Prevalence of NS1 Dengue Antigen among Blood Donors In Perak and Penang

By

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DECLARATION

I hereby declare that I am the sole author of the thesis entitled "Prevalence of NS1 Dengue Antigen among Blood Donors in Perak and Penang. I declare that this thesis is being submitted to Universiti Sains Malaysia (USM) for the purpose of the award of Master of Science in Transfusion Science. This dissertation is the result of my own research under the supervision of Prof Dr. Narazah binti Mohd Yusoff, except as cited in the references. The dissertation has been accepted for the study performed and is not concurrently submitted in candidature of any other degree.

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LIST OF ABBREVIATIONS

ABBREVIATIONS

Ag	: Antigen
CO	: Cut off value
DENV	: Dengue virus
DHF	: Dengue hemorrhagic fever
EDTA	: Ethylene diamine tetra acetic acid
Ig	: Immunoglobulin
IMR	: Institute for Medical Research
Mab	: Murine monoclonal antibodies
NS	: Nonstructural protein
OD	: Optical density
PCR	: Polymerase Chain Reaction
RNA	: Ribonucleic acid
RT-PCR	: Real time – Polymerase Chain Reaction

TMA : Transcription – mediated amplification

WPKL : Wilayah Persekutuan Kuala Lumpur

LIST OF SYMBOLS

SYMBOLS

%	: Percentage
µm	: Micrometer
µl	: Microliter
g	: Gram
ml	: Milliliter
ºC	: Degree Celsius

ABSTRAK

Virus denggi boleh berjangkit melalui pemindahan darah daripada penderma darah yang telah dijangkiti kepada penerima. Di Malaysia, peningkatan kes jangkitan denggi boleh menyumbang kepada kewujudan penderma darah yang asimptomatik dan meningkatkan risiko pemindahan darah yang tercemar dengan virus ini. Oleh itu, objektif kajian ini adalah untuk mengkaji prevalen NS1 denggi antigen di kalangan penderma darah dan menentukan data demografi penderma darah di Pulau Pinang dan Perak. Seramai 374 penderma darah secara sukarela telah direkrut daripada dua kempen derma darah yang dianjurkan oleh Hospital Pulau Pinang, Pulau Pinang dan Hospital Raja Permaisuri Bainun, Ipoh, Perak dari April hingga Mei 2016. Daripada setiap kempen, 187 penderma darah secara sukarela telah mendaftar dan ujian saringan NS1 denggi antigen telah dijalankan terhadap semua sampel. Hasil kajian mendapati kesemua 374 sampel adalah negatif untuk NS1denggi antigen. Umur purata penderma darah adalah 36 tahun. Penderma darah daripada kumpulan darah O Rh positif adalah paling ramai (42%, 157 daripada jumlah penderma darah) dan diikuti oleh penderma darah daripada kumpulan darah B Rh positif (29.7%, 111 daripada jumlah penderma darah), A Rh positif (23.5%, 88 daripada jumlah penderma darah) dan seterusnya AB Rh positif (4.8%, 18 daripada jumlah penderma darah). Majoriti penderma darah ialah lelaki (64.7%), berbanding dengan penderma darah perempuan (35.3%). Majoriti penderma darah terdiri daripada kaum Cina (338 penderma darah), diikuti oleh kaum Melayu (27 penderma darah) dan India (9 penderma darah). Kesimpulannya, NS1 denggi antigen denggi tidak dikesan di kalangan penderma darah di dua pusat pengumpulan darah di Pulau Pinang dan Perak. Ini

menunjukkan bahawa tiada penderma darah yang menderma darah pada peringkat viraemia dan juga program saringan penderma yang sedia ada adalah efektif bagi memastikan jangkitan denggi menerusi pemindahan darah adalah minimum. Data demografi menunjukkan penderma darah dengan kumpulan darah yang paling biasa adalah O Rh positif, penderma lelaki lebih banyak daripada penderma wanita dan penderma darah kaum Cina adalah paling ramai. Data yang dikumpulkan dalam kajian ini mungkin berguna dalam memastikan aspek keselamatan dalam aktiviti transfusi serta dalam perancangan aktiviti derma darah, saringan penderma dan pengurusan inventori darah.

