PREVALENCE OF POSITIVE DENGUE SEROLOGY AMONG THE BLOOD DONORS IN HOSPITAL UNIVERSITI SAINS MALAYSIA

BY

DR NOR NAZUHA BINTI MOHAMAD @ MANAS

Dissertation Submitted In Partial Fulfillment Of The Requirements For The Degree Of Masters Of Pathology (Haematology)

UNIVERSITI SAINS MALAYSIA

2017

ACKNOWLEDGEMENT

Firstly I would like to express all my praise and gratitude to Allah, the Most Gracious, Most Merciful, for giving me the strength, courage and wisdom in completing this research project.

In the process of completing this research project, I am indebted to many people for their assistance. I would like to express my sincere appreciation and thankfulness to every person who were involved directly or indirectly in helping me to finish out research project, hence my study. Without their support, guidance and encouragement, doubtfully I would manage to complete this project.

With great pleasure I would like to convey my sincere appreciation Dr Wan Haslindawani Wan Mahmood, my supervisor and as well as lecturer of Hematology Department, School of Medical Sciences, USM for her supervision, encouragement and support in this study. I also would like to acknowledge Assoc. Prof. Dr Noor Haslina Mohd Noor as my co-supervisor, Assoc. Prof. Dr Rapiaah Mustaffa, and all lecturers of Hematology Department, School of Medical Science, USM, for their supervision, guidance and encouragement in this research project. Not to be forgotten, I also want to thank my supervisor in Hospital Tengku Ampuan Afzan, Kuantan, Dr Rusmawati Ismail, for the support and being understanding. Special thanks to Dr Mohd Nazri and Dr Siti Azrin from Statistic Department, USM for their guidance in statistical work.

My great appreciation to all staffs from Transfusion Medicine Unit, especially Dr Marne, Dr Nur Ilya Syazwani, Dr Amran, Puan Khairiah and other staffs for

supporting me in sample collection and testing. I also would like to express my sincere thank you and appreciation to all my friends, for their support, guidance and advice in completing this research project.

Not to be forgotten, I also wish to thank En Mohd Aminuddin and other staffs from Department of Microbiology and Parasitology for helping me in the technical part of this research project, their advices and guidance.

Last but not least, my special thanks to my beloved parents, Haji Mohamad @ Manas bin Daud and Hajah Hasnah Abas, and my sister, Hasmawati and my brother, Haznan, for their love, sacrifice, support and encouragement throughout my study.

TABLE OF CONTENTS

LISTS OF TABLES

LISTS OF FIGURES

LISTS OF ABBREVIATIONS

AABB	American Association of Blood Banks
DENV	Dengue Virus
DF	Dengue Fever
DHF	Dengue Hemorrhagic Fever
DSS	Dengue Shock Syndrome
ELISA	Enzyme-Linked Immunosorbent Assay
FBC	Full Blood Count
FDA	Food and Drug Administration
HBV	Hepatitis B Virus
HCT	Hematocrit
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HUSM	Hospital Universiti Sains Malaysia
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IR	Incidence Rate
NAT	Nucleic Acid Testing
NS1	Nonstructural Protein 1
RT- PCR	Reverse transcription- polymerase chain reaction
SHOT	Severe Hazards of Transfusion
SOP	Standard Operating Procedure
WCC	White Cell Count

PREVALENS BAGI POSITIF SEROLOGI DENGGI DI KALANGAN PENDERMA DARAH DI HOSPITAL UNIVERSITI SAINS MALAYSIA

ABSTRAK

Pendahuluan

Unit perubatan transfusi bertanggung jawab untuk menyediakan bekalan darah yang selamat kepada pesakit. Kebelakangan ini, terdapat banyak penyakit yang mula menular dan semakin meningkat dalam populasi kita, yang menjadikan tugas Unit Perubatan Transfusi semakin mencabar. Sebagai contoh, penyakit denggi kini dilaporkan semakin meningkat dan menjadi ancaman di negara kita. Dalam tahun 2014, Kelantan dilaporkan sebagai sebagai penyumbang utama peningkatan kes denggi di Malaysia, terutamanya di daerah Kota Bharu. Penyakit denggi ini boleh terjadi tanpa tanda- tanda ataupun subklinikal. Oleh itu tujuan kajian ini dijalankan adalah untuk menentukan prevalens serologi positif bagi penyakit denggi di kalangan penderma darah yang sihat.

Metodologi

Kajian keratan rentas ini telah dijalankan dari Mac 2015 hingga February 2016 di Hospital Universiti Sains Malaysia (HUSM), Kubang Kerian. Sebanyak seratus dua puluh lapan (128) sampel darah telah diperolehi daripada penderma darah yang menderma darah di Unit Perubatan Transfusi HUSM dan program pendermaan darah bergerak. Sampel darah tersebut telah dijalankan ujian untuk mengenal pasti antigen NS1, dan antibodi IgM dan IgG bagi virus denggi dengan menggunakan kaedah ‘immunochromatography (rapid test)’.

Hasil Kajian

Hasil daripada kajian ini, didapati satu sampel (0.8%) daripada 128 sampel diuji positif untuk antibodi IgM bagi virus denggi, tetapi keputusan untuk ujian antigen NS1 dan antibodi IgG bagi virus denggi adalah negatif. Untuk antigen NS1 dan antibodi IgG bagi virus denggi, semua sampel daripada 128 sampel (100%) didapati negatif. Daripada kajian ini juga didapati tiada perkaitan yang signifikan di antara faktor- faktor seperti jantina, bangsa dan jenis penderma dengan serologi positif bagi antibody IgM untuk virus denggi.

