicity, and diabetes status.

[1] P-Interaction tests between the model without and with interaction by dengue fever phase.

[2] Dengue fever was categorised into Phase 1 (Fever day 0-3), Phase 2 (Fever day 4-6), and Phase 3 (Fever day ≥7).

Figure 4.3 describes the association between BMI and changes in hematocrit levels by dengue fever phase. At phase 1, we observed an increasing trend of hematocrit with increasing BMI. Similarly, at phase 2, the trend was increasing but plateauing with higher BMI. In contrast, at phase 3, there was an increasing trend of hematocrit at lower BMI but reduces as the BMI increases.

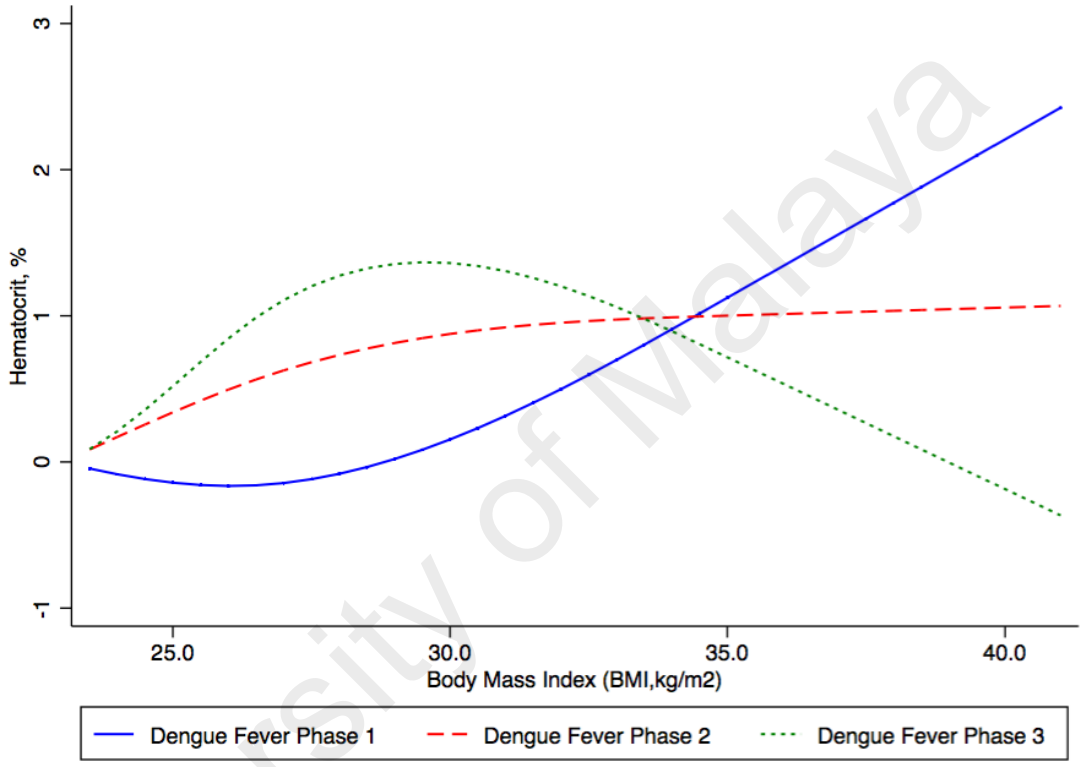

Figure 4.3: Association between BMI and changes in hematocrit levels by dengue fever phase

4.4 Diabetes and Dengue

4.4.1 Baseline Characteristics of Participants by Diabetes Status

To analyse the association between diabetes and dengue severity, we categorised participants into non-diabetics and diabetics based on the patient's latest HbA1c level at baseline and medical record. There were 302 participants (83.4%) in the non-diabetes group and 60 participants (16.6%) in the diabetes group. Table 4.17 described the general characteristics of participants with diabetes status at baseline. According to age, there was a significant ($p<0.001$) difference in the mean age between non-diabetes and diabetes group. We found a higher mean age of 49.0 ± 13.62 years among those with diabetes compared to non-diabetics with a mean of 33.2 ± 12.67 years.

Based on gender, there was no significant ($p=0.370$) difference in the distribution of males and females between the non-diabetes and diabetes group. There were more females in the non-diabetes group (49.7%) as compared to diabetes group (43.3%). The percentage of a male in the non-diabetes group was lower (50.3%) compared to diabetes group (56.7%). According to the ethnic group distribution, there was a higher percentage of Malay participants in the non-diabetes and diabetes group, followed by Chinese, Indian, and other ethnic groups. There were 68.2% Malay participants, 18.2% Chinese participants, 10.3% Indian participants, and 3.3% of other ethnic groups in the non-diabetes group. Comparatively, in the diabetes group, there were 63.3% Malay participants, 18.3% Chinese participants, 18.3% Indian participants, with no other ethnic groups participants. Comparing the ethnic distribution of participants with diabetes status, we found no significant difference between the non-diabetes and diabetes group ($p=0.198$).

Based on the status of previous dengue infection, 57 (18.9%) non-diabetic participants had a previous dengue infection and 51 participants (89.5%) were unaware. In the diabetes group, 21 participants (35.0%) had previous dengue infection with 18 participants (85.7%) were unaware. The was a significant difference in previous dengue infection status (p=0.006). We found no significant difference in the awareness of the previous dengue infection (p=0.696) between diabetes and non-diabetes group. Comparing the mean days of fever, we found no significant difference (p=0.720) between diabetes status. The mean days of fever for non-diabetes and diabetes group was 3.90 ± 1.67 days and 3.98 ± 1.42 days, respectively.

Table 4.17: Baseline characteristics of participants by diabetes status

General Characteristics	Non-Diabetes (n = 302)	Diabetes (n = 60)	P-value
	n (%) / mean ± SD	n (%) / mean ± SD	
Age, years	33.2 ± 12.67	49.0 ± 13.62	<0.001
Gender			0.370
Male	152 (50.3%)	34 (56.7%)	
Female	150 (49.7%)	26 (43.3%)	
Ethnicity			0.198
Malay	206 (68.2%)	38 (63.3%)	
Chinese	55 (18.2%)	11 (18.3%)	
Indian	31 (10.3%)	11 (18.3%)	
Others	10 (3.3%)	0 (0.0%)	
Previous Dengue Infection			
No	245 (81.1%)	39 (65.0%)	0.006
Yes	57 (18.9%)	21 (35.0%)	
Unaware	51 (89.5%)	18 (85.7%)	0.696
Aware	6 (10.5%)	3 (14.3%)	
Days of Fever, days	3.90 ± 1.67	3.98 ± 1.42	0.720

4.4.2 Baseline Clinical Characteristics of Participants by Diabetes Status

Table 4.18 compares the characteristics of the non-diabetes and diabetes group. Based on warning signs, the non-obese group had a higher percentage of vomiting (9.9%), abdominal pain (8.0%), and spontaneous bleeding (1.3%). We observed a higher percentage of diarrhea (18.3%), lethargy (28.3%), liver tenderness (1.7%), and raised hematocrits with a rapid drop in platelet (35.0%) in the diabetes group compared to the non-diabetes group, respectively. Statistically, we only found a significant difference in lethargy (p=0.018). Furthermore, there was no significant difference between BMI categories in platelet (p=0.159) and hematocrit (p=0.353). Nevertheless, we found a significantly (p=0.016) higher mean white blood cells of 4.3 ± 1.86 x10^3/µL among those with diabetes compared to 3.7 ± 1.63 x10^3/µL among non-diabetes.

Table 4.18: Baseline clinical characteristics of participants by diabetes status

General Characteristics	Non-Diabetes (n = 302) n (%) / mean ± SD	Diabetes (n = 60) n (%) / mean ± SD	P-value
Warning Signs			
Diarrhea			0.416
No	259 (85.8%)	49 (81.7%)	
Yes	43 (14.2%)	11 (18.3%)	
Vomiting			0.627
No	272 (90.1%)	56 (93.3%)	
Yes	30 (9.9%)	4 (6.7%)	
Abdominal Pain			>0.999
No	278 (92.1%)	56 (93.3%)	
Yes	24 (8.0%)	4 (6.7%)	
Lethargy			0.018
No	255 (84.4%)	43 (71.7%)	
Yes	47 (15.6%)	17 (28.3%)	
Liver Tenderness			0.420
No	300 (99.3%)	59 (98.3%)	
Yes	2 (0.7%)	1 (1.7%)	
Third Space Fluid Accumulation			-
No	302 (100.0%)	60 (100.0%)	
Yes	0 (0.0%)	0 (0.0%)	
Spontaneous Bleeding			>0.999
No	298 (98.7%)	60 (100.0%)	
Yes	4 (1.3%)	0 (0.0%)	
Raised HCT[a] with drop in PLT[b]			0.116
No	226 (74.8%)	39 (65.0%)	
Yes	76 (25.2%)	21 (35.0%)	
Laboratory Values			
WBC[a], x10^3 µL	3.7 ± 1.63	4.3 ± 1.86	0.016
PLT[b], x10^3 µL	135.8 ± 52.38	125.2 ± 58.67	0.159
HCT[c], %	42.0 ± 4.86	41.4 ± 5.02	0.353
RBS[d], mmol/L	5.8 ± 1.35	9.3 ± 4.16	<0.001

[a] WBC: White Blood Cells; [b] PLT: Platelets; [c] HCT: Hematocrits; [d]RBS: Random Blood Sugar.

4.4.3 Associations of RBS and Diabetes with Dengue Severity at Baseline

Table 4.19 describes the associations of random blood sugar (RBS) and status of diabetes with dengue severity. There was no significant association in the association between RBS and dengue severity (p=0.073). We observed that participants in the severe dengue infection group have a higher mean RBS of 6.6 ± 2.66 mmol/L compared to 6.15 ± 2.22 mmol/L for the non-severe dengue infection group. Similarly, we found no significant association between the status of diabetes and dengue severity (p=0.109). Based on the distribution, the severe dengue infection group had a higher percentage of participants with diabetes (19.8%) compared to the non-severe dengue infection group (13.5%).

Table 4.19: Association of RBS and diabetes status with dengue severity at baseline

Exposures	Non-Severe (n = 185)	Severe (n = 177)	P-value
	n (%) / mean ± SD	n (%) / mean ± SD	
RBS, mmol/L	6.15 ± 2.22	6.6 ± 2.66	0.073
Diabetes			0.109
No	160 (86.5%)	142 (80.2%)	
Yes	25 (13.5%)	35 (19.8%)	

Table 4.20 describes the crude and adjusted cross-sectional association between RBS, diabetes and dengue severity. In the crude association, there was no significant associations for RBS (p=0.078) and diabetes (p=0.111). When adjusted for age, gender, ethnicity, BMI categories, and diabetes status, we found no significant association between RBS and dengue severity (p=0.192). Similarly, when adjusted for age, gender, ethnicity, and BMI categories, no significant association between diabetes and dengue severity (p=0.065).

Table 4.20: Crude and adjusted associations between RBS and diabetes and the prevalence of dengue severity at baseline

Exposures	Dengue Severity			
	Crude	P-value	Adjusted	P-value
	OR (95% CI)		aOR (95% CI)	
RBS, mmol/L[1]	1.08 (0.99,1.18)	0.078	1.07 (0.96,1.20)	0.192
Diabetes[2]	1.58 (0.90,2.76)	0.111	1.82 (0.96,3.46)	0.065

[1] Adjusted for age, gender, ethnicity, BMI categories, and diabetes status.
[2] Adjusted for age, gender, ethnicity, and BMI categories.

4.4.4 Associations of RBS and Diabetes with The Incidence of Dengue Severity

Table 4.21 describes the crude and adjusted association of RBS and diabetes with the incidence of dengue severity. According to RBS, there was no significant crude (p=0.050) and adjusted (p=0.068) association between RBS and the incidence of dengue severity controlled for dengue fever phase. In the stratified analysis by dengue fever phases, we found significant crude (p=0.020) and adjusted (p=0.025) association between RBS and incidence of dengue severity at phase 2. For the crude association, with every 1 mmol/L increase in RBS at phase 2, the odds of developing severe dengue infection increases by 37% (OR=1.37; 95% CI:1.05,1.78).

For the adjusted association, with every 1 mmol/L increase in RBS at phase 2, the odds of developing severe dengue infection increases by 37% with regard to age, gender, ethnicity, BMI categories, and diabetes remain constant (aOR=1.37; 95% CI:1.04,1.81). Based on the P-interaction test, the effect of RBS on the incidence of dengue severity by dengue fever phase in the crude (p=0.308) and adjusted association (p=0.281) were not important. Thus, we selected the simple model without interaction for interpretation.