ABSTRACT

Dengue virus is one of the emerging agents that can be transmitted via blood transfusion from infected blood donors to recipients. In Malaysia, the increase in dengue infection may contribute to the existence of asymptomatic blood donors and increase the risk of blood transfusions contaminated with this virus. Thus, the objectives of this study were to investigate the prevalence of NS1 dengue antigen among blood donors and ascertain the demographic data of blood donors in Penang and Perak. A total of 374 voluntary blood donors were recruited from two blood donation campaigns organised by Hospital Pulau Pinang, Penang and Hospital Raja Permaisuri Bainun, Ipoh, Perak from April to May 2016. From each centre, 187 voluntary blood donors were enrolled, blood was collected and Dengue NS1 Ag was screened on all the samples. The study showed, all 374 samples were found to be negative for the Dengue NS1 antigen. The mean age for blood donors was 36 years. Blood group O Rh positive blood group was the commonest (42%, 157 of the total blood donors) followed by B Rh positive blood group (29.7 %, 111 of the total blood donors), A Rh positive (23.5%, 88 of the total blood donors) and AB Rh positive (4.8%, 18 of the total blood donors). There were more male blood donors (64.7%) compared to female blood donors (35.3%). Majority of these blood donors were Chinese (338 blood donors), followed by Malays (27 blood donors) and Indians (9 blood donors). In conclusion, there is no NS1 dengue antigen detected among blood donors in two blood collection centres in Penang and Perak. This indicates that none of the blood donor at the time of donation was in viraemic stage which translates that the established donor screening program is effective to ensure that dengue transmission through transfusion is

minimal. Demographic data of these blood donors showed that the most common blood group was O Rh positive, men donated more than women and Chinese blood donors were the commonest. Data collected in this study may be useful to ascertain the safety aspects of clinical transfusion and in the planning of blood donation activities, donor recruitment and blood inventory management.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Epidemiology of Dengue Virus

1.1.1 Global Health Problem

Dengue is a vector-borne disease that leads to health problems worldwide. Overall, 100 million dengue cases is estimated per year, with roughly 500 000 severe dengue patients requiring continuous treatment in hospital (Back *et al*, 2013). Dengue infection has been prevalent in more than 100 countries including Africa, the Americas, the eastern Mediterranean, Southeast Asia and the Western Pacific. In 2011, there is an increase in dengue cases documented in the United States and Brazil, with 1.1 million cases and 764 305 cases of severe dengue (Felix *et al*, 2012).

A widespread outbreak of dengue infection was recorded in Comoros, after time-to-time cases in the mild locales of North Africa and the Mediterranean in the 1990s. However, dengue has been common in tropical Africa, in spite of the fact that dengue hemorrhagic fever (DHF) is uncommon. Europe and Antarctica never come across with any dengue cases, but by the time, dengue infection has spread across boundaries. In Asia, DHF spread dramatically from the first epidemic country, Philippines, followed by Thailand, Malaysia, Singapore and Vietnam (D. Teo *et al*, 2009).

In Florida and Hawaii, the presence of the vector is the main cause of the epidemiology of dengue. All classified serotypes of dengue virus found in the prevalent areas in Puerto Rico, the Virgin Islands and American Samoa. In United State, most tourists who were back from the Caribbean, Latin America and Asia also experienced a sudden onset of fever caused by dengue (Vazquez J *et al*, 2013).

Dengue infection occurs in most periods in Queensland, although the virus is not commonly found in Australia. Virus transmission from infected humans to local population by primary vector causing epidemic of the disease. In the past 16 years, 3550 cases of infection have been reported, with approximately 200 cases a year in Australia. An average of 200 cases identified without clinical manifestations with 5 deaths due to dengue within this interval (Hilary J. Bambrick *et al*, 2009).

Treatment and deaths caused by dengue among children in Asia has exceeded malaria in the mid-70s (Gubler, 2002). In the most recent couple of years, climate changes have led to the extension of mosquito breeding sites. Gradual increase of people, the social process whereby cities grow and societies become urban, insufficient water administration, ineffective vector management and globalization, contribute to expand mosquito habitat. A rapid spread of dengue, very likely could extend and alter antigenic properties of the virus, including new serotypes and genetic make-up with a larger outbreaks possibility (D. Teo *et al*, 2009). Moreover, dengue transmitted rapidly, in the suburban and rural areas (Gubler, 2002).

1.1.2 Dengue In Malaysia

After an epidemic of dengue infections in 1973, many dengue cases are reported in Malaysia. In the early 1990s, approximately 5000 cases were documented each year. A dramatically increase pattern of dengue cases were reported in 1999 to 2007, with 44.3 cases to 181 cases per 100,000 population (Nizal *et* al, 2012). Unpublished ongoing systematic collection and data analysis, from the Ministry of Health Malaysia, showed an increase of 29,803 dengue cases in 2010 compared with the past 9 years (Cheah *et al*, 2014). The rate of dengue infection has increased continuously, within rapidly developing and population growth area (Nizal *et* al, 2012).