Kesimpulan

Kajian ini menunjukkan bahawa terdapat serologi denggi yang positif di kalangan penderma darah yang sihat. Walaupun masih belum dapat dipastikan sama ada terdapat demam denggi yang berjangkit melalui pemindahan darah di Malaysia, namun hasil kajian ini menimbulkan persoalan sama ada penyakit denggi boleh menjadi ancaman kepada bekalan darah yang selamat. Oleh itu, kajian yang berskala besar dan lebih komprehensif dengan ujian pengesahan seperti ujian isolasi virus, ujian asid nukleik, dan ujian molekular bagi virus denggi perlu dijalankan bagi menentukan risiko penyebaran penyakit denggi secara pemindahan darah.

PREVALENCE OF POSITIVE DENGUE SEROLOGY AMONG THE BLOOD DONORS IN HOSPITAL UNIVERSITI SAINS MALAYSIA

ABSTRACT

Introduction

Blood bank has important duty to provide safe blood to the patients. As many infections have emerging and re-emerging in our populations, the duty of blood bank has become tougher. For example, dengue infection has become a serious threat to our country recently. In 2014, Kelantan has become one of the main contributors to the increase number of dengue cases in Malaysia, especially in Kota Bharu. Dengue infection can be subclinical or asymptomatic. Therefore, the aim of our study was to determine the prevalence of positive dengue serology among the asymptomatic blood donors.

Methodology

A cross-sectional study was conducted starting from March 2015 till February 2016 in Hospital Universiti Sains Malaysia (HUSM), Kubang Kerian. Hundred and twenty-eight samples were collected from blood donors who donated blood at blood bank and mobile blood donation programs. The samples were tested for dengue NS1 antigen, IgM, and IgG using immunochromatography method (rapid test).

Results

From this study, there was one sample (0.8%) out of 128 samples was positive for dengue IgM antibody. However the sample was negative for dengue IgG antibody and NS1 antigen.

For dengue IgG antibody and NS1 antigen, all of 128 samples (100%) were negative. There were no significant association between gender, age, race and type of donors with positive dengue IgM antibody.

Conclusion

This study showed that dengue serology can be positive in asymptomatic donor. Although it still cannot be confirmed if any dengue transmission by blood transfusion in Malaysia, but these results suggest that dengue can be a possible threat to the blood supply. However, large scale study, supported by confirmatory test like viral isolation test, nucleic acid testing or molecular test should be conducted to determine the risk of transfusion- related dengue infection.

Chapter 1

Introduction

1.0 GENERAL INTRODUCTION

Blood transfusions can be a life-saving treatment but it is not without the risks. The possible complications of blood transfusion can be divided into infectious and non-infectious complications. Therefore, procedures and protocols to ensure the safety of the blood and blood products have become stringent to ensure the benefits to the patients, and to reduce the risks, especially in ill-patients.

When the blood transfusion was first introduced, transfusion- transmitted viral infections such as Human Immunodeficiency Virus (HIV), Hepatitis B (HBV) and Hepatitis C (HCV) were high in prevalence. However after the introduction of the serological screening of these viruses for blood and blood products, the prevalences for these infections were significantly reduced. But, with the emerging and re-emerging diseases that spreading in our population, there is a need to assess the risks of transfusion- transmission of these diseases and the suitable methods for screening. The American Association of Blood Banks (AABB)'s Transfusion Transmitted Diseases committee has identified 69 infectious diseases that were potentially to be transfusion- transmitted infections in the United States and Canada. Among them, *Babesia*, dengue virus, and the prion responsible for variant Creutzfeldt-Jakob disease represent high risk agents (Trimble *et al.*, 2010).

The current concern was dengue fever had become serious outbreaks in Malaysia with the number of dengue deaths were increasing year by year. Kelantan also had serious outbreaks in 2014 with 14,456 cases with 17 dengue deaths were reported compared to 1,427 cases with 2 dengue deaths in 2013. Among that, majority of the cases were reported in Kota Bharu (MOH, 2015b). In a study reviewing the dengue fever trend in Kota Bharu during the years 1998- 2003, they found that the highest percentage of cases were in the age group of 15 to 29 years old, which accounts for 40.1% of cases (Hussin *et al.*, 2005).

As generally known, dengue infection is a mosquito-borne disease, namely *Aedes* species (especially *Ae.aegypti*, *Ae.albopictus* and *Ae polynesiensis*) (McBride and Bielefeldt-Ohmann, 2000). However other modes of transmission have been reported. Dengue virus (DENV) can be transmitted from mother to fetus in utero or to infants at parturition or also known as perinatal transmission (Tan *et al.*, 2008; Pouliot *et al.*, 2010). It is even more alarming since a few cases of dengue infections after receipt of blood and blood products had been reported. The blood products that were used in these cases included platelet concentrates, fresh frozen plasma and packed red cell from viraemic asymptomatic blood donors (Chuang *et al.*, 2008; Tambyah *et al.*, 2008). Other than transfusion- related, dengue infections related to organ/ tissue transplant and after occupational exposure in a health care setting also have been reported (Chuang *et al.*, 2008; Tambyah *et al.*, 2008; Wilder-Smith *et al.*, 2009). Risk is high in endemic areas as most DENV infections are asymptomatic and the viraemia is high titered, long lasting and detectable among asymptomatic individuals. The donor infectivity rate was estimated to be nearly 1 in 1000 donation (Mohammed *et al.*, 2008; Seed *et al.*, 2009).