When stratified by diabetes status, there was no significant crude (p=0.977) and adjusted (p=0.813) association between RBS among diabetes patients and dengue severity controlled by dengue fever phase. Similarly, in the stratified analysis by dengue fever phase, there was no significant crude association between RBS and dengue severity at phase 1 (p=0.826), phase 2 (p=0.896), and phase 3 (p=0.495) of dengue fever. We also found no significant adjusted stratified association between RBS and dengue severity at phase 1 (p=0.986), phase 2 (p=0.558), and phase 3 (p=0.644) of dengue fever. Based on the p-interaction test, the effect of RBS on the incidence of dengue severity by dengue fever phase in the crude (p=0.733) and adjusted association (p=0.734) were not important. Thus, we selected the simple model without interaction for interpretation.

However, among those without diabetes, we found a significant association in the crude (p=0.010) and adjusted (p=0.007) association between RBS and dengue severity controlled by dengue fever phase. In the crude association, with every 1 mmol/L increase in RBS among non-diabetic patients, the odds of dengue severity increase by 54% with regard to dengue fever phase remains constant (OR=1.54; 95% CI:1.11,2.13). In the adjusted association, with every 1 mmol/L increase in RBS among non-diabetic patients, the odds of dengue severity increase by 57% with regard to age, gender, ethnicity, and BMI categories remain constant (aOR=1.57; 95% CI:1.13,2.18).

In the stratified analysis by dengue fever phase, we found a significant crude (p=0.003) and adjusted (p=0.003) association between RBS and dengue severity among non-diabetic patients at phase 2 of dengue fever. In the crude association, with every 1 mmol/L increase in RBS among non-diabetic patients at phase 2 of dengue fever, the odds of dengue severity increase by 97% with regard to dengue fever phase remains constant (OR=1.97; 95% CI:1.26,3.08). In the adjusted association, with every 1 mmol/L increase in RBS among non-diabetic patients at phase 2 of dengue fever, the odds of dengue severity increase by 97% with regard to age, gender, ethnicity, and BMI categories remain constant (aOR=1.97; 95% CI:1.27,3.05). Based on the p-interaction test, the effect of RBS on the incidence of dengue severity by dengue fever phase in the crude (p=0.142) and adjusted association (p=0.205) were not important. Thus, we selected the simple model without interaction for interpretation.

Based on the association between diabetes and dengue severity, there were no significant crude (p=0.260) and adjusted (p=0.104) associations between diabetes and the incidence of dengue severity controlled for dengue fever phase. In the stratified analysis by dengue fever phases, we found no significant crude association between diabetes and the incidence of dengue severity at phase 1 (p=0.096), phase 2 (p=0.328), and phase 3 (p=0.238).

However, there was a significant association between diabetes and the incidence of dengue severity at phase 1 where the odds of dengue severity were 19.9 times higher among diabetes compared to non-diabetes (aOR=20.89; 95% CI:1.08,403.36; p=0.044). No significant adjusted association between diabetes and the incidence of dengue severity at phase 2 (p=0.149) and phase 3 (p=0.410) was found. Based on the P-interaction test, the effect of diabetes on the incidence of dengue severity by dengue fever phase in the crude (p=0.027) and adjusted association (p=0.027) were important. Thus, we selected the complex model with interaction for interpretation.

Table 4.21: Crude and adjusted association of RBS and diabetes with the incidence of dengue severity

Exposures	Dengue Severity			
	Crude OR (95% CI)	P-value	Adjusted aOR (95% CI)	P-value
RBS, Centred at 5 mmol/L[1]				
RBS at All Phases	1.23 (1.00,1.51)	0.050	1.23 (0.98,1.54)	0.068
RBS at Phase 1	1.18 (0.79,1.77)	0.422	1.20 (0.80,1.80)	0.255
RBS at Phase 2	1.37 (1.05,1.78)	0.020	1.37 (1.04,1.81)	0.025
RBS at Phase 3	0.99 (0.65,1.50)	0.952	0.98 (0.65,1.48)	0.913
P-Interaction[3]	-	0.308	-	0.281
RBS among Diabetes				
RBS at All Phases	1.01 (0.71,1.41)	0.977	0.96 (0.70,1.32)	0.813
RBS at Phase 1	0.94 (0.55,1.60)	0.826	1.00 (0.62,1.60)	0.986
RBS at Phase 2	0.97 (0.64,1.48)	0.896	0.88 (0.59,1.34)	0.558
RBS at Phase 3	1.32 (0.59,2.94)	0.495	1.19 (0.57,2.49)	0.644
P-Interaction[3]	-	0.733	-	0.734
RBS among Non-Diabetes				
RBS at All Phases	1.54 (1.11,2.13)	0.010	1.57 (1.13,2.18)	0.007
RBS at Phase 1	0.96 (0.47,1.94)	0.901	1.05 (0.52,2.11)	0.896
RBS at Phase 2	1.97 (1.26,3.08)	0.003	1.97 (1.27,3.05)	0.003
RBS at Phase 3	1.24 (0.64,2.41)	0.523	1.27 (0.66,2.46)	0.478
P-Interaction[3]	-	0.142	-	0.205
Diabetes[2]				
All Phases				
No	1.00	Ref	1.00	Ref
Yes	3.03 (0.44,20.87)	0.260	5.59 (0.70,44.59)	0.104
Phase 1				
No	1.00	Ref	1.00	Ref
Yes	11.65 (0.65,209.78)	0.096	20.89 (1.08,403.96)	0.044
Phase 2				
No	1.00	Ref	1.00	Ref
Yes	3.18 (0.31,32.35)	0.328	6.12 (0.52,71.59)	0.149
Phase 3				
No	1.00	Ref	1.00	Ref
Yes	0.12 (0.003,4.09)	0.238	0.22 (0.01,7.88)	0.410
P-Interaction[3]	-	0.027	-	0.027

[1] Adjusted for age, gender, ethnicity, BMI categories, and diabetes status.
[2] Adjusted for age, gender, ethnicity, and BMI categories.
[3] P-Interaction tests between the model without and with interaction by dengue fever phase.
[4] Dengue fever was categorised into Phase 1 (Fever day 0-3), Phase 2 (Fever day 4-6), and Phase 3 (Fever day ≥7).

Figure 4.4 shows the association between diabetes and dengue severity by dengue fever phases. There was a reducing trend of odds ratio for dengue severity as dengue fever phase progress. We observed a higher odds of dengue severity among those with diabetes at phase 1 of dengue fever. Similarly, the odds ratio of dengue severity was lower at phase 2 compared to phase 1 of dengue fever. Furthermore, at phase 3 of dengue fever, the odds ratio of dengue severity was the lowest compared to phase 1 and phase 2 of dengue fever.

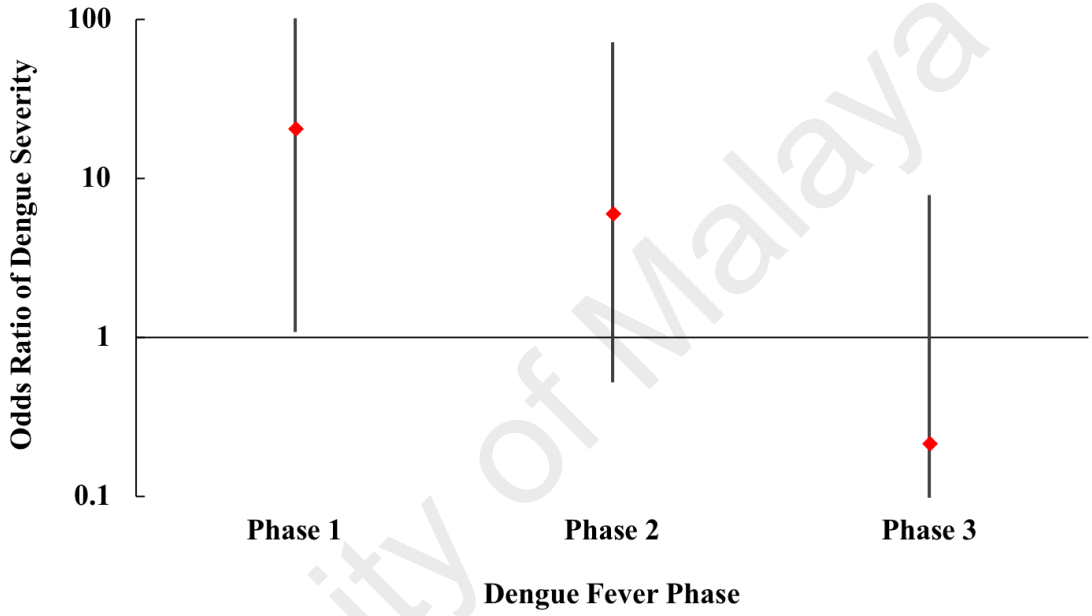

Figure 4.4: Association between diabetes and dengue severity by dengue fever phase

4.4.5 Associations of RBS and Diabetes with Platelet

Table 4.22 describes the associations between RBS, diabetes, and platelet levels in quartiles at baseline. There was no significant association found between RBS and different quartiles of platelets (p=0.053). Similarly, we found no significant association between diabetes and different quartiles of platelets (p=0.613). However, there was a decreasing trend observed in the mean RBS between different quartiles. There was a higher mean of RBS in quartile 1 with 6.8 ± 2.19 mmol/L, followed by 6.6 ± 2.46 mmol/L in quartile 2, 6.2 ± 2.83 mmol/L in quartile 3, and 5.9 ± 2.04 mmol/L in quartile 4. Moreover, we found a higher percentage of participants with diabetes in quartile 1 with 20.5%, followed by 16.8% in quartile 2, 16.2% in quartile 3, and 12.7% in quartile 4.

Table 4.22: RBS and diabetes characteristics by platelet quartile at baseline

Exposures	Platelet Quartiles				P-value
	Q1 (n = 83)	Q2 (n = 95)	Q3 (n = 105)	Q4 (n = 79)	
	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	
RBS, mmol/L	6.8 ± 2.19	6.6 ± 2.46	6.2 ± 2.83	5.9 ± 2.04	0.053
Diabetes					0.613
No	66 (79.5%)	79 (83.2%)	88 (83.8%)	69 (87.3%)	
Yes	17 (20.5%)	16 (16.8%)	17 (16.2%)	10 (12.7%)	

Table 4.23 describes the crude and adjusted cross-sectional association based on the multivariable linear regression analysis. According to the crude association, we found a significant association between RBS and platelet levels (p=0.003). With every 1 mmol/L increase in RBS value, the platelet level decreases by 3.44 $x10^3/\mu L$ (β=-3.44; 95% CI:-5.67,-1.20). In the adjusted association, there was a significant association between RBS and platelet levels (p=0.037). With every 1 mmol/L increase in RBS value, the platelet level decreases by 2.49 $x10^3/\mu L$ ($a\beta$=-2.49; 95% CI:-4.83,-0.16) with regards to age, gender, ethnicity, BMI categories, and diabetes status remains constant.

According to diabetes status, no significant crude association between the status of diabetes and platelet levels was found (p=0.159). Similarly, there was no significant adjusted association between diabetes and platelet levels (p=0.419).

Table 4.23: Crude and adjusted association of RBS and diabetes with platelet levels at baseline

Exposures	Platelet, x10^3/µL			
	Crude	P-value	Adjusted	P-value
	β (95% CI)		aβ (95% CI)	
RBS, mmol/L[1]	-3.44 (-5.67,-1.20)	0.003	-2.49 (-4.83,-0.16)	0.037
Diabetes[2]	-10.65 (-25.51,4.21)	0.159	-5.87 (-20.15,8.40)	0.419

[1] Adjusted for age, gender, ethnicity, BMI categories, and diabetes status.
[2] Adjusted for age, gender, ethnicity, and BMI categories.

Table 4.24 describes the crude and adjusted association between RBS, diabetes and changes in platelet levels. According to RBS, there was no significant crude (p=0.077) and adjusted (p=0.111) association between RBS and changes in platelet levels controlled for dengue fever phase. In the stratified analysis, we found a significant crude (p=0.009) and adjusted (p=0.014) association between RBS and changes in platelet levels at phase 2 of dengue fever. In the crude association, with every 1 mmol/L increase in RBS at phase 2, the platelet levels were -2.63 x10^3/µL lower (β=-2.63; 95% CI:-4.62,-0.65).

In the adjusted association, with every 1 mmol/L increase in RBS at phase 2, the platelet levels decrease by -2.74 x10^3/µL with regard to age, gender, ethnicity, BMI categories, and diabetes status remain constant (aβ=-2.74; 95% CI:-4.93,-0.54). Based on the P-interaction test, the effect of RBS on the changes in platelet levels by dengue fever phase in the crude (p=0.010) and adjusted association (p<0.001) were important. Thus, we selected the complex model with interaction by dengue fever phase for interpretation.