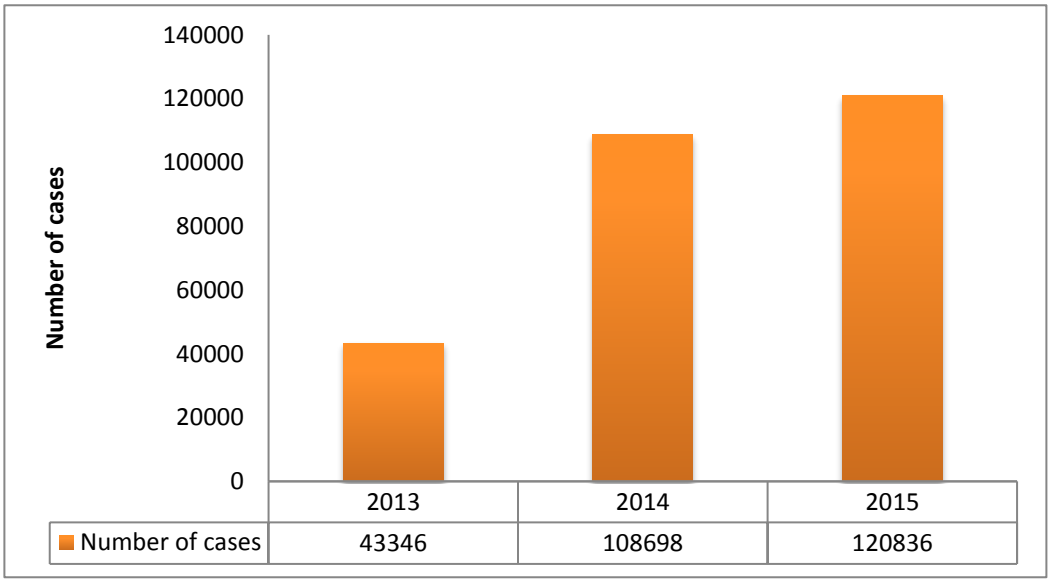

Figure 1: Dengue cases In Malaysia

An increase of dengue cases was recorded in Malaysia from 2013 to 2015. An increase of 65 352 cases were reported from 2013 to 2014, while from 2014 to 2015, an increase of 12 138 cases documented (Ministry of Health, 2015).

Continuous increase in dengue cases were documented in the country, since November 2015 until the first week of 2016. The number of cases increased by 826 cases (33%), with 2511 cases in the last week of 2015 to 3337 cases of dengue early 2016. In the past two years, dengue cases increased dramatically, but the number of cases reported for the first week of 2016 (3337 cases) was higher than the number of cases reported for the same period in 2015 (2633 cases). Overall, there was 12,138 cases (11.2%) were reported in 2015 compared to 108,698 cases in 2014 (Ministry of Health Malaysia, 2016).

Dengue cases were reported increased in all 13 states of Malaysia. In the first week of 2016, 4 deaths were accounted from Kuala Lumpur, Selangor, Putrajaya, Negeri Sembilan and Terengganu. The number of affected areas increased to 1,044 in the early weeks of 2016 compared to 907, the previous week. Specifically, seven states, namely Selangor (122), Johor (22), Perak (8), Pulau Pinang (4), Sabah (2), Negeri Sembilan (2) and Wilayah Persekutuan Kuala Lumpur (WPKL) & Putrajaya (1) showed an increase in hotspots area, with an expansion from 145 to 161 localities (Ministry of Health Malaysia, 2016).

Provincial studies around Malaysia demonstrated that the measure of raindrop, temperature, and moistness were all straightforwardly connected to epidemic of dengue infection. A reported case of dengue infection is highest in the first quarter of the year and between June and November. Even so, monthly systematic observation shows dengue infection can occur throughout the year. A few geological observation and a schematic description have exhibited, that the expanding urbanization in Malaysia was a main factor for the frequency of dengue infection nationwide (Zaki *et* al 2014).