The person infected with DENV can be either symptomatic or asymptomatic. It is estimated that 50-80% of DENV infected cases are asymptomatic, leading to potential transfusion-transmitted cases of DENV from infected donors (Mohammed *et al.*, 2008; Munoz-Jordán *et al.*, 2009). It is problematic if the asymptomatic person comes to donate blood because he still can transmit the virus into the recipient since he is still in viraemic state. Exclusion of donors in endemic areas during the high- incidence dengue season or during an outbreak is not feasible since the entire population is at risk of DENV infection, and the need for blood components is typically high during outbreaks and outbreaks can be long lasting (Tomashek and Margolis, 2011).

Therefore, the aim of this study was to address this issue and to determine the prevalence of positive dengue serology in the healthy blood donor population.

Chapter 2

Literature Review

2.1 Blood donation and transfusion

2.1.1 Blood donation process

The blood donation process is started with blood donor procurement. Blood donation should be procured from voluntary non renumerated blood donors. Blood donor recruitment activities should be active and continuous to ensure adequate blood supply (PDN, 2008). However, to ensure the safety of blood donors and blood recipients, selection of blood donors should be guided by strict donor criteria. The criteria can vary among the blood bank centres.

The blood donors who donate the blood should be healthy, free of diseases or medical illness, and also do not have any risk factors for HIV, HBV, HCV, and Syphilis. The criteria for donor acceptance includes age (between 18 to 65 years old), weight (more than 45 kg), in good health with no medical history that can harm themselves or the blood recipient (including bleeding disorders, medications, or recent illness), adequate rest (sleep more than 5 hours), haemoglobin level between 13.5 to 18.0 g/dL for male and 12.5 to 18.0 g/dLfor female, and appropriate blood donation interval (more than 8 weeks for whole blood donor, and more than two weeks for apheresis donor) (NBC, 2016).

All candidates for blood donors should undergo pre-donation questionnaire and counselling by trained blood bank staffs (include doctors and nurses). The counselling process is to assess the donor's health and to explain regarding the process of blood donation. During this process, the donors are educated and emphasized regarding the importance of disclosing any risk factors to ensure blood safety. This is then proceeded by screening the donors for any risks, either medical or behavioural risks, that can cause harm from the blood donation, either to the recipient later, or even to the donor himself. Usually this is done by screening the donors using a standard questionnaires. The content of the questionnaires may vary from

a blood bank centres to one another, especially in different countries. This is due to different prevalence of adverse reactions, cultural influences and other factors in one centre may differ from another centre (Karp and King, 2010).

After the counselling, the consent should be obtained. These candidates should be deferred if they are having conditions as mentioned in the donor deferral criteria. The detailed Guidelines for Donor Deferral are as in Appendix 1 (NBC, 2016).

All blood donors should be checked for blood pressure, haemoglobin level and blood group. Once the donors are qualified, the donors' identity should be verified before blood is collected, and ensured that all the labels of the blood bags and specimen bottles for infection screening are correct. The blood collection starts with venepuncture, which should follow strict Standard Operating Procedure (SOP) such as cleaning at the venepuncture site to reduce the risk of contamination during blood collection. For a successful blood collection, it requires successful venepuncture and proper mixing of blood. The ideal duration of bleeding process should not exceed 10 minutes (NBC, 2016). Post donation, the donors should have adequate rest before discharge. The donors also should be advised for adequate fluid intake after being discharged.

The blood that has been collected must have ABO and Rhesus blood grouping to be done, as well as screening for transfusion- transmitted infection. Other than that, they must be tested for infectious screening. In Malaysia, it includes HIV, Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), and Syphilis, as per recommendation by World Health Organization for blood safety (WHO, 2016a). Any blood which is tested positive will be discarded to prevent transfusion transmitted diseases. The implicated donor will be called for further testing and confirmation.

2.1.2 Demographic Characteristics of Blood Donors

The blood donors can be grouped into voluntary non- remunerated blood donors, directed or replacement blood donors and paid blood donors. As much as possible, voluntary non-remunerated blood donors are preferred to ensure safe and reliable blood supply (WHO, 2016a). Many studies have shown that the prevalence of transfusion transmitted infection is the lowest in this group (Zou *et al.*, 2012).

Female gender has been reported to have slightly higher proportion (~50%) compared to male gender among the new blood donors. However, the successful blood donation and repeat donations were more likely contributed by male gender (Zou *et al.*, 2012; Lattimore *et al.*, 2015). Repeat donors, who donated more than 1 time, accounted for majority (>70%) of blood donors, compared to first time donors. The mean number of donation was about 2 donations (Zou *et al.*, 2012; Lattimore *et al.*, 2015).

Regarding the infectious diseases, the prevalence of positive serology is persistently higher among first time blood donors compared to repeat donors. This is partly contributed by the facts that repeat donors are aware of self-deferral and the benefits of donor testing and deferrals of donors with previous confirmed positive serology of the tested infectious diseases. Among the positive serology donors, the male gender was more prominent compared to female gender (Zou *et al.*, 2012).

2.1.3 Blood Component Processing

Blood that is collected can be either as whole blood or by apheresis. Apheresis is a collection of specific blood component either plasma or platelets. After collection, the blood bags should be kept at appropriate temperature to maintain the quality. For component preparation from whole blood, the blood bag will be centrifuged at different speed and temperature,

depending on the types of component to be prepared. From one whole blood, the components that can be prepared include packed red cells and platelet concentrates, or packed cells and fresh frozen plasma, cryoprecipitate and cryosupernatant. All products must be adequately labelled based on the SOP; consist of the bag number, date of collection, ABO and Rhesus blood group, name of the component, and date of expiry (NBC, 2016).