When stratified by diabetes status, we found no significant crude (p=0.612) and adjusted (p=0.641) association between RBS among diabetes patients and changes in platelet levels controlled by dengue fever phase. Similarly, in the stratified analysis by dengue fever phase, we found no significant crude association between RBS and changes

in platelet levels at phase 1 (p=0.682), phase 2 (p=0.800), and phase 3 (p=0.729) of dengue fever. We also found no significant adjusted stratified association between RBS and changes in platelet levels at phase 1 (p=0.911), phase 2 (p=0.908), and phase 3 (p=0.393) of dengue fever. Based on the p-interaction test, the effect of RBS among diabetes on the changes in platelet levels by dengue fever phase in the crude (p=0.979) association was not important. In contrast, the adjusted association (p<0.001) was important. Thus, we selected the simple model without interaction for interpretation of the crude association and the complex model with interaction for interpretation of the adjusted association.

Among those without diabetes, we found a significant association in the crude (p=0.006) and adjusted (p=0.005) association between RBS and changes in platelet levels controlled by dengue fever phase. In the crude association, with every 1 mmol/L increase in RBS among non-diabetic patients, the platelet level decreases by 3.55 $x10^3/\mu L$ with regard to dengue fever phase remains constant (β=-3.55; 95% CI:-6.08,-1.01). In the adjusted association, with every 1 mmol/L increase in RBS among non-diabetic patients, the platelet level decreases by 3.79 $x10^3/\mu L$ with regard to age, gender, ethnicity, and BMI categories remain constant (aβ=-3.79; 95% CI:-6.43,-1.15). Based on the p-interaction test, the effect of RBS among non-diabetics on the changes in platelet levels by dengue fever phase in the crude (p=0.489) association was not important. In contrast, the adjusted association (p<0.001) was important. Thus, we selected the simple model without interaction for interpretation of the crude association and the complex model with interaction for interpretation of the adjusted association.

According to diabetes status, there was no significant crude (p=0.559) and adjusted (p=0.858) association between diabetes and changes in platelet levels controlled for dengue fever phase. In the stratified crude analysis by dengue fever phases, no significant crude association was found between diabetes and changes in platelet levels

at phase 1 (p=0.177) and phase 2 (p=0.238) of dengue fever. Similarly, we found no significant adjusted association between diabetes and changes in platelet levels at phase 1 (p=0.369) and phase 2 (p=0.687) dengue fever.

In contrast, we found a significant crude (p=0.001) and adjusted (p<0.001) association between diabetes and the changes in platelet levels at phase 3. For the crude association, among those with diabetes at phase 3, the platelet levels were 39.14 $x10^3/\mu L$ higher compared to non-diabetes (OR=39.14; 95% CI:16.83,61.45). For the adjusted association, among those with diabetes at phase 3, the platelet levels were 44.29 $x10^3/\mu L$ higher compared to non-diabetes with regard to age, gender, ethnicity, and BMI categories remain constant (aOR=44.29; 95% CI:21.28,67.30).

Based on the P-interaction test, the effect of diabetes on the changes in platelet levels by dengue fever phase in the crude (p=0.001) and adjusted association (p=0.001) were important. Thus, we selected the complex model with interaction by dengue fever phase for interpretation.

Table 4.24: Crude and adjusted association of RBS and diabetes with the changes in platelet levels

Exposures	Platelet, x10^3/µL			
	Crude β (95% CI)	P-value	Adjusted aβ (95% CI)	P-value
RBS, Centred at 5 mmol/L[1]				
RBS at All Phases	-1.42 (-3.00,0.16)	0.077	-1.55 (-3.42,0.32)	0.103
RBS at Phase 1	-2.17 (-4.87,0.54)	0.116	-2.10 (-4.95,0.75)	0.149
RBS at Phase 2	-2.63 (-4.62,-0.65)	0.009	-2.74 (-4.93,-0.54)	0.014
RBS at Phase 3	2.58 (-0.46,5.62)	0.097	2.41 (-0.71,5.53)	0.129
P-Interaction[3]	-	0.010	-	<0.001
RBS among Diabetes				
RBS at All Phases	-0.68 (-3.30,1.94)	0.612	0.67 (-2.15,3.50)	0.641
RBS at Phase 1	-0.84 (-4.86,3.18)	0.682	0.23 (-3.75,4.21)	0.911
RBS at Phase 2	-0.45 (-3.91,3.01)	0.800	-0.20 (-3.66,3.25)	0.908
RBS at Phase 3	-0.96 (-6.35,4.44)	0.729	-2.31 (-7.61,2.99)	0.393
P-Interaction[3]	-	0.979	-	<0.001
RBS among Non-Diabetes				
RBS at All Phases	-3.55 (-6.08,-1.01)	0.006	-3.79 (-6.43,-1.15)	0.005
RBS at Phase 1	-2.54 (-7.95,2.86)	0.357	-1.75 (-7.26,3.75)	0.532
RBS at Phase 2	-4.67 (-7.84,-1.49)	0.004	-4.53 (-7.72,-1.34)	0.005
RBS at Phase 3	-1.24 (-6.53,4.05)	0.646	-1.06 (-6.36,4.24)	0.695
P-Interaction[3]	-	0.489	-	<0.001
Diabetes[2]				
All Phases				
No	1.00	Ref	1.00	Ref
Yes	-3.55 (-15.45,8.36)	0.559	1.20 (-11.99,14.39)	0.858
Phase 1				
No	1.00	Ref	1.00	Ref
Yes	-12.74 (-31.23,5.75)	0.177	-8.75 (-27.84,10.35)	0.369
Phase 2				
No	1.00	Ref	1.00	Ref
Yes	-8.11 (-21.59,5.36)	0.238	-3.04 (-17.84,11.75)	0.687
Phase 3				
No	1.00	Ref	1.00	Ref
Yes	39.14 (16.83,61.45)	0.001	44.29 (21.28,67.30)	<0.001
P-Interaction[3]	-	0.001	-	0.001

[1] Adjusted for age, gender, ethnicity, BMI categories, and diabetes status.
[2] Adjusted for age, gender, ethnicity, and BMI categories.
[3] P-Interaction tests between the model without and with interaction by dengue fever phase.
[4] Dengue fever was categorised into Phase 1 (Fever day 0-3), Phase 2 (Fever day 4-6), and Phase 3 (Fever day ≥7).

Figure 4.5 shows the association between diabetes and changes in platelet levels by dengue fever phase. We observed an increasing trend of platelet levels as the dengue fever phase progress to a later phase. At phase 1 of dengue fever, the platelet level was lower compared to phase 2 and phase 3 of dengue fever. At phase 2 of dengue fever, the platelet level remained low but showed an increasing trend compared to phase 1. At phase 3 of fever, the platelet level was higher compared to phases 1 and 2.

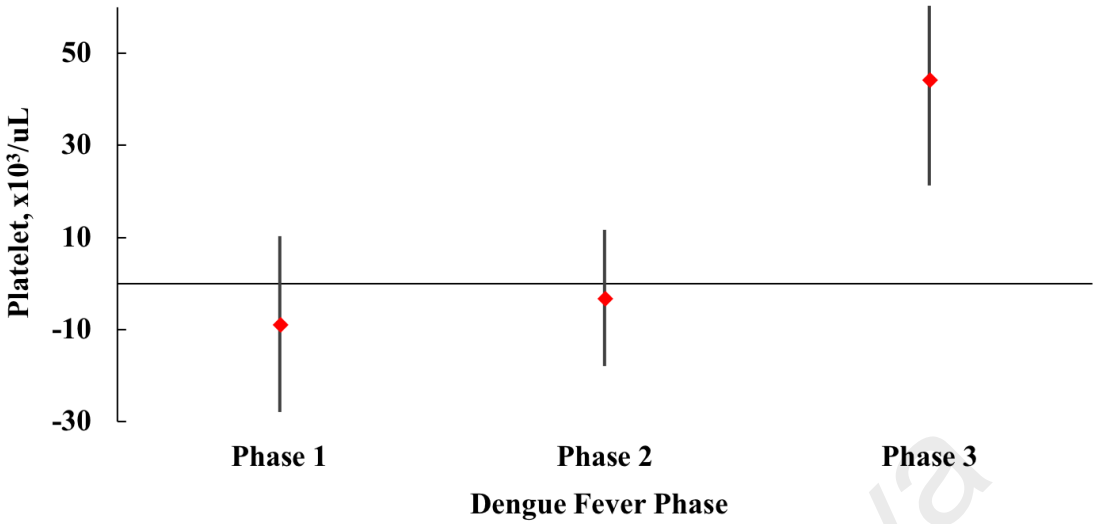

Figure 4.5: Association between diabetes and changes in platelet levels by dengue fever phase

4.4.6 Associations of RBS and Diabetes with Hematocrit

Table 4.25 describes the association between RBS, diabetes and hematocrit levels in quartiles at baseline. According to the RBS, there was no significant association between RBS values and different quartiles of hematocrit levels (p=0.062). Similarly, we found no significant association between the status of diabetes and different quartiles of hematocrit levels (p=0.750).

Table 4.25: RBS and diabetes characteristics by hematocrit quartiles at baseline

Exposures	Hematocrit Quartiles				P-value
	Q1 (n = 109)	Q2 (n = 76)	Q3 (n = 93)	Q4 (n = 84)	
	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	
RBS, mmol/L	6.5 ± 2.88	6.3 ± 1.87	6.6 ± 2.85	6.1 ± 1.75	0.062
Diabetes					0.750
No	89 (81.7%)	64 (84.2%)	76 (81.7%)	73 (86.9%)	
Yes	20 (18.4%)	12 (15.8%)	17 (18.3%)	11 (13.1%)	

We performed the multivariable linear regression analysis to determine the association between RBS, diabetes, and hematocrit levels. Table 4.26 describes the crude and adjusted cross-sectional association based on the multivariable linear regression analysis. According to the crude association, there was no significant association found between RBS (p=0.539), diabetes (p=0.427), and hematocrit levels. Similarly, when adjusted for age, gender, ethnicity, BMI categories, and diabetes status, there was no significant association between RBS (p=0.538), diabetes (p=0.440), and hematocrit levels at baseline.

Table 4.26: Crude and adjusted association of RBS and diabetes with hematocrit levels at baseline

Exposures	Hematocrit, %			
	Crude β (95% CI)	P-value	Adjusted aβ (95% CI)	P-value
RBS, mmol/L[1]	-0.06 (-0.25,0.13)	0.539	-0.03 (-0.22,0.16)	0.753
Diabetes[2]	-0.51 (-1.76,0.75)	0.427	-0.46 (-1.63,0.71)	0.440

[1] Adjusted for age, gender, ethnicity, BMI categories, and diabetes status.
[2] Adjusted for age, gender, ethnicity, and BMI categories.

Table 4.27 describes the crude association of RBS and diabetes with the changes in hematocrit levels. In terms of RBS, we found no significant crude (p=0.651) and adjusted (p=0.895) association between RBS and changes in hematocrit levels controlled for dengue fever phase. Similarly, we found no significant crude and adjusted association in the stratified analysis by dengue fever phase. Based on the p-interaction test, the effect of RBS on the changes in hematocrit levels by dengue fever phase in the crude (p=0.394) and adjusted association (p=0.611) were not important. Thus, we selected the simple model without interaction by dengue fever phase for interpretation.

When stratified by diabetes status, we found no significant crude (p=0.409) and adjusted (p=0.445) association between RBS among diabetes patients and changes in hematocrit levels controlled by dengue fever phase. Similarly, in the stratified analysis by dengue fever phase, there was no significant crude association between RBS and changes in hematocrit levels at phase 1 (p=0.713), phase 2 (p=0.054), and phase 3 (p=0.247) of dengue fever. We also found no significant adjusted stratified association between RBS and changes in hematocrit levels at phase 1 (p=0.526), phase 2 (p=0.076), and phase 3 (p=0.196) of dengue fever. Based on the p-interaction test, the effect of RBS among diabetes on the changes in hematocrit levels by dengue fever phase in the crude (p=0.056) and adjusted (p=0.063) association was not important. Thus, we selected the simple model without interaction for interpretation of the crude and adjusted association.

Among those without diabetes, there was no significant crude (p=0.462) and adjusted (p=0.443) association between RBS among non-diabetic patients and changes in hematocrit levels controlled by dengue fever phase. Similarly, in the stratified analysis by dengue fever phase, we found no significant crude association between RBS and changes in hematocrit levels at phase 1 (p=0.930), phase 2 (p=0.429), and phase 3 (p=0.705) of dengue fever.

We also found no significant adjusted stratified association between RBS and changes in hematocrit levels at phase 1 (p=0.536), phase 2 (p=0.219), and phase 3 (p=0.882) of dengue fever. Based on the p-interaction test, the effect of RBS among non-diabetes on the changes in hematocrit levels by dengue fever phase in the crude (p=0.899) and adjusted (p=0.482) association was not important. Thus, we selected the simple model without interaction for interpretation of the crude and adjusted association.

According to diabetes status, there was no significant crude (p=0.175) and adjusted (p=0.177) association between diabetes and changes in hematocrit levels controlled for dengue fever phase. Similarly, there was no significant crude and adjusted association in the stratified analysis by dengue fever phase. Based on the p-interaction test, the effect of diabetes on changes in hematocrit levels by dengue fever phase in the crude (p=0.585) and adjusted association (p=0.584) were not important. Thus, we selected the simple model without interaction by dengue fever phase for interpretation.