1.2 Dengue Virus

1.2.1 Genome and Structural Features

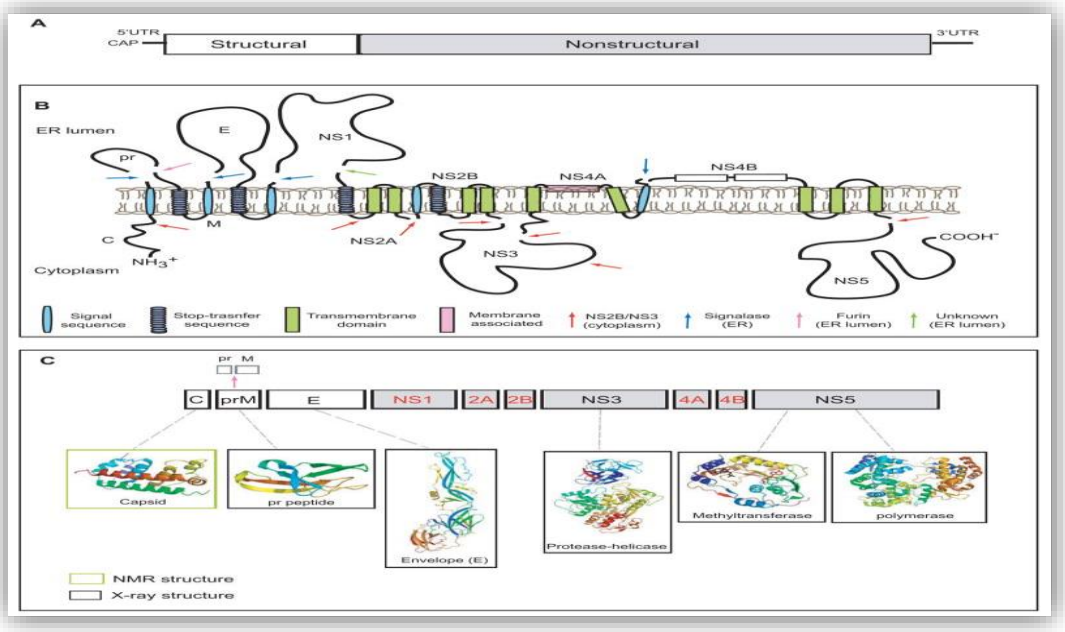

Figure 2: Dengue Virus Genome (Adapted from: Perera *et* al, 2008)

Dengue virus consists of single stranded RNA genomes and surrounded by an envelope. The genome corresponds to a range of nucleotides, which encode 3411 amino acids. The entire genome contains seven proteins, with three structural proteins, membrane protein, core and envelope protein, besides seven nonstructural proteins, including NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5 (Castro-Jorge LA *et al.* 2010). The viral structural proteins are part of a complete virus structure and not directly involved in viral replication. The nonstructural proteins present in cells infected with virus and not at the detectable level in a complete virus structure (Back *et al*, 2013).

The nonstructural protein 1 (NS1) consist of glycoprotein with two glycosylation sites. NS1 is produced in the endoplasmic reticulum and then processed in the Golgi apparatus (Winkler G. *et* al. 1989). The presence of NS1 protein in the process of viral replication is not known, but it gives the impression in terms of virus infection and the development of a disease or morbid condition (Libraty *et* al. 2002). NS1 protein also produced by cells infected by the virus, known as NS1 secreted from infected cells (sNS1). sNS1 plays a role in structure and function of the immune system to produce antibodies. Production of antibodies induces changes in endothelial cell function caused by the reaction between the host protein and endothelial cells (Back *et al*, 2013).

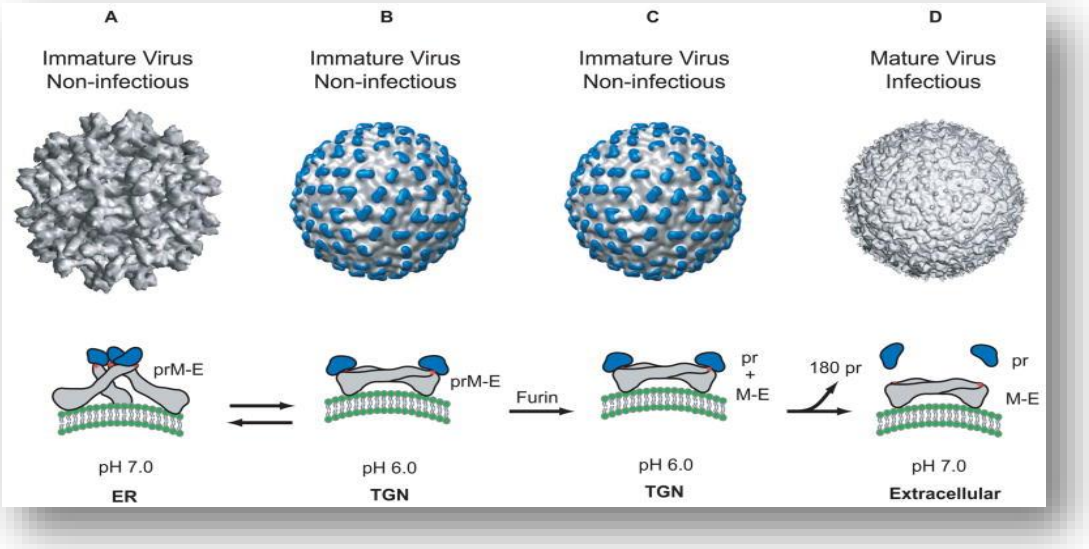

Figure 3: Maturation of dengue virus (Adapted from: Perera *et* al, 2008)

Dengue virus can be in the form of infectious or non-infectious in the body. Immature and mature virus is different from the structure of the membrane and envelope. Changes in the structure of the immature to mature occurred when the virus travel through the Golgi apparatus

1.2.2 Circulating Serotypes

Dengue virus is divided into four serotypes, namely, DENV 1-4 with differences in structure and function in the immune system. The four serotypes of dengue rise from forest in Southeast Asia (Wang *et* al. 2000). Infection of a specific serotypes only provide immunity against that serotypes only, without giving immunity to infection by other serotypes. In general, all serotypes are associated with dengue outbreaks. Recent studies have shown, genotype diversity between the four serotypes contribute to disparity in dengue outbreaks (D. Teo *et al*, 2009).