2.1.4 Blood Transfusion Process

The decision for blood transfusion should be justified to benefit the patient and to avoid unnecessary transfusion. Unnecessary blood transfusion will cause inappropriately exposed the patient to infectious and non-infectious risks of blood transfusion. The patient should be explained regarding the blood transfusion, including the benefits, risks and alternatives, and consent should be obtained before the procedure (Farrugia, 2009; Vamvakas and Blajchman, 2009).

The request for blood transfusion should be accompanied by request form and sample, which are taken according to the SOP, to avoid unnecessary errors. Pre transfusion testing will be done by the blood bank laboratory which includes ABO and Rhesus blood grouping, and antibody screening. If the antibody screening test is positive, the test will be followed by antibody identification test. Upon request, the cross matching between the patient's blood and the donor's blood will be done. Only compatible blood or with specific requirement if any is supplied to the patient (NBC, 2016).

Before administering the blood to the patient, identity check should be carried out and verify the correct patient with the compatibility label by the blood bank. After identifying the correct patient and the correct blood, the transfusion can be started. The patient should be monitored closely including vital signs and any adverse reactions of blood transfusion. If any adverse reactions occur, appropriate action and resuscitation of the patient should be taken.

All adverse reactions should be reported to the blood bank and should be investigated. In all steps of blood transfusion, adequate and appropriate documentation should comply according to the SOP of the respective blood transfusion unit (NBC, 2016).

2.2 Blood safety

Blood safety is the main concern in the modern blood banking. It encompasses actions aimed at ensuring that everyone has access to blood and blood products that are as safe as possible, available at reasonable cost. At the same time, it is adequate to meet the needs of patients, only transfuse when necessary, and provide as part of a sustainable blood program within the existing health care system (WHO, 2016a). The threats in blood safety can be infectious and non- infectious agents. As many emerging and re-emerging infections have increased, more stringent measures are required to ensure the safety of blood and blood components supplied to the patients. However this task has become tougher with globalisation of blood and blood products. This is especially true for plasma fractionation products as they were made global compared to red cells which usually are used locally (Farrugia, 2009).

2.2.1 Non-Infectious Risks

With the effectiveness of screening methods of blood and blood components, the major cause of allogeneic blood transfusion complication and mortality is contributed by non-infectious risk. In United States (US), the leading causes of transfusion related mortality are currently included transfusion-related acute lung injury (TRALI) and hemolytic transfusion reactions (HTRs) (Vamvakas and Blajchman, 2009). The incidence of blood transfusion–related deaths in US and United Kingdom (UK) are as in Figure 2.1.