Table 4.27: Crude and adjusted association of RBS and diabetes with the changes in hematocrit levels

Exposures	Hematocrit, %			
	Crude β (95% CI)	P-value	Adjusted aβ (95% CI)	P-value
RBS, Centred at 5 mmol/L[1]				
RBS at All Phases	-0.03 (-0.14,0.09)	0.651	0.01 (-0.10,0.12)	0.895
RBS at Phase 1	0.01 (-0.18,0.20)	0.921	-0.002 (-0.19,0.19)	0.984
RBS at Phase 2	-0.07 (-0.21,0.06)	0.270	-0.02 (-0.15,0.11)	0.773
RBS at Phase 3	0.05 (-0.13,0.24)	0.582	0.08 (-0.10,0.26)	0.387
P-Interaction[3]	-	0.394	-	0.611
RBS among Diabetes				
RBS at All Phases	-0.07 (-0.25,0.10)	0.409	-0.07 (-0.24,0.11)	0.445
RBS at Phase 1	-0.05 (-0.31,0.21)	0.713	-0.08 (-0.34,0.18)	0.526
RBS at Phase 2	-0.20 (-0.40,0.004)	0.054	-0.18 (-0.39,0.02)	0.076
RBS at Phase 3	0.17 (-0.12,0.46)	0.247	0.19 (-0.10,0.47)	0.196
P-Interaction[3]	-	0.056	-	0.063
RBS among Non-Diabetes				
RBS at All Phases	0.06 (-0.10,0.22)	0.462	0.06 (-0.09,0.21)	0.443
RBS at Phase 1	-0.02 (-0.39,0.35)	0.930	-0.11 (-0.46,0.24)	0.536
RBS at Phase 2	0.08 (-0.12,0.27)	0.429	0.12 (-0.07,0.31)	0.219
RBS at Phase 3	0.06 (-0.24,0.36)	0.705	0.02 (-0.27,0.32)	0.882
P-Interaction[3]	-	0.899	-	0.482
Diabetes[2]				
All Phases				
No	1.00	Ref	1.00	Ref
Yes	-0.83 (-2.04,0.37)	0.175	-0.76 (-1.87,0.34)	0.177
Phase 1				
No	1.00	Ref	1.00	Ref
Yes	-0.39 (-1.86,1.08)	0.604	-0.35 (-1.71,1.02)	0.619
Phase 2				
No	1.00	Ref	1.00	Ref
Yes	-1.02 (-2.29,0.25)	0.116	-0.96 (-2.14,0.22)	0.109
Phase 3				
No	1.00	Ref	1.00	Ref
Yes	-1.03 (-2.66,0.59)	0.213	-0.89 (-2.43,0.64)	0.253
P-Interaction[3]	-	0.585	-	0.584

[1] Adjusted for age, gender, ethnicity, BMI categories, and diabetes status.
[2] Adjusted for age, gender, ethnicity, and BMI categories.
[3] P-Interaction tests between the model without and with interaction by dengue fever phase.
[4] Dengue fever was categorised into Phase 1 (Fever day 0-3), Phase 2 (Fever day 4-6), and Phase 3 (Fever day ≥7).

Figure 4.6 shows the association between diabetes and changes in hematocrit levels by dengue fever phase. The hematocrit levels were lower among those with diabetes at three phases of dengue fever. At phase 1 of dengue fever, the hematocrit level was the highest compared to phase 2 and phase 3 of dengue fever. In contrast, the hematocrit level was the lowest at phase 2 of dengue fever among diabetes compared to phase 1 and phase 3 of dengue fever. We also observe that the hematocrit levels were decreasing with increasing phase of dengue fever.

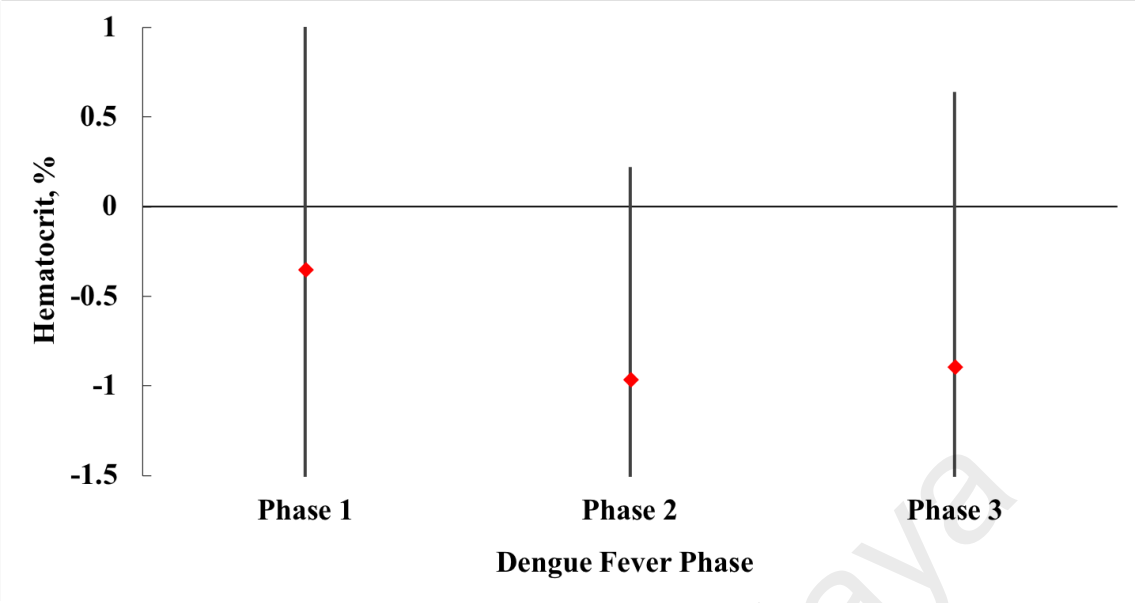

Figure 4.6: Association between diabetes and changes in hematocrit levels by dengue fever phase

4.5 Hypertension and Dengue

4.5.1 Baseline Characteristics of Participants by Hypertension Status

To analyse the association between hypertension and dengue severity, we categorised participants into non-hypertension and hypertension based on the average systolic and diastolic blood pressure at baseline and as well as the recorded diagnosis of hypertension in the medical record. There were 319 participants (88.1%) in the non-hypertension group and 43 participants (11.9%) in the hypertension group. Table 4.28 describes the general characteristics of participants by hypertensive status at baseline. According to age, there was a significant difference in the mean age between non-hypertension and hypertension group ($p<0.001$). We found a higher mean age of 45.3 ± 13.84 years among those with hypertension compared to non-hypertension with 34.5 ± 13.66 years. Based on gender, there was no significant difference in the distribution of male and female between non-hypertension and hypertension group ($p=0.536$). In the non-hypertension group, there were 162 male participants (50.8%) compared to 157 female participants (49.2%). In the hypertension group, there were 24 male participants (55.8%) compared to 19 female participants (44.2%).

According to ethnic distribution, there was a higher percentage of Malay participants in the non-hypertension and hypertension group, followed by Chinese, Indian, and other ethnic groups. There were 213 Malay participants (66.8%), 58 Chinese participants (18.2%), 38 Indian participants (11.9%), and 10 other ethnic groups participants (3.1%) in the non-hypertension group. Comparatively, in the hypertension group, there were 31 Malay participants (72.1%), 8 Chinese participants (18.6%), 4 Indian participants (9.3%), and no other ethnic groups participants. Comparing the ethnic distribution of participants by ethnic groups, we found no significant difference between the non-hypertension and hypertension group ($p=0.845$).

Based on the status of previous dengue infection, 68 (21.3%) non-hypertension participants had a previous dengue infection, and 60 participants (88.2%) were unaware of previous dengue infection. In the hypertension group, 10 participants (23.3%) had previous dengue infections and 9 participants (90.0%) were unaware of previous dengue infection. The was no statistically significant difference in previous dengue infection status (p=0.772) and the awareness of the previous dengue infection (p>0.999). Furthermore, we found no significant difference (p=0.741) in the mean days of fever between hypertension status. The mean days of fever for non-hypertension and hypertension group were 3.90 ± 1.64 days and 3.80 ± 1.51 days, respectively.

Table 4.28: Baseline characteristics of participants by hypertension status

General Characteristics	Non-HPT (n = 319)	HPT (n = 43)	P-value
	n (%) / mean ± SD	n (%) / mean ± SD (n, %)	
Age, years	34.5 ± 13.66	45.3 ± 13.84	<0.001
Gender			0.536
Male	162 (50.8%)	24 (55.8%)	
Female	157 (49.2%)	19 (44.2%)	
Ethnicity			0.845
Malay	213 (66.8%)	31 (72.1%)	
Chinese	58 (18.2%)	8 (18.6%)	
Indian	38 (11.9%)	4 (9.3%)	
Others	10 (3.1%)	0 (0.0%)	
Previous Dengue Infection			
No	251 (78.7%)	33 (76.7%)	0.772
Yes	68 (21.3%)	10 (23.3%)	
Unaware	60 (88.2%)	9 (90.0%)	>0.999
Aware	8 (11.8%)	1 (10.0%)	
Days of Fever, days	3.9 ± 1.64	3.8 ± 1.51	0.741

4.5.2 Baseline Clinical Characteristics of Participants by Hypertension Status

Table 4.29 compared the clinical characteristics of the non-hypertension and hypertension. The hypertension group had a higher percentage of diarrhea (16.3%), vomiting (16.3%), abdominal pain (9.3%), lethargy (25.6%), and raised hematocrits with rapid drop in platelet (37.2%) compared to 14.7%, 8.5%, 7.5%, 16.6%, and 25.4% in non-hypertension, respectively. There were no participants with liver tenderness, third space fluid accumulation, and spontaneous bleeding among the hypertension group. According to the laboratory values, no significant difference was found between BMI categories in white blood cells count (p=0.066), platelets level (p=0.149), and hematocrits level (p=0.110).

Table 4.29: Baseline clinical characteristics of participants by hypertension status

General Characteristics	Non-HPT (n = 319)	HPT (n = 43)	P-value
	n (%) / mean ± SD	n (%) / mean ± SD	
Warning Signs			
Diarrhea			0.789
No	272 (85.3%)	36 (83.7%)	
Yes	47 (14.7%)	7 (16.3%)	
Vomiting			0.099
No	292 (91.5%)	36 (83.7%)	
Yes	27 (8.5%)	7 (16.3%)	
Abdominal Pain			0.682
No	295 (92.5%)	39 (90.7%)	
Yes	24 (7.5%)	4 (9.3%)	
Lethargy			0.148
No	266 (83.4%)	32 (74.4%)	
Yes	53(16.6%)	11 (25.6%)	
Liver Tenderness			>0.999
No	316 (99.1%)	43 (100.0%)	
Yes	3 (0.9%)	0 (0.0%)	
Third Space Fluid Accumulation			-
No	319 (100.0%)	43 (100.0%)	
Yes	0 (0.0%)	0 (0.0%)	
Spontaneous Bleeding			>0.999
No	315 (98.8%)	43 (100.0%)	
Yes	4 (1.3%)	0 (0.0%)	
Raised HCT[a] with drop in PLT[b]			0.100
No	238 (74.6%)	27 (62.8%)	
Yes	81 (25.4%)	16 (37.2%)	
Laboratory Values			
WBC[c], x10^3 µL	3.8 ± 1.64	4.3 ± 1.95	0.066
PLT[b], x10^3 µL	135.6 ± 53.56	123.0 ± 52.61	0.149
HCT[a], %	41.8 ± 4.95	43.0 ± 4.33	0.110

[a] HCT: Hematocrits; [b] PLT: Platelets; [c]WBC: White Blood Cells.

4.5.3 Associations between Hypertension and Dengue Severity at Baseline

Table 4.30 describes the associations between hypertension and dengue severity at baseline. There were no significant associations of systolic blood pressure (p=0.303), diastolic blood pressure (p=0.097), and hypertension (p=0.052) with the prevalence of dengue severity. In addition, we found a higher percentage of hypertensive participant in the severe dengue infection group (15.3%) compared to the non-severe dengue infection group (8.7%).

Table 4.30: Blood pressure and hypertension characteristics by dengue severity at baseline

Exposures	Non-Severe (n = 185)	Severe (n = 177)	P-value
	n (%) / mean ± SD	n (%) / mean ± SD	
Systolic BP[a], mmHg	116.1 ± 13.60	117.7 ± 15.99	0.303
Diastolic BP[a], mmHg	73.7 ± 9.65	75.5 ± 11.06	0.097
Hypertension			0.052
No	169 (91.4%)	150 (84.8%)	
Yes	16 (8.7%)	27 (15.3%)	

[a] BP: Blood Pressure

Table 4.31 describes the crude and adjusted association between hypertension and dengue severity at baseline. According to the crude association, there were no significant association between hypertension and dengue severity (p=0.055). Similarly, we found no significant adjusted association between hypertension and dengue severity (p=0.061).