In Malaysia, a high and persistent incident of dengue cases is documented in the presence of infection by all serotypes. Even so, some serotypes of dengue infection became dominant for a long period. Dengue infection caused by DENV 3 is high between the years 1992-1995 and 2001-2002 (Ravindran *et* al. 2001). DENV 2 caused the dengue epidemic between years 1998-2000, while DENV 1 contributed to an increase of dengue cases between 2004-2006. In the past 2 years, until 1969, dengue serotypes 4 become dominant. After that, dengue cases caused by DENV 4 decreased throughout of the year, until an increase in 2001. The Institute for Medical Research (IMR), Malaysia, reported that, DENV 3 contributed to dengue cases between 2008 and 2009 (Cheah *et* al 2014). According to a research conducted in India, within all four serotypes, DENV 2 and DENV 3 contributed to the increase in cases of severe dengue and dengue hemorrhagic fever infection among Asians (Nizal *et* al, 2012)

1.2.3 Life Cycle of Dengue Virus

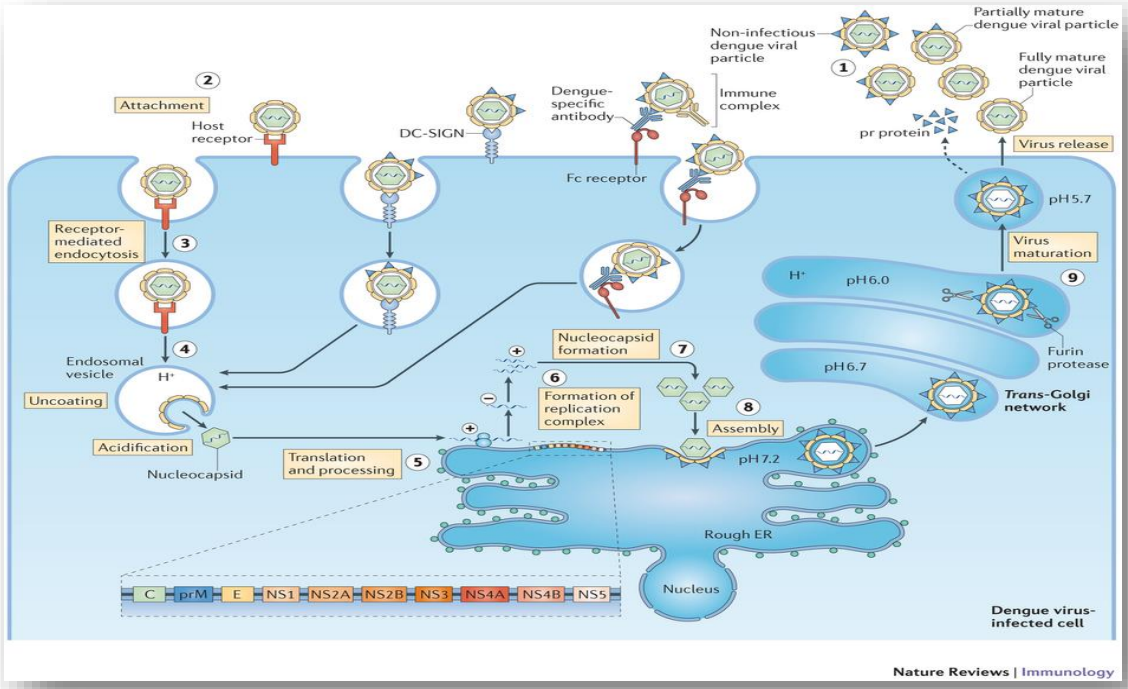

Figure 4: Life Cycle of Dengue Virus (Adapted from: Screaton *et* al. 2015)

The virus envelope protein binds to receptors molecules and trigger cellular process called receptor-mediated endocytosis. Then, the virus internalized into a structure called endosomes. The viral responds to lower pH in the endosomes, and form spike like structure. The spike allows penetration of endosome membrane and release capsid to cytoplasm. The capsid breaks apart and releases viral RNA. Viral RNA travel to rough endoplasmic reticulum. The whole viral genome is translated to a single long polyprotein chain. The capsid protein is in the cytoplasmic site on the endoplasmic reticulum. The envelope and premembrane protein are in the lumen site and activated by host peptidase enzyme (Back *et al*, 2013).