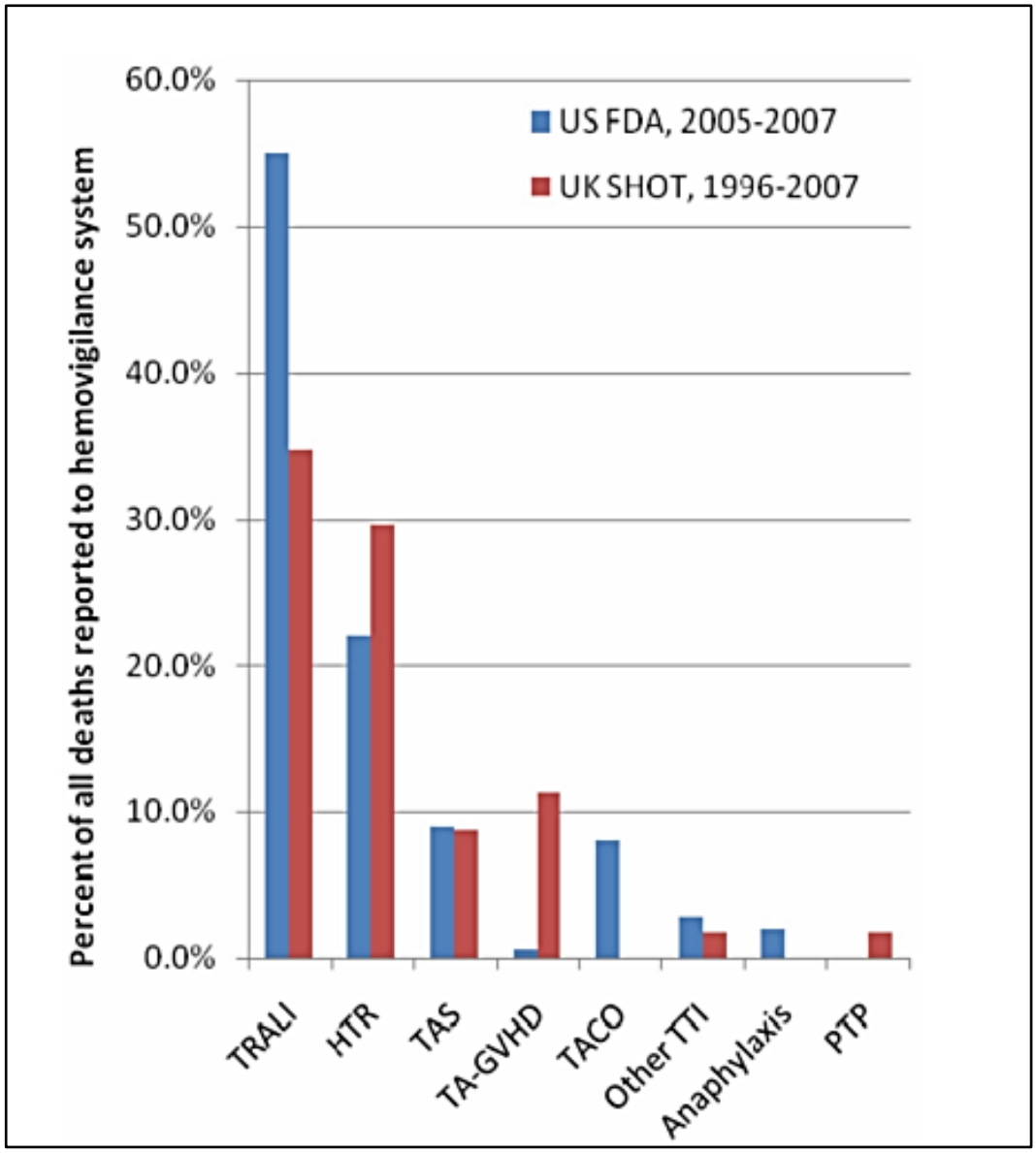

Figure 2.1. Causes of allogeneic blood transfusion–related deaths as a percent-age of all deaths reported to Severe Hazards of Transfusion (SHOT) (1996-2007) or the Food and Drug Administration (FDA) (2005-2007). (Adapted from (Vamvakas and Blajchman, 2009)).

The other potentially fatal complications of blood transfusion includes transfusion-associated graft-versus-host disease (TA-GVHD), transfusion associated circulatory overload (TACO), anaphylaxis, and post transfusion purpura (PTP). The definition of these complications is as below in Table 2.1.

Table 2.1. Potentially fatal non-infectious complications of allogeneic blood transfusion

Complication	Definition	Mechanism
Transfusion-related acute lung injury (TRALI)	New acute lung injury (ALI) occurring within 6 hours after a transfusion, with a clear temporal relationship to the transfusion, in patients without risk factors for ALI other than transfusion*	Donor anti-WBC antibodies attacking the recipient's WBCs in the microcirculature of the lungs "Two-hit" hypothesis implicating biologic response modifiers accumulating in supernatant plasma during storage†
Hemolytic transfusion reactions (HTRs)	Immune destruction of the transfused donor RBCs which are attacked by the recipient's: "naturally occurring" antibodies to the A or B antigens of the ABO blood group system, and/or alloantibodies to other RBC antigens produced following immunization through a previous transfusion or pregnancy	Acute HTR: Destruction of "incompatible" donor RBCs intravascularly or extravascularly (in the liver and/or spleen) by preexisting circulating antibody within 24 hours of a transfusion‡ Delayed HTR: Destruction of "compatible" donor RBCs 7-10 days after a transfusion, following an anamnestic immune response to a donor RBC antigen to which the recipient has been alloimmunized by a previous transfusion or pregnancy
Transfusion-associated graft-versus-host disease (TA-GVHD)	Immune attack against the recipient's tissues and organs by donor lymphocytes which engraft, proliferate, and mount an immune assault against the recipient	Donor lymphocytes not cleared by: immunocompromised patients and patients who receive components from donors (eg, relatives) with whom they partially share HLA haplotypes survive and engraft in the recipient
Transfusion-associated circulatory overload (TACO)	Acute pulmonary edema secondary to congestive heart failure precipitated by transfusion of a volume of blood greater than what the recipient's circulatory system can tolerate	Usually rapid infusion or massive transfusion of blood in patients with diminished cardiac reserve, chronic anemia, infants, and the elderly, although no patient is immune
Anaphylaxis	Anaphylactic response of a presensitized patient to various proteins contained in donor plasma	Often, donor IgA infused into an IgA-deficient recipient with preexisting circulating anti-IgA triggers anaphylaxis

Table 2.1 Continued

Posttransfusion purpura (PTP)	Sudden, severe thrombocytopenia occurring 7-10 days after transfusion in a patient previously alloimmunized (by pregnancy or transfusion) to a platelet-specific antigen	Production of potent alloantibody to a platelet-specific antigen through an anamnestic immune response that follows reexposure to the antigen on the donor's platelets. Paradoxically, the antibody destroys the recipient's own (antigen-negative) platelets as well

*A category of possible TRALI encompasses cases in which patients have other risk factors for ALI temporally related to the transfusion.
†See text in the "TRALI" subsection.
‡Hemolysis often starts early during the transfusion in the case of an ABO-incompatible transfusion. (Adapted from (Vamvakas and Blajchman, 2009))

Other complications of blood transfusion include febrile non-hemolytic transfusion reaction and allergic reaction. These are usually non-fatal, and can be prevented by giving premedication to the recipient prior administering the blood or blood products.

2.2.2 Infectious Risks

An emerging infection is the infection that shows increasing trend within two decades or threatens to increase in the future (Allain *et al.*, 2009; Trimble *et al.*, 2010). This infection can become transfusion risks as more people in the population are exposed to the infectious agent especially during endemic and epidemic period. Among the transfusion risks, there are the risks that are known and unknown (Trimble *et al.*, 2010).

The known transfusion risks include Hepatitis B, Hepatitis C, HIV and Syphilis. Over the past 30 years, the world has seen that there was marked decreased in the incidence of post-transfusion by these viruses, currently about less than 1 in 1 million transfusion, due to effective screening for these viruses. However, even with the known transfusion risks which

have established and effective methods of screening, there are still problems in detecting the infection during the window period (Trimble *et al.*, 2010).