Table 4.31: Crude and adjusted association between hypertension and prevalence of dengue severity at baseline

Exposures	Dengue Severity			
	Crude	P-value	Adjusted*	P-value
	OR (95% CI)		aOR (95% CI)	
Hypertension	1.90 (0.99,3.66)	0.055	1.94 (0.97,3.87)	0.061

* Adjusted for age, gender, ethnicity, and BMI categories.

4.5.4 Associations between Hypertension and The Incidence of Dengue Severity

Table 4.32 describes the crude and adjusted association between hypertension and incidence of dengue severity. According to the hypertension status, we found no significant crude (p=0.359) and adjusted (p=0.338) association between hypertension and the incidence of dengue severity controlled for dengue fever phase. Similarly, we found no significant crude and adjusted association in the stratified analysis by dengue fever phase.

Based on the p-interaction test, the effect of hypertension on the incidence of dengue severity by dengue fever phase in the crude (p=0.055) and adjusted association (p=0.053) were not important. Thus, we selected the simple model without interaction by dengue fever phase for interpretation.

Table 4.32: Crude and adjusted association between hypertension and incidence of dengue severity

Exposures	Dengue Severity			
	Crude	P-value	Adjusted*	P-value
	OR (95% CI)		aOR (95% CI)	
Hypertension				
All Phases				
No	1.00	Ref	1.00	Ref
Yes	3.10 (0.27,34.82)	0.359	3.23 (0.29,35.34)	0.338
Phase 1				
No	1.00	Ref	1.00	Ref
Yes	22.35 (0.75,667.29)	0.073	22.35 (0.82,608.58)	0.065
Phase 2				
No	1.00	Ref	1.00	Ref
Yes	2.57 (0.17,38.35)	0.494	2.75 (0.19,39.19)	0.456
Phase 3				
No	1.00	Ref	1.00	Ref
Yes	0.15 (0.002,8.48)	0.352	0.15 (0.003,7.98)	0.345
P-Interaction[2]	-	0.055	-	0.053

[*] Adjusted for age, gender, ethnicity, and BMI categories.
[1] P-Interaction tests between the model without and with interaction by dengue fever phase.
[2] Dengue fever was categorised into Phase 1 (Fever day 0-3), Phase 2 (Fever day 4-6), and Phase 3 (Fever day ≥7).

Figure 4.7 shows the association between hypertension and dengue severity by dengue fever phases. We observed that there was a reducing trend in the odds of dengue severity as dengue fever progresses to the later phase. At phase 1, the odds of dengue severity were the highest. At phase 2 of dengue fever, the odds of dengue severity decrease to a lower value compared to phase 1. At phase 3 of dengue fever, the odds of dengue severity were at the lowest value compared to phase 1 and phase 2 of dengue fever.

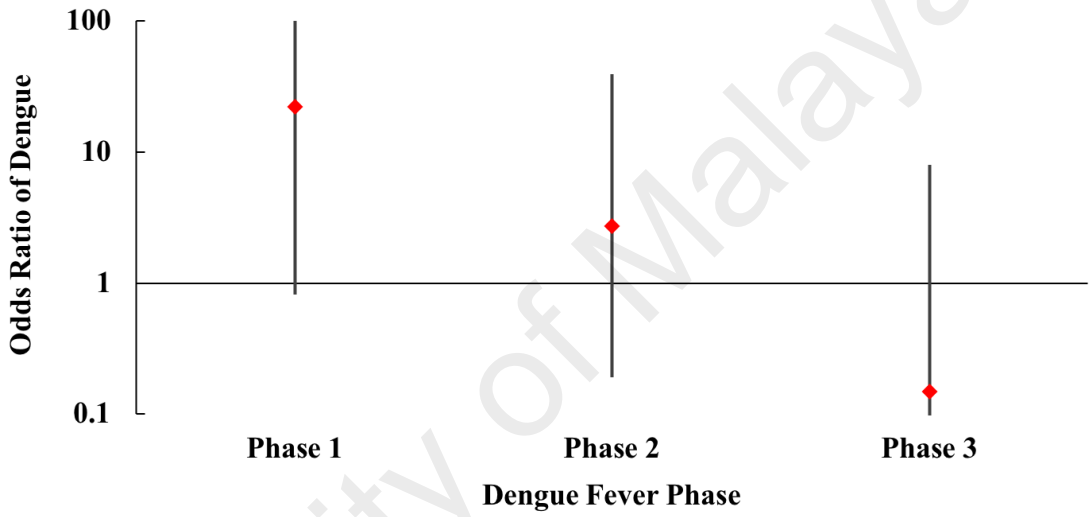

Figure 4.7: Association between hypertension and dengue severity by dengue fever phase

4.5.5 Associations between Hypertension and Platelet

Table 4.33 describes the associations between hypertension and platelet levels in quartiles at baseline. According to systolic blood pressure, diastolic blood pressure, and hypertension status, we found no significant associations in the mean systolic blood pressure (p=0.523), mean diastolic blood pressure (p=0.194), and status of hypertension (p=0.633) between different quartiles of the platelet.

Table 4.33: Association between hypertension and platelet levels in quartile at baseline

Exposures	Platelets Quartiles				P-value
	Q1 (n = 83)	Q2 (n = 95)	Q3 (n = 105)	Q4 (n = 79)	
	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	
Systolic BP[a], mmHg	117.3 ± 16.29	117.4 ± 14.78	117.7 ± 13.33	114.7 ± 15.20	0.523
Diastolic BP[a], mmHg	75.0 ± 10.78	74.4 ± 10.51	75.9 ± 8.89	72.6 ± 11.51	0.194
Hypertension					0.633
No	70 (84.3%)	84 (88.4%)	95 (90.5%)	70 (88.6%)	
Yes	13 (15.7%)	11 (11.6%)	10 (9.5%)	9 (11.4%)	

[a] BP: Blood Pressure.

We performed the multivariable linear regression analysis to determine the association between and hypertension platelet levels at baseline. Table 4.34 described the crude and adjusted cross-sectional association between hypertension status and platelet levels at baseline. According to the crude association, we found no significant association between hypertension and platelet levels (p=0.274). We also found no significant adjusted association between hypertension (p=0.742) and platelet levels at baseline.

Table 4.34: Crude and adjusted association between hypertension and platelet levels at baseline

Exposures	Platelet, $x10^3/\mu L$			
	Crude	P-value	Adjusted*	P-value
	β (95% CI)		aβ (95% CI)	
Hypertension	-8.16 (-22.82,6.49)	0.274	-2.56 (-17.89,12.76)	0.742

* Adjusted for age, gender, ethnicity, and BMI categories.

Furthermore, we performed the crude and adjusted random intercept multivariable linear regression analysis to determine the association between hypertension and changes in platelet levels. Table 4.35 describes the crude and adjusted association between hypertension and changes in platelet levels. According to the hypertension status, there was no significant crude (p=0.609) and adjusted (p=0.941) association between hypertension and changes in platelet levels controlled for dengue fever phase. In the stratified analysis by dengue fever phase, we found a significant crude association between hypertension and changes in platelet levels at phase 2 (p=0.014) and phase 3 (p=0.009) of dengue fever. Among those with hypertension at phase 2, the platelet levels were 19.35 $x10^3/\mu L$ lower compared to non-hypertension (β=-19.35; 95% CI:-34.71,-3.99). Among those with hypertension at phase 3, the platelet levels were 34.87 $x10^3/\mu L$ higher compared to non-hypertension (β=34.87; 95% CI:8.81,60.93). When adjusted for age, gender, ethnicity, and BMI categories, we only found a significant association between hypertension and changes in platelet levels at phase 3 (p=0.005). Among those with hypertension at phase 3, the platelet levels were 37.70 $x10^3/\mu L$ higher compared to non-hypertension (aβ=37.70; 95% CI:11.50,63.91). Based on the p-interaction test, the effect of systolic blood pressure on the changes in platelet levels by dengue fever phase in the crude (p<0.001) and adjusted association (p<0.001) were important. Thus, we selected the complex model with interaction by dengue fever phase for interpretation.

Figure 4.8 shows the association between hypertension and changes in platelet levels by dengue fever phases. We observed higher platelet levels among those with hypertension at phase 1 and phase 3 of dengue fever. In contrast, at phase 2 of dengue fever, the platelet levels were lower compared to phase 1 and phase 2 of dengue fever. No trend was observed between the association between hypertension and changes in platelet levels.

Table 4.35: Crude and adjusted association between hypertension and changes in platelet levels by dengue fever phase

Exposures	Platelet, x10^3/µL			
	Crude β (95% CI)	P-value	Adjusted* aβ (95% CI)	P-value
Hypertension				
All Phases				
No	1.00	Ref	1.00	Ref
Yes	-3.54 (-17.11,10.03)	0.609	0.53 (-13.54,14.60)	0.941
Phase 1				
No	1.00	Ref	1.00	Ref
Yes	11.27 (-9.46,32.01)	0.287	15.26 (-5.70,36.22)	0.154
Phase 2				
No	1.00	Ref	1.00	Ref
Yes	-19.35 (-34.71,-3.99)	0.014	-15.34 (-31.17,0.50)	0.058
Phase 3				
No	1.00	Ref	1.00	Ref
Yes	34.87 (8.81,60.93)	0.009	37.70 (11.50,63.91)	0.005
P-Interaction[2]	-	<0.001	-	<0.001

[*] Adjusted for age, gender, ethnicity, and BMI categories.
[1] P-Interaction tests between the model without and with interaction by dengue fever phase.
[2] Dengue fever was categorised into Phase 1 (Fever day 0-3), Phase 2 (Fever day 4-6), and Phase 3 (Fever day ≥7).

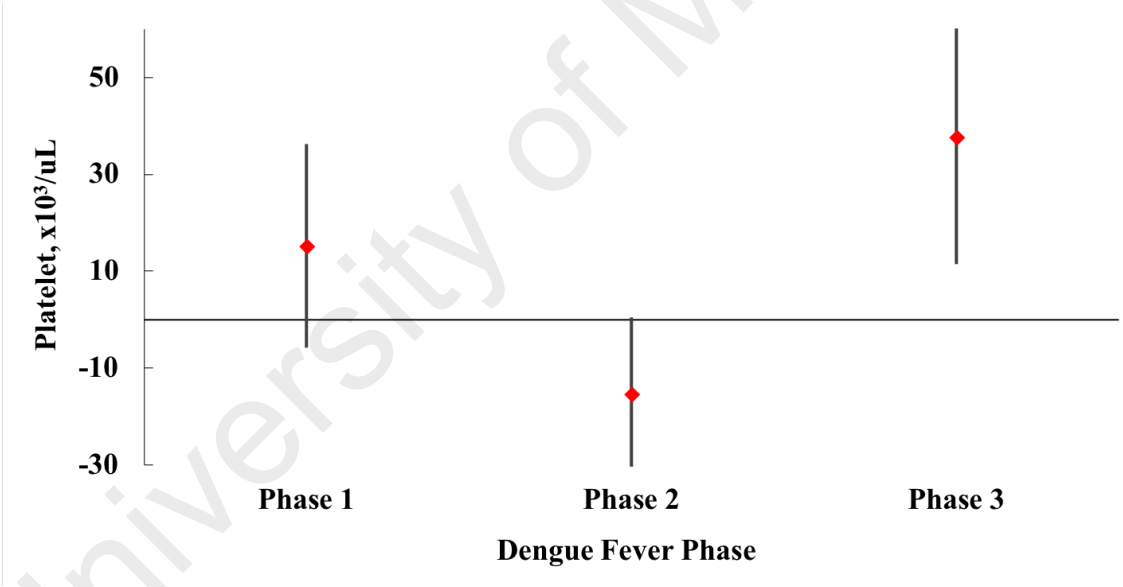

Figure 4.8: Association between hypertension and changes in platelet levels by dengue fever phase

4.5.6 Associations between Hypertension and Hematocrit

Table 4.36 describes the associations between systolic blood pressure, diastolic blood pressure, hypertension, and hematocrit levels in quartiles at baseline. According to systolic blood pressure, there was no significant association for the mean systolic blood pressure (p=0.104). Based on the diastolic blood pressure, we found a significant association in the mean diastolic blood pressure between quartiles of hematocrit levels (p=0.007). There was a higher mean diastolic blood pressure among those in the fourth quartile with a mean of 77.3 ± 11.09 mmHg, followed by 75.7 ± 10.32 mmHg in the third quartile, 74.6 ± 9.67 mmHg in the second quartile, and 71.5 ± 9.70 mmHg in the first quartile. According to the hypertension status, there is no significant association between quartiles of hematocrit levels (p=0.576). Moreover, we observed a higher percentage of participants with hypertension in the fourth quartile (15.5%) compared to the percentage in the third (12.9%), first (10.1%), and second (9.2%) quartiles.