In cytoplasm, viral protein activates other protein in polyprotein chain. This protein aggregates to form RNA replication complex. Viral RNA attaches to replication complex to start synthesis positive sense strand of virus and translated to make viral protein. Viral RNA binds to capsid protein and packed into new immature virus particle. The virus travels through Golgi apparatus and continue to cell surface. Before reaching cell surface, premembrane protein is processed and virus becomes mature. New dengue virus released from cells (Back *et al*, 2013).

1.3 Dengue Transmission

1.3.1 Vector

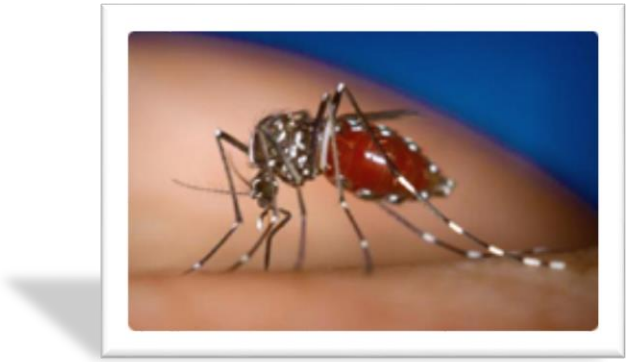

Figure 5: *Aedes aegypti* (Adapted from : WHO 2009)

Aedes aegypti is the primary vector for dengue virus. In spite of that, there is a possibility of infections caused by *Aedes albopicturs* and *Aedes polynesiensis*.

Humans infected with dengue virus will be the host for virus replication. Female mosquitoes captured dengue virus by biting human with the presence of virus particles in the bloodstream. Female mosquitoes spread dengue virus during each consumption (D. Teo *et al*, 2009). Environment with stagnant water facilitates female mosquitoes to lay eggs. This indirectly contributes to the increase in the number of mosquitoes during the rainy season and an increase in dengue cases (Back *et al*, 2013). Dissemination of dengue virus in some areas, indirectly gives an overview of the vector distribution (WHO 1997). The incubation period and virus replication in the vector depends on the temperature of the external environment and the type of virus involved (D. Teo *et al*, 2009).

1.3.2 Transplant

Dengue infections through transplants have been documented in Singapore. The recipient received a renal transplant from a living donor. The recipient developed an immune response following the transplant procedure, with an abnormal decrease in the number of platelets (Tan *et* al. 2005). Transmission of dengue through bone marrow transplants was reported during the dengue outbreak in Puerto Rico. The donor showed symptoms of dengue infection such as fever and headache while the recipient had fever, four days after the transplant and died (Rigau-Pereze *et* al.,2001).

In German, dengue transmission was reported through peripheral blood stem cell transplant. The recipient experienced abdominal pain, with oxygen deficiency and high acidity. Dengue screening performed on the recipient's blood, after three days of the transplant procedure. The result was negative for dengue antibodies but positive for dengue antigen and dengue RNA. The recipient had circulatory arrest and died nine days after transplant. Further investigation revealed that the donor had just returned from Sri Lanka, a place with a high prevalence of dengue fever and other infectious diseases (Punzel *et* al. 2014).

1.3.3 Nosocomial

Community acquired dengue transmission via the skin mucosa membrane and needle stick injury were also recorded. Transmission via mucocutaneous occurs when the blood of patients infected with dengue virus splashed onto face of a health personnel. The health worker showed signs and symptoms of dengue virus infection. Further screening, found that patients and health care workers infected with dengue virus serotypes 3 (Chen & Wilson, 2005)

In Hungary, there are cases of dengue infection through needle stick injury that occurred in the hospital, where a patient was infected with dengue after a mosquito bite while on holiday in Bangkok. The medical officer who took the blood of the infected patient, accidently punctured her finger. A week later, she showed a vague feeling of discomfort or unease, fever, visible lesions on the skin and muscular rheumatism (Nemes *et* al 2004). There are also a few articles published related to dengue infection through needlestick injury. Community acquired dengue transmission in endemic areas through needlestick injury is still in obscurity. Therefore, laboratory personnel and front line need to take precaution when dealing with patients (Chen & Wilson, 2005)

1.3.4 Vertical Transmission

There are documented cases of dengue infection involving transmission from mother to baby. Infected baby had fever a day after birth. There were also cases where transmission of dengue virus occured from a pregnant woman, found to suffer from dengue hemorrhagic fever before delivery (D. Teo *et al*, 2009).

In Sri Lanka, a number of cases related to dengue infection through vertical transmission had been reported, despite most cases overlooked it as complications during pregnancy and not well diagnosed. There were cases where the newborn showed symptoms of dengue. Dengue screening test results was strongly positive for dengue antigen and weakly positive for dengue Ig M. The mother presented with fever during delivery and dengue screening tests were positive for both dengue antibodies (Sinhabahu *et* al. 2014).