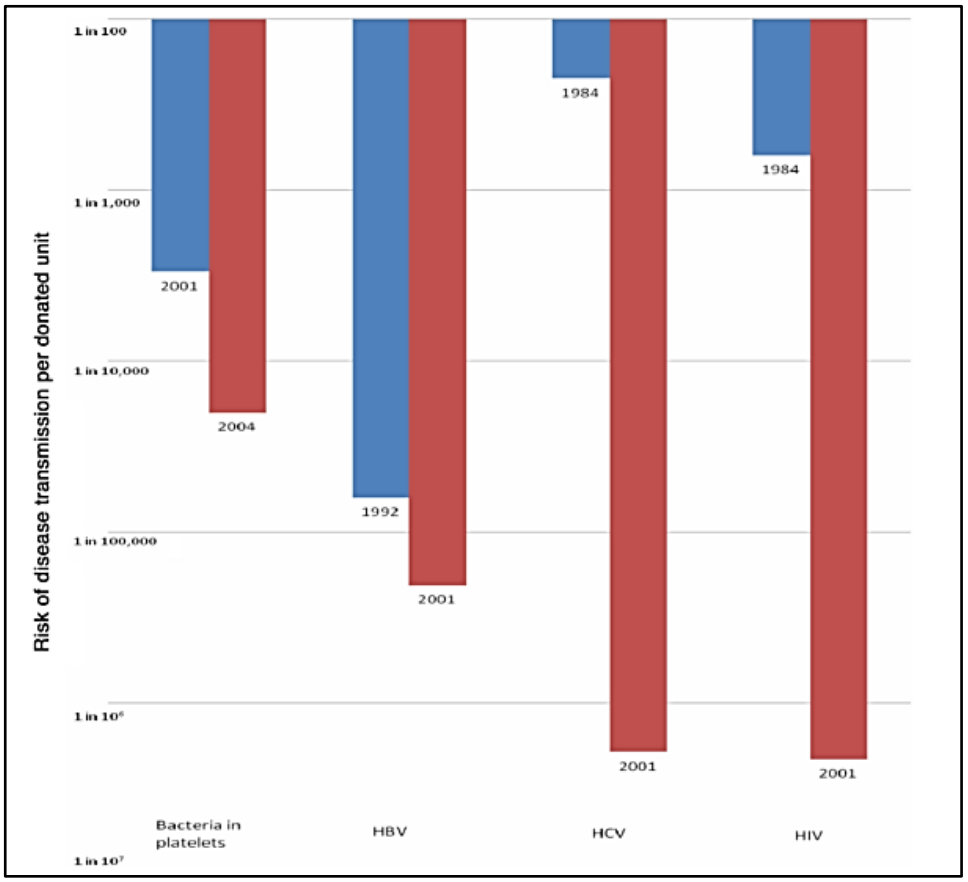

Figure 2.2. Reduction in the risk of transmission of the 4 most frequently transmitted, potentially fatal, transfusion-acquired infections in the US since the mid-1980s. (Adapted from (Vamvakas and Blajchman, 2009).

Among the unknown risks, there are pathogens which are predictable to be a transfusion risk, such as West Nile Virus (WNV) in US and DENV in Puerto Rico (Klein, 2010). The AABB's Transfusion Transmitted Diseases committee has identified significant numbers of infectious diseases that were potentially to be transmitted by transfusion in the United States and Canada, including *Babesia*, dengue virus, and the prion responsible for variant Creutzfeldt-Jakob disease is considered as high risk agents (Allain *et al.*, 2009; Trimble *et al.*, 2010). However, these risks may differ from one country to another based on many factors, for example the geography, socioeconomic status and quality of healthcare system. Therefore, evaluation of the transfusion risks related to these possibly new threat for each

country should be done periodically by proactive strategies and recipient surveillance (Trimble *et al.*, 2010).

2.2.3 Strategies for blood safety

The steps in ensuring blood safety include donor selection, laboratory testing for infective agents, and aseptic technique as well as on proper storage. Many strategies are developed to ensure blood safety, which include promoting voluntary non-remunerated donors, supporting repeated donations, emphasizing on self-deferral, adhering to strict donor screening criteria, and sensitive and specific serological tests for screening for viral markers on donated blood (Nafishah *et al.*, 2014).

In many countries, questionnaires are used as screening tools during donor selection process. Deferring the candidate donors based on risks factors of these transmissible infections can prevent collecting the contaminated blood. This is not only can save time and resources from testing the contaminated unit but also reduced the risks of exposure to the laboratory personnel and possible transfusion error. However, it has low sensitivity and specificity that can lead to deferring otherwise healthy donors (Epstein, 2010; Klein, 2010). In a study conducted in Canada showed that the face to face interview during screening for high risk behaviour before blood donation did not change the incidence rate of HIV or HCV among first time blood donors (Zou *et al.*, 2012).A small proportion of the blood donors, especially the young and first time donors, they tend to hide the risk factors possible due to several reasons. In a study by Vahidnia *et a.l*, 2016, they found that there were a proportion of donors, thought that current donor health screening questionnaires and deferral policies were unfair in which their reasons included ineligibility of “some groups of people”, travel restrictions, or a long questionnaire for repeat donors (Vahidnia *et al.*, 2016). These reasons may lead to non-disclosure of deferrable risk factors.

From previous studies, demographic characteristics can give prediction regarding which group are more likely to have the risk factors. In a study regarding donor testing and and risk in US allogenic donations, younger age group, male gender, and first time blood donors are potential factors to predict the risks for transfusion-related infections, especially HIV, Hepatitis B and C viruses, Syphilis, HTLV and West Nile Virus (Zou *et al.*, 2012; Custer *et al.*, 2015). Similar findings were also observed in a study conducted in National Blood Centre, Kuala Lumpur (Nafishah *et al.*, 2014).