Table 4.36: Association between hypertension and hematocrit levels in quartiles at baseline

Exposures	Hematocrit				P-value
	Q1 (n = 109)	Q2 (n = 76)	Q3 (n = 93)	Q4 (n = 84)	
	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	n (%) mean ± SD	
Systolic BP[a], mmHg	114.7 ± 15.88	115.7 ± 13.38	118.1 ± 15.59	119.3 ± 13.48	0.140
Diastolic BP[a], mmHg	71.5 ± 9.70	74.6 ± 9.67	75.7 ± 10.32	77.3 ± 11.09	0.007
Hypertension					0.576
No	98 (89.9%)	69 (90.8%)	81 (87.1%)	71 (84.5%)	
Yes	11 (10.1%)	7 (9.2%)	12 (12.9%)	13 (15.5%)	

[a] BP: Blood Pressure.

Table 4.37 describes the crude and adjusted association between hypertension and hematocrit levels at baseline. According to the crude association, there was no significant association between hypertension and hematocrit levels at baseline (p=0.110). Similarly, no significant adjusted association was found between hypertension and hematocrit levels at baseline (p=0.087).

Table 4.37: Crude and adjusted association between hypertension and hematocrit levels at baseline

Exposures	Hematocrit, %			
	Crude	P-value	Adjusted*	P-value
	β (95% CI)		aβ (95% CI)	
Hypertension	1.27 (-0.29,2.83)	0.110	1.22 (-0.18,2.61)	0.087

* Adjusted for age, gender, ethnicity, and BMI categories.

Table 4.38 describes the crude association between hypertension and changes in hematocrit levels. According to the hypertension status, we found no significant crude (p=0.149) and adjusted (p=0.103) association between hypertension and changes in hematocrit levels controlled for dengue fever phase. In the stratified analysis, we found no significant crude association between hypertension and changes in hematocrit levels at phase 1 (p=0.265), phase 2 (p=0.092), and phase 3 (p=0,942) of dengue fever. When adjusted for age, gender, ethnicity, and BMI categories, we found no significant association between hypertension and changes in hematocrit levels at phase 1 (p=0.230), phase 2 (p=0.057), and phase 3 (p=0.934) of dengue fever. Based on the p-interaction test, the effect of hypertension on the changes in hematocrit levels by dengue fever phase in the crude (p=0.255) and adjusted association (p=0.253) were not important. Thus, we selected the simple model without interaction by dengue fever phase for interpretation.

Figure 4.9 shows the association between hypertension and changes in hematocrit levels by dengue fever phases. The hematocrit levels were higher among those with hypertension at phase 1 dengue fever and increases at phase 2 of dengue fever. At phase 3 of dengue fever, the hematocrit level decreases to a lower level compared to phase 1 and phase 2 of dengue fever. There was no trend observed between hypertension and changes in hematocrit levels by dengue fever phase.

Table 4.38: Crude and adjusted association between hypertension and changes in hematocrit levels by dengue fever phase

Exposures	Hematocrit, %			
	Crude β (95% CI)	P-value	Adjusted* aβ (95% CI)	P-value
Hypertension				
All Phases				
No	1.00	Ref	1.00	Ref
Yes	1.09 (-0.39,2.57)	0.149	1.06 (-0.21,2.34)	0.103
Phase 1				
No	1.00	Ref	1.00	Ref
Yes	1.02 (-0.77,2.80)	0.265	0.98 (-0.62,2.58)	0.230
Phase 2				
No	1.00	Ref	1.00	Ref
Yes	1.34 (-0.22,2.89)	0.092	1.32 (-0.04,2.68)	0.057
Phase 3				
No	1.00	Ref	1.00	Ref
Yes	0.07 (-1.92,2.07)	0.942	0.08 (-1.74,1.90)	0.934
P-Interaction[2]	-	0.255	-	0.253

[*] Adjusted for age, gender, ethnicity, and BMI categories.
[1] P-Interaction tests between the model without and with interaction by dengue fever phase.
[2] Dengue fever was categorised into Phase 1 (Fever day 0-3), Phase 2 (Fever day 4-6), and Phase 3 (Fever day ≥7).

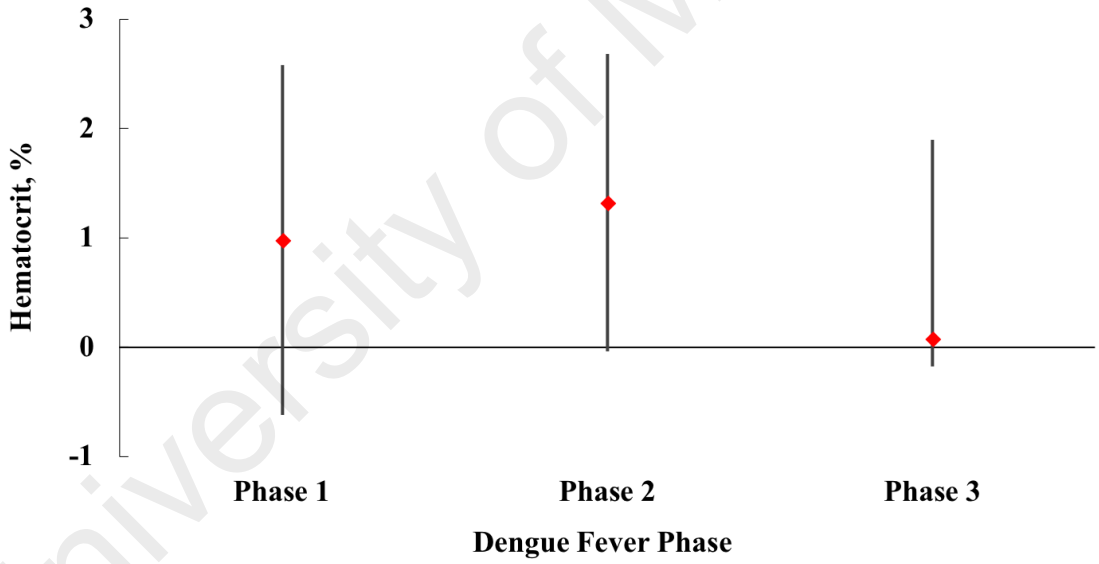

Figure 4.9: Association between hypertension and changes in hematocrit levels by dengue fever phase

4.6 Additional Analysis - Mediation and Interaction Analysis

We perform mediation analysis to identify mediating effects of diabetes on the association between BMI, dengue severity, platelet, and hematocrit. According to Table 4.39, diabetes act as a total mediator for the association between BMI and hematocrit with 66.67% of total effect mediated. Thus, we adjusted the association between BMI and hematocrit levels for diabetes.

Table 4.39: Mediation analysis of diabetes between BMI, dengue severity, platelet, and hematocrit

Variable	Indirect Effect, 95% CI	Total Effect, 95% CI	Proportion of Total Effect Mediated, %
BMI and Dengue Severity			
Diabetes	0.001 (-0.0003,0.001)	0.004 (-0.002,0.010)	12.73%
P-value	0.227	0.185	
BMI and Platelet			
Diabetes	-0.007 (-0.108,0.095)	0.001 (-0.733, 0.736)	-700%
P-value	0.898	0.997	
BMI and Hematocrit			
Diabetes	-0.010 (-0.018,-0.002)	-0.015 (-0.072,0.043)	66.67%
P-value	0.018	0.614	

In the interaction analysis, we tested the effect of secondary dengue infection on the association between BMI, RBS, Diabetes at Phase 1 and Dengue Severity. Based on the analysis results in Table 4.40 below, we found that secondary infection was not an interaction between BMI, RBS, Diabetes, and Dengue Severity.

Table 4.40: Interaction analysis of secondary infection between BMI, RBS, diabetes, and dengue severity

Association	Adjusted* aOR (95% CI)	P-value
BMI at All Phase and Dengue Severity		
Without Interaction with Secondary Infection	1.15 (1.03,1.28)	0.016
With Interaction with Secondary Infection	1.11 (1.00,1.21)	0.055
P-Interaction[1]	-	0.927
RBS among Non-Diabetes and Dengue Severity		
Without Interaction with Secondary Infection	1.57 (1.13,2.18)	0.007
With Interaction with Secondary Infection	1.52 (1.06, 2.17)	0.024
P-Interaction[1]	-	0.6211
Diabetes at Phase 1 and Dengue Severity		
Without Interaction with Secondary Infection	20.89 (1.08,403.96)	0.044
With Interaction with Secondary Infection	62.45 (2.57,1517.96)	0.011
P-Interaction[1]		0.5818

1 P-Interaction tested the simple model without interaction by secondary infection and model with interaction by secondary infection

4.7 Summary of Results

4.7.1 Obesity and dengue severity

In determining the association between obesity and dengue severity, the prevalence of obesity among dengue cases aged 15 years and above for Malaysia was 17.4%. The study found that dengue patients with higher BMI have higher odds of dengue severity. Those with a higher waist circumference and abdominal obesity have higher platelet levels at phase 3 of dengue fever. Moreover, dengue patients with higher BMI have higher hematocrit levels in the prevalence and incidence association. Obese dengue patients have higher hematocrit levels compared to normal weight dengue patients in the prevalence and incidence association. In conclusion, there was a significant association between BMI, obesity and dengue severity.

4.7.2 Diabetes and dengue severity

In determining the association between diabetes and dengue severity, the prevalence of diabetes among dengue cases aged 15 years and above for Malaysia was 16.6%. The research findings demonstrated that the odds of dengue severity increase with increasing random blood sugar in the incidence association. There were higher odds of dengue severity at phase 1 of dengue fever among those with diabetes. We also found that non-diabetes patient has higher incidence of dengue severity with increasing RBS value. The platelet level decreases with higher RBS level in the prevalence association and phase 2 of dengue fever in the incidence association, which reflects a more severe form of dengue infection. Also, among non-diabetes, the platelet levels were lower compared to diabetes at phase 2 of dengue fever.

At phase 3 of dengue fever, diabetic patients have higher platelet levels compared to non-diabetes. Thus, there is a significant association between higher random blood sugar, diabetes and dengue severity.

4.7.3 Hypertension and dengue severity

In determining the association between hypertension and dengue severity, the prevalence of diabetes among dengue cases aged 15 years and above for Malaysia was 11.9%. The findings show no association between hypertension and dengue severity. However, hypertensive dengue patients have higher platelet levels compared to non-hypertensive patients at phase 3 of dengue fever. Thus, there is no significant association between blood pressure, hypertension, and dengue severity.

CHAPTER 5: DISCUSSION

5.1 Introduction

This study determines the association of obesity, diabetes, and hypertension with the development of dengue severity among confirmed dengue patients aged 15 years and above in the district of Melaka Tengah, Seremban, Hulu Langat, Petaling, Klang, and Gombak. We performed a cross-sectional and study to estimate on the proportion of dengue severity among dengue patients with obesity, diabetes, and hypertension aged 15 years and above. This study obtained the proportion of severe dengue infection that was attributable to confirmed dengue patients with obesity, diabetes, and hypertension.

A national and large community study to determine the proportion of severe dengue infection among all confirmed dengue cases would be more accurate. However, due to limited resources and time, it was quite impossible for us to perform such a significant scale study. The proportion of dengue patients with warning signs and severe dengue in this study were similar compared to other studies conducted in the South-East Asia region, which meant that this study was not under reporting nor overreporting the severe dengue infection as a major disease burden in Malaysia.

This is the first prospective study that investigates the association between obesity, diabetes, hypertension, and the development of dengue severity among individuals aged 15 years and above. The study provides new information on the association between obesity, diabetes, hypertension, and dengue. Additionally, this study highlighted the importance of obesity, diabetes, and hypertension in the progression of dengue infection.

With increasing prevalence of obesity, diabetes, and hypertension as well as expanding areas that were at high risk for dengue infection, these risk factors could play a role in increasing the burden and mortality related to dengue infection. Such evidence could help clinicians and front liners for better risk stratification and identification of high-risk dengue patients. The subsequent sections discuss the findings in more detail.

5.2 Dengue Infection

A total of 362 confirmed dengue patients aged 15 years and above had sought treatment at the 24 primary health care clinics in the district of Melaka Tengah, Seremban, Hulu Langat, Klang, Gombak, and Petaling from May 2016 to November 2017. The mean age of patients with dengue infection in this study was 35.8 ± 14.10 years old. Typically, dengue infection affects all age groups. However, this study found that the mean age of patients with dengue infection was higher compared the mean age reported in previous dengue study (32.2 ± 15.8 years) (Ahmad Nizal MG et al., 2010). Similarly, the mean age of dengue patients was higher than the reported mean age of dengue patients from the Malaysia National Dengue Registry in 2013 (30.0 ± 15.7 years old) (Liew et al., 2016). Thus, the higher mean age of dengue patients in this study was expected.