Another case was reported in Sri Lanka, where the newborn had a fever a few hours after birth. The mother showed an abnormal decrease in the number of platelets. Dengue antibody tests carried out on blood samples from newborn and the mother. Both were positive for dengue Ig M and Ig G. Vertical transmission of dengue is quite difficult to identify unless the mother or newborn shows symptoms of infection. Most positive cases of dengue, through vertical transmission was diagnosed, when the mother has fever during labor (Lokuarachchi & Jayasekaran, 2007).

1.3.5 Transfusion Associated

Dengue infection can lead a person to have a high viral load up to 10^9 copies/ml. Patients with dengue hemorrhagic fever will have a higher viral load than patients with dengue fever. Approximately 50-80% of reported cases of infection are asymptomatic. Therefore, there is a possibility of dengue infection through transfusion from asymptomatic donors to recipients (Mohammed *et* al 2008).

In Mawan, Hong Kong, dengue infection through blood transfusion was reported in areas of low prevalence of dengue. The blood donor showed symptoms of dengue fever, a day after donating blood. Dengue screening was performed using blood samples from donated blood, by using reverse-transcriptase PCR. The presence of dengue antigens identified through screening tests that had been carried out. During epidemics of dengue in Puerto Rico, Brazil, dengue screening was performed on donated bloods (Stramer *et al*, 2009). Results through nucleic acid testing, found 12 donors out of 16521 donors were positive for dengue (Mohammed *et* al 2008).

Dengue infection through blood transfusion has also been reported in Singapore. Donors had a fever after giving blood. Screening of dengue found that donor was infected with dengue serotype 2. Both recipients of packed cells and plasma products (fresh frozen plasma), developed fever after transfusion. Screening tests that had been carried out to the recipient, using PCR, gave positive results for dengue antigen (Tambyah *et* al, 2008).

A dengue screening conducted among 329 volunteer donors in an area with a high number of dengue cases in Thailand. Dengue screening was found on 29 donors positive to dengue Ig M, while 2 donors were positive for dengue RNA (Poblap *et* al, 2006). In Indonesia, a study of dengue was carried out on 785 volunteer blood donors. Of these, 8 dengue infected asymptomatic donors have been identified (Beckett *et* al, 2005). Dengue screening was also carried out in areas with a high prevalence of dengue in Colombia. Out of 3189 blood donors, 215 donors were asymptomatic dengue virus infection (Mendez *et* al, 2006).

Table 1: Dengue and Donor Deferral (Adapted from: D. Teo *et al*, 2009)

Country	Donor deferral measures for dengue
Singapore*	6 months deferral for history of dengue infection 3 weeks deferral for history of fever No travel-related deferral for dengue
Hong Kong*	6 months deferral for history of dengue infection 2 weeks deferral for history of fever No travel-related deferral for dengue
Sri Lanka*	No specific deferral for history of dengue infection 2 weeks deferral for history of fever No travel-related deferral for dengue
Australia†	4 weeks deferral for history of dengue infection No travel-related deferral for dengue
New Zealand‡	4 weeks deferral for history of dengue infection No travel-related deferral for dengue
UK‡	2 weeks deferral for history of dengue infection No travel-related deferral for dengue
United States‡	4 weeks deferral for history of dengue infection No travel-related deferral for dengue

*Endemic for dengue. †Non-endemic except parts of Northern Australia. ‡Non-endemic.

Generally, history of infections, health condition and travelling history of blood donors are points to consider when differing blood donor. In Malaysia blood donors infected with the dengue virus will be deferred for a period of 6 months after full recovery (National Blood Centre, 2014).

1.4 Dengue Infection

1.4.1 Pathogenesis

Early signs and symptoms of dengue infection present between 4 to 7 days after infection. A person infected with dengue may be asymptomatic or show signs of chronic infection. Symptoms of dengue infection, characterized by clinical manifestation are variable according to severity of the infection. Symptoms of infection may fluctuate in a short period especially patients with plasma leakage. Dengue virus can be detected day before the appearance of signs and symptoms of infection. Virus detectable in the blood stream until the fifth day of infection for primary infection and until day four for secondary infection (Ministry Of Health, 2015).

The possibility of dengue hemorrhagic fever cases is high with persistent occurrence of multiple serotypes of dengue. The presence of dengue antibody can be detected in the bloodstream before infection, namely through the transmission of maternal antibodies or the effects of previous dengue infection. Dengue infection can be more severe, with the presence of dengue antibody before infection, namely through the transmission of maternal antibodies or the effects of previous dengue infection. In a study, involving medical examination of a deceased, dengue antigen found in phagocytic cells of the reticuloendothelial system, lymph and connective tissue (Cheah *et* al, 2014).