The laboratory testing for infectious diseases are very crucial and highly cost effective, even though their performances are largely depending on the methods used and their costs. Other than to detect these diseases, they also serve as a platform for donor education, treatment, and preventive measures as the positively tested donors are managed and treated accordingly and they are deferred either temporarily or permanently based on type of the diseases (Epstein, 2010). However, to screen for all potential transfusion risks is impossible and not cost effective. Therefore only selected infectious diseases are tested; namely Hepatitis B, Hepatitis C, HIV and Syphilis in many part of the world. Screening for these infectious agents is also highlighted as basic requirement for blood bank by WHO (WHO, 2016a). The testing methods have been improved remarkably with the introduction of nucleic acid testing (NAT) together with serological testing, in which these test shorten the detectable period to days or weeks. However, they still cannot completely eradicate the possibility of transmission by these infectious agents during window period (Klein, 2010; O'Brien *et al.*, 2012). Despite the introduction of nucleic acid testing (NAT), the residual risks of transfusion-related infection for each agent is estimated to be 1 in 300 000 to 1 million units (Zou *et al.*, 2012; Custer *et al.*, 2015). This is because that there is still a period of time that the viral markers for these agents are remain undetectable probably due to low titre eventhough it has been markedly reduced by this test (Zou *et al.*, 2012).

2.2.4 Dengue threat to the blood supply

Dengue infection is an endemic in tropical and subtropical countries, including Malaysia. Dengue infection has become a serious threat to the public health as the reported cases have been increased in trends. The nature of dengue infection is also variable, from asymptomatic infection, mild febrile illness to severe illness complicated by shock and even death (McBride and Bielefeldt-Ohmann, 2000).

Previously, dengue infection is only known as vector-borne infection, transmitted from human to human by mosquitoes, namely *Aedes* sp. However, there was increased awareness of the possibility of other mode transmission of dengue infection. The more serious concern is the healthcare-associated transmission. There were a few reported cases of dengue infection transmitted by percutaneous, mucocutaneous, blood transfusion, bone marrow transplant and renal transplant (Wilder-Smith *et al.*, 2009). For example, there was a case reported in Hong Kong in 2002 of transfusion associated dengue infection in a lady who developed febrile illness after receiving blood contaminated by dengue virus (Chuang *et al.*, 2008). There was also another case reported in Singapore in 2007 of a donor who reported to develop febrile illness which later confirmed to dengue infection a days after whole blood donation. The three recipients who received his blood and blood products (packed red cell, fresh frozen plasma and platelets) were all positive for dengue serology (Tambyah *et al.*, 2008).

A few studies were also conducted in endemic area to identify the risk of transfusion-associated dengue infection. In a study in Puerto Rico, they found that as high as 59% of donors were positive for anti-DENV IgG and 16 (2%) were positive for anti-DENV IgM antibodies (Rodríguez Rodríguez *et al.*, 2009). In different studies, the results showed there were significant rate of viraemia (about 0.07%) in asymptomatic blood donors in endemic or epidemic areas (Rodríguez Rodríguez *et al.*, 2009; Seed *et al.*, 2009; Teo *et al.*, 2009). These

findings suggest that there is presence of risk to collect the blood from viraemic donor during asymptomatic or subclinical dengue infection.

The impact of dengue infection towards blood donors and blood supply is also significant. In some countries, the donor who has been infected with dengue is deferred up to 6 months. This will cause a loss of potential blood donation frequency as many as 2- 3 times of whole blood donation, or 12- 13 times of apheresis donation in regular blood donors (Teo *et al.*, 2009). The table 2.2 illustrates the dengue and deferral period in selected countries.

Table 2.2. Dengue and donor deferral.

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.
(Adapted (Teo *et al.*, 2009)

In Malaysia, previously the deferral period for a person who have dengue infection is 4- 6 weeks after recovery (PDN, 2008). However, due to increasing evidence of transfusion

associated dengue infection and recent outbreaks, the deferral period has been extended to 6 months after full recovery, and 14 days after acute febrile illness (NBC, 2016).

The requests for blood and blood products, especially platelets are also increased during dengue outbreaks. Combination of donor deferrals and increased requests for blood and blood products will affect the blood bank services. Therefore, judicious use of blood and blood products are strictly emphasized to avoid unnecessary transfusion and subsequently unnecessary risks (Teo *et al.*, 2009; Tomashek and Margolis, 2011).

2.3 Dengue

2.3.1 Dengue Virus

Dengue fever is caused by DENV, which belongs to family Flaviviridae, genus Flavivirus. DENV is a spherical, lipid-enveloped virus which contains a positive strand RNA genome that codes for three structural proteins, which are capsid, membrane and envelope. It also encodes for seven non-structural (NS) proteins namely the NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5 (Chawla *et al.*, 2014). DENV have 4 known serotypes, which are DENV-1, DENV-2, DENV-3, and DENV-4. It is a vector-borne disease, which is transmitted by female *Aedes* mosquitoes of the subgenus *Stegomyia* (especially *Ae. aegypti*, *Ae. albopictus* and *Ae. polynesiensis*) as the primary mosquito vectors, with human as its primary hosts (McBride and Bielefeldt-Ohmann, 2000).