According to a systematic review in 2014 on the epidemiology of dengue disease in Malaysia from 2000 to 2012, Mohd-Zaki et al. concluded that there was a shift in the age predominance of dengue infection from children to adults age group (Mohd-Zaki et al., 2014). The changes in the age predominance were due to the demographic transition (Cummings et al., 2009). With lower birth and death rates, there was a decreased flow of new susceptible individuals into the population and increases the longevity of older immune individuals in the population (Cummings et al., 2009). With increases in the proportion of the older immune population, the likelihood that an infectious mosquito will feed on some older immune individual increases. Thus, it leads to a higher number of the older population infected with dengue infection. There were also more male dengue patients (51.4%) compared to female dengue patients (48.6%) with a ratio of 1.1 male to 1.0 female. The higher percentage of male dengue patients was similar to the reported percentage of male dengue patients in Malaysia ranging from 54.4% to 61.5% (Anker & Arima, 2011).

Two earlier studies on dengue also found that there was a significant predominance of male dengue patients in Malaysia (Liew et al., 2016; Mohd-Zaki et al., 2014). Furthermore, the higher percentage of male dengue patients was similar and consistent over several years across six culturally and economically diverse countries in Asia (Anker & Arima, 2011). Thus, regarding the gender distribution, we were confident that the higher distribution of male dengue patients in this study was not over-reported and could provide a valid inference of the study results to the general population.

According to ethnic group, the majority of dengue patients in this study were Malay, followed by Chinese, Indian, and the others ethnicity group. The distribution of dengue cases by ethnic group broadly reflects the ethnic distribution of Malaysia as a whole (World Health Organization: Western Pacific Region, 2012). 21.6% of dengue patients were seropositive at baseline. The seroprevalence of dengue infection among dengue patients was lower compared to other studies done in Malaysia (Muhammad Azami et al., 2011; Vinomarlini et al., 2011).

The lower seroprevalence recorded could be due to the exclusion of dengue patients with only IgG positive during the enrolment process. At baseline, 88.5% of patients with previous dengue infection were unaware of their previous infection. The higher percentage of those who were unaware of previous dengue infection indicates that there might be a higher number of asymptomatic dengue infection in the population.

Approximately three-quarters of patients with dengue infections were asymptomatic (Bhatt et al., 2013; Burke, Nisalak, Johnson, & Scott, 1988; Endy et al., 2011; Mammen et al., 2008). Asymptomatic dengue infection was, therefore, a significant part of the dengue burden.

Asymptomatic dengue infection might have a role in dengue transmission although there has been no clear data on viremia in these asymptomatic cases as well as the impact of asymptomatic infection on dengue transmission (Duong et al., 2015). The findings demonstrated that there might be a higher percentage of asymptomatic dengue infection in the population.

The mean days of fever at baseline was 3.9 ± 1.63 days. On average, the symptoms of dengue infection start to appear after the incubation period of 4 to 10 days. The most common presentation of dengue infection during the first 3 to 4 days was high fever (World Health Organization, 2017a). Typically, patients will seek medical treatment once they have the symptoms. Thus, the mean days of fever recorded in this study at baseline for this study was appropriate as it reflects the time when dengue patients seek medical treatment for the first time.

Our study reported a higher percentage of dengue with warning signs patients compared to the percentage of dengue hemorrhagic fever patients diagnosed using the WHO 1997 classification in earlier studies (Liew et al., 2016; Y. P. Lin et al., 2016; Mohd-Zaki et al., 2014; Pang et al., 2012). 51.1% of dengue patients were classified as dengue without warning signs, 48.1% as dengue with warning signs, and 0.8% as severe dengue. In contrast, the percentage of dengue with warning signs patients in this study were similar with the reported percentage of 45.5% to 62.2% in studies using the WHO 2009 dengue classification (Basuki et al., 2010; L. K. Lee et al., 2012; van de Weg et al., 2012). The differences in the distribution of dengue infection classification were due to the utilisation of the latest WHO 2009 classification in this study. The new WHO 2009 classification were useful in detecting severe dengue cases and allow precise detection of severe dengue patients (Basuki et al., 2010; Narvaez et al., 2011). Thus, the higher percentage of dengue with warning signs patients in our study was expected and does not overreport the occurrence of severe dengue infection in the population.

Comparing the general characteristics of dengue patients between the non-severe and severe dengue infection group, there is no significant difference in age, gender, ethnicity, previous dengue infection, awareness of previous dengue infection, and days of fever.

5.3 Obesity and Dengue Severity

The prevalence of obesity among dengue patients was 17.4%. According to the previous study among pediatric dengue patients, the prevalence of obesity was lower (14.0%) than the prevalence recorded in this study (Maria Mahdalena Tri Widiyati et al., 2013). The Malaysia National Health and Morbidity Survey (NHMS) in 2015 reported that the prevalence of obesity in the Malaysian population was 30.6% (National Institute of Health Malaysia, 2016). The reported NHMS 2015 prevalence was higher compared to the prevalence of obesity among dengue patients in this study. Due to the focus on the association between obesity and dengue severity, we only reported the prevalence of obesity among dengue patients aged 15 years and above. Currently, no available primary study reported on the prevalence and association between obesity and dengue severity in adults. Thus, our finding serves as a primary result of the prevalence of obesity among dengue patients in Malaysia.

There was a significant difference in the mean age of dengue patients between different categories of BMI. We found a higher mean age of 39.0 ± 12.59 years among overweight patients, and 38.4 ± 13.36 years among obese patients. There was a lower mean age of 33.7 ± 14.13 years found among those with normal weight. Among those with underweight, the mean age was even lower at 29.6 ± 17.32 years. The differences in the mean age of dengue patients in this study are expected. A similar trend was reported in previous studies where age increased significantly with increasing prevalence of overweight or obesity (National Institute of Health Malaysia, 2016; Villareal et al., 2005).

There were no significant differences in the distribution of gender, ethnicity, previous dengue infection, and days of fever between different BMI categories. Similarly, there were no significant differences in the presentation of warning signs and laboratory values between different BMI categories.

Comparing between the non-severe and severe dengue infection group, we found a significantly higher BMI mean of 25.8 ± 6.42 years in the severe dengue infection group compared to the BMI mean of 24.6 ± 4.83 years in the non-severe group. We also found a higher percentage of obese patients (20.3%) among those in the severe group compared to the non-severe group (14.6%). In contrast, there was no significant association between different categories of BMI as well as waist circumference and abdominal obesity status. Our findings on the distribution of BMI categories were similar with the findings of earlier studies where higher percentage of obese patients was found among the severe dengue infection group compared to non-severe dengue infection group (Basuki, 2003; Bongsebandhu-Phubhakdi et al., 2008; Chuansumrit et al., 2000; Maria Mahdalena Tri Widiyati et al., 2013).

This study determines the prevalence and incidence association between obesity and dengue severity. It found a significant association between BMI and the prevalence of dengue severity as well as with the incidence of dengue severity. In the prevalence association, with every 1 kg/m^2 increase in BMI value, the odds of developing severe dengue infection increases by 4% with regard to age, gender, and ethnicity remain constant.

Similarly, in the incidence association, with every 1 kg/m^2 increase in BMI value, there were 15% higher odds of developing dengue severity with regard to age, gender, ethnicity, and dengue fever phase remains constant. In contrast, there was no significant association in the prevalence and incidence associations of underweight, overweight, obesity, and dengue severity compared to normal weight.

Also, there was no significant association of waist circumference with the prevalence and incidence associations with dengue severity. Based on the effect of BMI on dengue severity, at phase 1 of dengue fever, the higher the BMI value, the higher the odds of dengue severity.

This combination of increasing odds of dengue severity was supported with the reduction in platelet as well as increased in hematocrit, which were signs of severe dengue. The findings are similar with previous studies on the association between obesity and dengue severity among pediatric patients (Bongsebandhu-Phubhakdi et al., 2008; Campbell et al., 2015; Maria Mahdalena Tri Widiyati et al., 2013). Similarly, in 2018, Zulkipli et al. in his systematic review and meta-analysis concluded that there were 38% higher odds of developing severe dengue infection among obese pediatric dengue patients (Zulkipli et al., 2018).

Hypothetically, among those with obesity, the deposition of white adipose tissue was higher compared to non-obese individuals (Bosch et al., 2002; Calabro et al., 2005; Juffrie et al., 2001). According to Jorge et al., an obese person with higher BMI shows a higher IL-6 and TNF-α expression in the WAT (Jorge et al., 2016). The higher expression of IL-6, IL-8, TNF-α leads to increased capillary permeability in obese individuals, which promotes plasma leakage. Thus, our findings were consistent with the underlying hypothesis on the development of plasma leakage in patients with higher BMI.

Lower platelet counts in dengue fever may lead to a life-threatening condition known as dengue hemorrhagic fever. Commonly, spontaneous bleeding resulted from lower platelet counts characterised the development dengue hemorrhagic fever and its severe form, dengue shock syndrome. According to the characteristics of BMI, BMI categories, waist circumference, and abdominal obesity between different quartiles of platelets, we only found a significant difference by waist circumference. In the incidence association, there was a significant association between waist circumference, abdominal obesity, and changes in platelet levels at phase 3 of dengue fever.

Based on waist circumference, with every 1 cm increase in waist circumference, the platelet level increases by 0.84 $x10^3/\mu L$ at phase 3 of dengue fever with regard to age, gender, and ethnicity remain constant. Among those with abdominal obesity, the platelet levels were 21.08 $x10^3/\mu L$ higher compared to those without abdominal obesity at phase 3 of dengue fever with regard to age, gender, and ethnicity remain constant.

Rising hematocrit levels are a marker of the critical phase of dengue infection. This study found a significant association between BMI and hematocrit levels. With every 1 kg/m^2 increase in BMI, the hematocrit level increases by 0.09% with regard to age, gender, ethnicity, and diabetes status remain constant. Moreover, there was a significant association between obese and hematocrit levels. Among obese dengue patients, the hematocrit levels were 1.77% higher compared to non-obese dengue patients with regard to age, gender, ethnicity, and diabetes status remain constant. No significant association was found found between underweight, overweight, waist circumference, abdominal obesity, and hematocrit levels.

In the incidence association, we found a significant association between BMI and changes in hematocrit levels where with every 1 kg/m^2 increase in BMI, the hematocrit level increases by 0.07%. Similarly, there was a significant association between obese and changes in hematocrit levels with regard to age, gender, ethnicity, diabetes status, and dengue fever phase remains constant. Among obese dengue patients, the hematocrit levels were 1.39% higher compared to non-obese dengue patients with regard to age, gender, ethnicity, diabetes status, and dengue fever phase remains constant. In contrast, we found no significant associations between underweight, overweight, waist circumference, abdominal obesity, and changes in hematocrit levels. Also, there are no significant effects of underweight, overweight, obese, waist circumference, and abdominal obesity with the changes in hematocrit levels between different phases of dengue infection.

In dengue infection, an increase in hematocrits levels higher than 20% from the baseline hematocrit levels was considered a sign of hemoconcentration and precedes shock. Hemoconcentration results from an increased in the capillaries permeability, which causes the plasma to leak out and increases the concentration of blood.

Our findings indicated that there was a higher level of hematocrit among obese dengue patient. Based on our study findings, we can link now the hypothesis where higher BMI leads to higher risk of dengue severity. An increased capillary permeability among individuals with higher BMI was due to the increased production of immune mediators, the interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor alpha (TNF-α) resulted from the higher deposition of white adipose tissue compared to dengue patients with lower BMI. The predispose plasma leakage among those with higher BMI may have rapidly worsened the hemoconcentration caused by dengue infection that leads to dengue severity. Moreover, a higher level of hematocrits among dengue patients with higher BMI paired with the reduction of platelets in dengue infection increases the severity of dengue infection.

Currently, no study has been conducted on the association between obesity and dengue severity in adults. To the best of our knowledge, this is the first study to demonstrate the association between obesity and dengue severity as well as its association with changes in platelet and hematocrit levels.

5.4 Diabetes and Dengue Severity

Regarding the association between diabetes and dengue severity, the prevalence of diabetes among dengue patients was 16.6%. The prevalence of diabetes in this study was higher compared to the prevalence of diabetes (6.0% to 6.9%) among dengue hemorrhagic fever patients reported from a study in Singapore (Pang et al., 2012). The differences in the prevalence of diabetes between studies might be due to the differences in the study population. Pang J et al. included more Chinese patients compared to other ethnic groups. Furthermore, the prevalence of diabetes among Singaporean Chinese was at 9.7% compared to the overall prevalence of diabetes in Malaysia at 17.9% (Ministry of Health Singapore, 2014; National Institute of Health Malaysia, 2016). With the high prevalence of diabetes in the population, a large number of patients with dengue infection were likely to have concomitant diabetes. Thus, we expect that the prevalence of diabetes in our study to be higher and does not over-report the prevalence of diabetes in dengue infection.