1.4.2 Symptoms and Clinical Manifestations

Indications and signs of dengue infection vary from asymptomatic infections to dengue fever without having any distinguishing features, infections in critical phases and complications that capable of causing death (Ministry of Health, 2010). Symptoms of dengue fever include abnormal high body temperature, headache, muscular rheumatism, neuralgic pain, nausea and lesions on the skin. There are also patients with dengue hemorrhagic fever indicates presence of blood in the urine, nosebleed and abnormally dark tarry stools containing blood (Nizal *et* al, 2012).

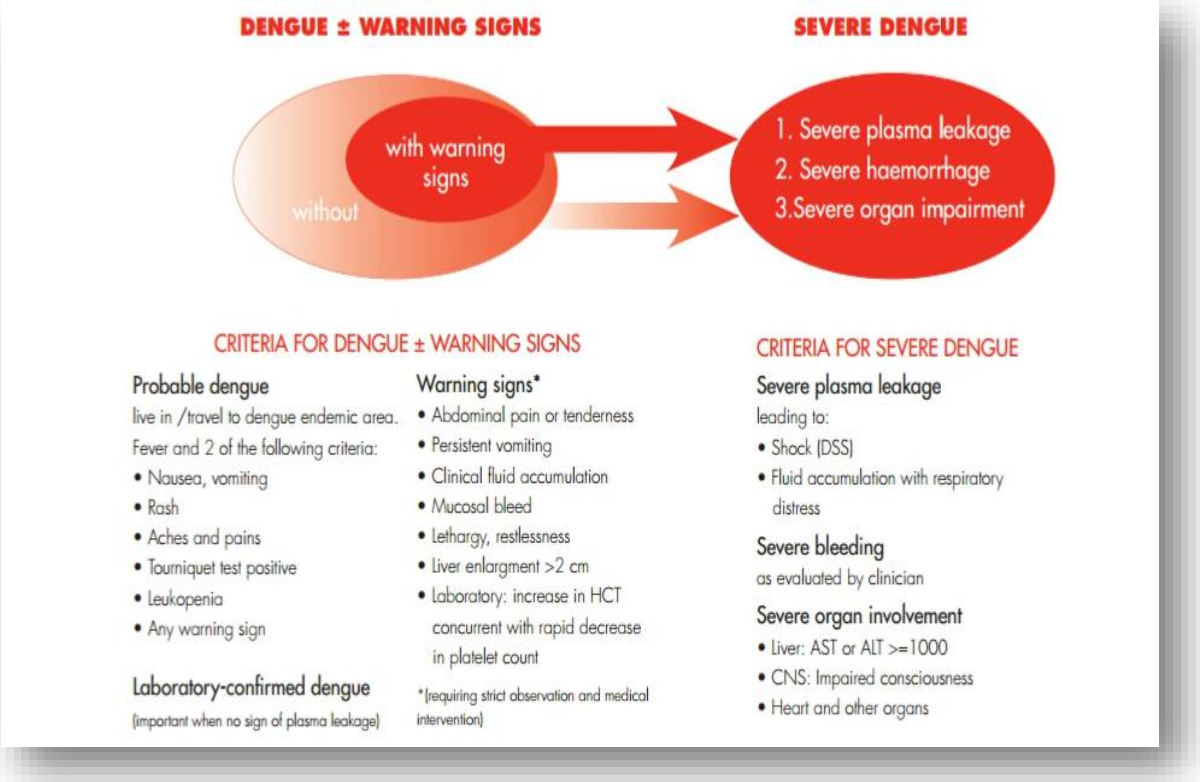

Figure 6: Dengue case classification and levels of severity (Adapted from: WHO Dengue Guidelines for Diagnosis, Treatment, Prevention and Control, 2009)

1.4.3 Treatment

Nowadays, treatment of dengue patients focused more on the management of body fluids. Even so, patients should closely monitored during treatment to prevent excessive fluid (Cheah *et* al, 2014). Until now, the treatment is more to treat symptoms of dengue infection because there are no specific medicines to treat dengue. Complications of dengue infection at the critical level can be reduced if intravascular fluid leakage detected early and treated promptly (D. Teo *et al*, 2009). A sufficient and comprehensive treatment can reduce mortality up to 1% compared with untreated patients (WHO, 1997).

Development of sophisticated treatment for dengue focuses on the different stages of viral replication and interaction between the envelope and membrane proteins. There are studies done to prevent the production of new copies of the virus, by inhibiting the formation of structure through mechanism or interaction of membrane and envelope proteins (Back *et al*, 2013).

Blood transfusion in dengue patients should only be considered, if the patient shows signs of bleeding. Justification for platelet count as an indication of platelet transfusions for dengue patients were still in obscurity. Nevertheless, most clinical guidelines have been set platelet transfusion should be performed when patients experience severe bleeding and if the platelet level drops accompanied by bleeding (D. Teo *et al*, 2009).