There were significant differences in the mean age and previous dengue patients among the non-diabetes and diabetes group. A higher mean age of diabetes patients was found among those with diabetes (49.0 ± 13.62 years) compared to non-diabetes (33.2 ± 12.67 years) (Twig et al., 2013). The higher mean age of diabetes patients was similar to the reported value from Malaysia National Health and Morbidity Survey (National Institute of Health Malaysia, 2016). We also found a higher percentage of patients with previous dengue infection among those with diabetes (35.0%) compared to non-diabetes (18.9%). According to the clinical characteristics, we found significant differences in the presence of lethargic and levels of white blood cells. In earlier studies on the association between WBC and diabetes, a higher WBC was found among those with diabetes compared to non-diabetes (Twig et al., 2013).

Comparing the characteristics of RBS and diabetes, there were no significant differences found between the non-severe and severe dengue infection. Similarly, there were no significant differences found between RBS, diabetes, and study visits. Based on the prevalence association, we found no significant association of RBS and diabetes with the prevalence of dengue severity adjusted for age, gender, ethnicity, and BMI categories.

In the incidence association, there was a significant association between RBS among non-diabetes and the incidence of dengue severity controlled by dengue fever phase. With every 1 mmol/L increase in the RBS value, there were 57% higher odds of developing severe dengue infection with regard to age, gender, ethnicity, BMI categories, and dengue fever phase remains constant. Similarly, at phase 1 of dengue fever, diabetes increases the odds of dengue severity by 19.9 times compares to non-diabetic patients with regard to age, gender, ethnicity, and BMI categories remain constant. Additionally, we also found that the odds of dengue severity were higher at phase 1 of dengue fever and lower at phase 3 of dengue fever.

We did not find any significant difference in the characteristics of RBS and diabetes between different quartiles of platelets. In the prevalence association, we found a significant association between RBS and platelet levels. With every 1 mmol/L increase in RBS value, the average value of platelets decreases by 2.49 $x10^3/\mu L$ with regards to age, gender, ethnicity, BMI categories, and diabetes status remain constant. There was no significant association found between diabetes and platelet levels. Furthermore, in the incidence association, we found a significant association between RBS and changes in platelet levels at phase 2 of dengue fever. With every 1 mmol/L increase in RBS value at phase 2 of dengue fever, the average value of platelets decreases by 2.74 $x10^3/\mu L$ with regards to age, gender, ethnicity, BMI categories, and diabetes status remain constant. Similarly, we also found that among non-diabetic patients, the platelet levels were 4.53 $x10^3/\mu L$ lower with regards to age, gender, ethnicity and BMI categories remain constant.

In contrast, among those with diabetes, the platelet levels were 44.29 $x10^3/\mu L$ higher at phase 3 of dengue fever compared to non-diabetes. In the prevalence and incidence association of RBS and diabetes with hematocrit levels, no significant association was found.

Diabetic patients were known to have higher blood glucose levels compared to non-diabetic patients. Our findings are consistent with the results of a systematic review and meta-analysis published in 2013 where the presence of a severe clinical presentation of dengue was positively associated with the presence of diabetes (Huy et al., 2013). Furthermore, on the association between diabetes and platelet, our findings were also similar with the previous study in 2015 where lower platelet levels were found among those with diabetes and those with higher blood glucose value (C. Y. Chen et al., 2015). However, we did not find any evidence on the effect of platelets at phase 3 of dengue fever.

Platelets in diabetic individuals adhere to vascular endothelium and aggregate more readily than those in healthy people. Prostacyclin (PGI_2) and nitric oxide (NO) generated by vascular endothelium exerted the main defect in the platelet function that leads to loss of sensitivity to the regular restraints (Akai, Naka, Okuda, Takemura, & Fujii, 1983; Chin, Azhar, & Hoffman, 1992; Modesti et al., 1991). Furthermore, insulin is a natural antagonist of platelet hyperactivity. It sensitises the platelet to PGI_2 and enhances the endothelial generation of PGI_2 and NO (Akai et al., 1983).

Thus, the defects in insulin action in diabetes create a milieu of disordered platelet activity conducive to macrovascular and microvascular events that may lead to a more severe dengue presentation. With the advantage of being a study, our study provides a stronger impact as well as a valid causal relationship on the association between diabetes and dengue severity.

5.5 Hypertension and Dengue Severity

The prevalence of hypertension among dengue patients in this study was 11.9%. This is similar to the finding from a previous case-control study in Singapore where the authors found that the prevalence of hypertension among dengue cases was 11.2% (Pang et al., 2012). Comparing with the prevalence of hypertension in the Malaysian population in 2015, our prevalence of hypertension was lower compared to the 30.3% prevalence of hypertension in Malaysia (National Institute of Health Malaysia, 2016). As the Malaysian population prevalence includes both dengue and non-dengue patients, we based our prevalence of hypertension among dengue patients with the prevalence in the previous study by Pang et al. Thus, we were confident that our finding does not overreport the prevalence of hypertension among dengue-infected patients and provide a similar proportion of patients with hypertension in dengue.

There was a higher mean age of dengue patients with hypertension (45.3 ± 13.84 years) compared to non-hypertension (34.5 ± 13.66 years). Based on the NHMS 2015 report, there was a higher prevalence of hypertension among older age group compared to the lower age group (National Institute of Health Malaysia, 2016). Thus, we expect a higher mean age of participant with hypertension in this study. There was no difference in regard to gender, ethnicity, previous dengue infection, and days of fever. Similarly, there was no significant difference between hypertension and non-hypertension groups in regard to the presence of warning signs and laboratory values.

The study found no significant prevalence and incidence association between hypertension and dengue severity. This is supported by a previous study where the authors found that there was no significant adjusted association between hypertension and dengue hemorrhagic fever (Pang et al., 2012). Furthermore, we did not find any significant prevalence association between hypertension and platelet levels.

In contrast, there is a significant association between hypertension and the changes in platelet levels at phase 3 of dengue fever. Among those with hypertension at phase 3 of dengue fever, the platelet levels were 37.70 $x10^3/\mu L$ higher compared to non-hypertension patients with regard to age, gender, ethnicity, BMI categories, and diabetes status remains constant.

Studies reported that an individual with hypertension has higher hematocrit level compared to non-hypertension (Letcher, Chien, Pickering, & Laragh, 1983; Safar, Weiss, Levenson, London, & Milliez, 1973; Tibblin, Bergentz, Bjure, & Wilhelmsen, 1966). Hypertensive patients were also reported to have higher mean platelet volumes (Gang, Yanyan, Zhongwei, & Juan, 2017). Previous studies found a positive correlation between systolic blood pressure, diastolic blood pressure, and hematocrit levels (Cirillo, Laurenzi, Trevisan, & Stamler, 1992). Based on the findings, hypertension does not affect the severity of dengue infection.

5.6 Limitations and Strengths

There were several limitations during data collection in this study. First, we enrolled dengue patients from the central region of Peninsular Malaysia due to limited time and budget. The extrapolation of these association to other regions is unknown. However, there has been no reported differences in characteristics of dengue patients between geographical regions in Peninsular Malaysia.

Second, the analysis did not model the joint effects of diabetes and hypertension on dengue severity. To address this limitation, we performed mediation analysis to identify mediators between the association between exposures and dengue severity. We found that diabetes was a total mediator for the association between BMI and hematocrit levels. Thus, we applied an adjustment that includes diabetes to control for the mediator effect of diabetes on the association between BMI and hematocrit level.

Third, the study has a higher percentage of severe percentage of severe dengue cases at baseline. This might be due to the usage of the WHO 2009 dengue classification. Usage of the WHO 2009 was known to detect more severe patients compared to the old WHO 1997 classification. Thus, the higher percentage of severe dengue infection was expected.

Fourth, this study did not differentiate between type-1 and type-2 diabetes. Thus, we could not ascertain the effect of different types of diabetes on the dengue severity. However, based on the hypothesized pathway, the effect of type-1 and type-2 diabetes on dengue severity should be similar. Furthermore, we acknowledge that we did not capture how well was the glycemic control of participants with diabetes. Among those with history / record of previous diagnosis with diabetes, we still perform the HbA1c at baseline. Thus, based on the HbA1c among those with history of diabetes at baseline, the control of diabetes can be assessed. We did additional exploratory analysis and found that among those with diabetes, only 15.2% had good glycemic control and 84.8% had poor

glycemic control. When tested with dengue severity, no significant association was found on both good and poor glycemic control diabetic patient. Thus, we did not include the results of the analysis as the determination of diabetic control was merely based on HbA1c, of which does not reflect the daily sugar control but more to previous 3-month sugar control.

Fifth, we acknowledge that our study did not account the clustering between districts. This is because we are interested in the association and not in point estimates. By using conditional analysis of random intercept modelling, it should correct the variance estimation for association at individual level. Furthermore, we did not perform stratification by primary health care clinics. The stratification of results by individual primary health care clinics will results in smaller sampling units that may have result in poorer bias-variance trade-off.

Sixth, we acknowledge that the sample size calculation in this study was perform priory based on a closed cohort design instead of longitudinal design. The process of sample size calculation using longitudinal modelling is based on simulation, of which will involve more inputs and a complex statistical calculation, can make sample size selection, a critical step in designing a successful study, more difficult.

Seventh, in regard to the sub-types of dengue infection, we acknowledge that there was no data on the sub-type of dengue infection. We could not ascertain the sub-types of dengue of each participant due to the nature of the dengue rapid combo test that only provide results based on the Ns1, IgG, and IgM only.

This was the first study on the association between obesity, diabetes, hypertension, and dengue severity among dengue patients aged 15 years and above in Malaysia. Previous studies on obesity and dengue severity only focused on the association between obesity and dengue severity among pediatric patients. Also, most of the previous

studies used the old WHO 1997 classification, which was not specific in detecting more severe dengue cases.

This study design combined with the use of the latest WHO 2009 classification provided more strength in the associations found between obesity, diabetes, hypertension, and dengue severity. Moreover, the findings provided the first insights into the association between obesity, diabetes, hypertension, and dengue severity among individuals aged 15 years and above.

Also, the proportion of dengue with warning signs and severe dengue patients was similar to other studies conducted in the South-East Asia region, which meant that this study was not underreporting nor overreporting the severe dengue infection cases as the disease burden in Malaysia. Moreover, this study reported the first recorded prevalence of obesity, diabetes, and hypertension among dengue infection in Malaysia. Previously, the prevalence of the obesity, diabetes, and hypertension among dengue patients in Malaysia had not been documented. We can generalise the findings to all Malaysia as well as other countries at risk of dengue infection due to the similar characteristic of dengue patients and the representativeness of the study population.

Also, this was the first study to identify the risk factor for dengue severity and the first to link the association between non-communicable disease and communicable disease. With the increasing prevalence of obesity, diabetes, and hypertension as well as expanding areas that were at high risk for dengue infection, these risk factors could play a role in increasing the burden and mortality related to dengue infection. This evidence could assist clinicians and front liners for better risk stratification and identification of high-risk dengue patients.

5.8 Recommendations

Obesity, diabetes, hypertension, and dengue have reached epidemic dimensions and pose a common threat to the more significant proportion of populations in countries with high risk of dengue transmission. Dengue is no longer a disease primarily affecting children. The shift in age predominance warrants further research among the adult population. Therefore, the influence of non-communicable diseases that includes obesity, diabetes, and hypertension, which are increasingly prevalent in adults, on the clinical presentation of a dengue episode is a public health priority.

This study documented the first reported prevalence of obesity, diabetes, and hypertension among dengue infection. Similarly, our findings provide strong evidence on the association between risk factor of dengue severity. However, we would still suggest for future studies with larger multi-national scale involving hospital-based dengue patients to understand better the pathophysiology that leads to the development of severe dengue infection among those with obesity, diabetes, and hypertension.

Furthermore, using these findings, we can educate the public on the relationship between non-communicable disease and dengue infection. These would lead to a better understanding on dengue infection as well as increasing awareness on the progression of dengue infection among those with obesity, diabetes mellitus, and hypertension. This study strongly recommended incorporating obesity, diabetes, and hypertension as the risk factors for severe dengue infection. Early identification of these risk factors among dengue patients would allow better risk stratification, closer monitoring, and better management during the critical phase of dengue infection. Furthermore, future studies could explore the possibility of using RBS and diastolic blood pressure as simple and readily available clinical tools for risk stratification dengue cases.

CHAPTER 6: CONCLUSION

This study highlighted the importance and association of obesity, diabetes, and hypertension with dengue severity. With increasing prevalence of obesity, diabetes, and hypertension individual as well as expanding areas that were at high risk for dengue infection, these risk factors could play a role in increasing the burden and mortality related to dengue infection.

Overall, there were significant associations of BMI, obesity, high random blood glucose, and diabetes with dengue severity. With the available evidence, clinicians can better risk stratify dengue patients and identify high-risk dengue patients for closer monitoring as well as preventing the severe complication of dengue infection.

In the future, we hope that more studies will focus on the usage of random blood glucose as simple tools to identify high-risk dengue patients. The application of the new and cost-effective method to identify dengue severity will result in the reduction of the dengue burden due to its complication.

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