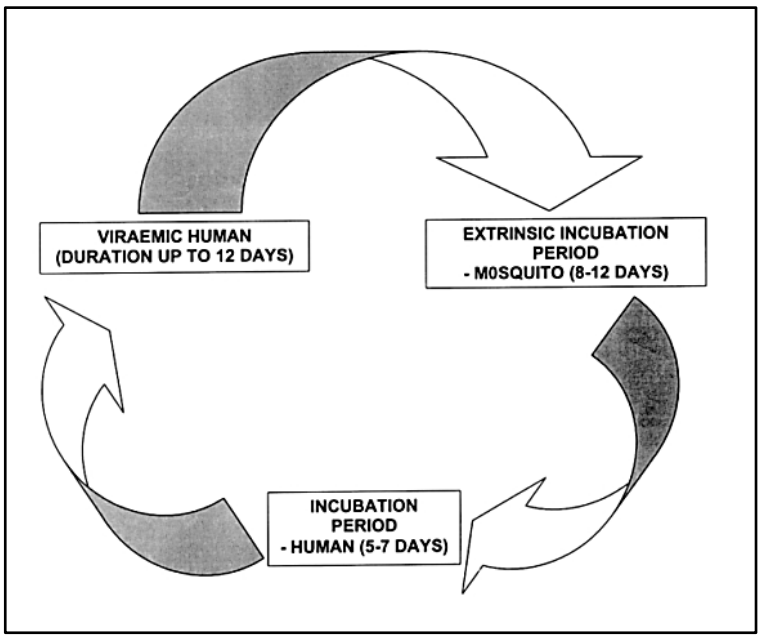

Figure 2.3. Stages in the transmission of dengue fever from individual to individual. A period of between 13 and 31 days is estimated to elapse between successive cases in an epidemic. (Adapted from (McBride and Bielefeldt-Ohmann, 2000)

The female *Ae. aegypti* must bite an infected human during the viraemic phase of the illness for the transmission to occur. The viraemic phase is generally last 4 to 5 days but may last up to 12 days. The extrinsic incubation period refers to the time required from when a viraemic human is bitten to when the mosquito itself becomes infective. This period is about 8 to 12 days. Figure 2.3 illustrates the time periods in the cycle of dengue virus transmission (McBride and Bielefeldt-Ohmann, 2000).

Ingestion of the infected blood by the mosquitoes causes the infection of the epithelial lining of the midgut in the mosquitoes. The virus escapes from the midgut epithelium into the haemocele before infecting the salivary gland and being secreted in the saliva. The genital tract can also be infected and the virus can enter the fully developed egg at the time of oviposition (McBride and Bielefeldt-Ohmann, 2000).

The feeding behaviour of the mosquito is characterized by easily interrupted feeding and repeated probing of one or several hosts. Therefore the persistence of dengue virus depends on the development of high viral titres in hosts to ensure transmission in mosquitoes (McBride and Bielefeldt-Ohmann, 2000).

2.3.2 Epidemiology

WHO recently estimated that there might be 50-100 million dengue infections every year worldwide (Chawla *et al.*, 2014). However some reviews estimated up to 390 million dengue infections per year including asymptomatic individuals with 96 million with clinical manifestations (Bhatt *et al.*, 2013). Dengue has been endemics in tropical and subtropical countries, especially in the urban area. The Asian countries is estimated to bear about 70% of dengue burden (Wilson and Chen, 2015). However, due to few factors such as global travelling, urbanization and climate changes, dengue cases have spread worldwide and

become a global health problem. Increased dengue cases have been reported in non-endemic area such as in Middle East (Wilson and Chen, 2015).

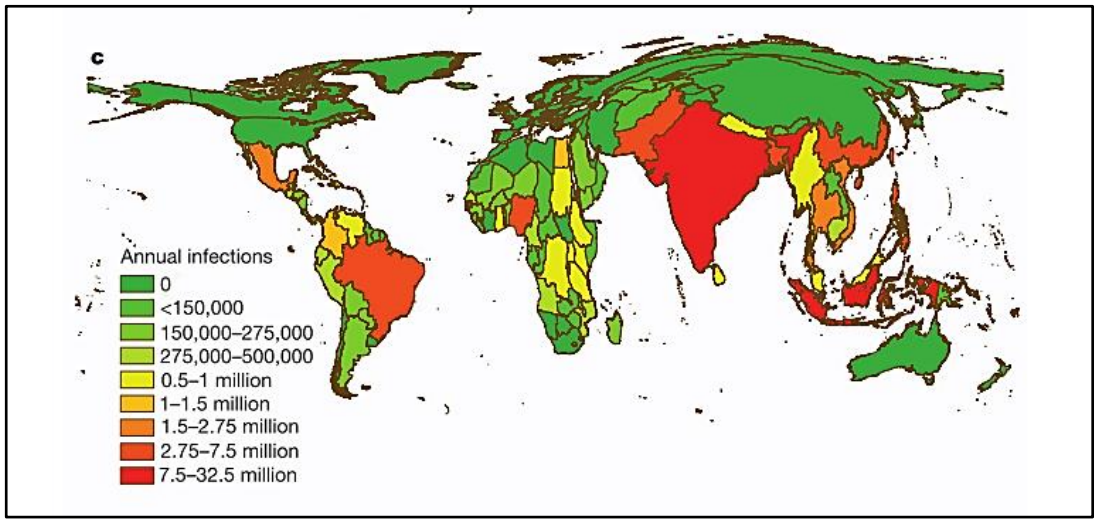

Figure 2.4. Cartogram of the annual number of infections for all ages as a proportion of national or subnational (China) geographical area. (Adapted from (Bhatt *et al.*, 2013).

Before 1970, less than 10 countries were reported to have severe dengue epidemics. However, the number has risen to more than 100 countries in the WHO regions; including America, Africa, South-East Asia, Eastern Mediterranean, and Western Pacific. Among these regions, America, South-East Asia and Western Pacific were most seriously affected (Pawar and Patravale, 2015).

Dengue has been endemic in Malaysia since the 1970s, but the incidence has also been increased in recent decades. It has been studied that the incident rate (IR) in Malaysia increased from 32 cases per 100,000 population in 2000 to 361 cases in 2014, though a temporary reverse trend was observed in 2011 and 2012 (Hii *et al.*, 2016). The dengue was affecting mainly in the age group of 15 and above in which IR is higher. The urban areas were documented to report most of the dengue cases (70%–80%) where the factors such as high density population and rapid development made dengue to be more transmissible. The dengue case fatality rate was reduced from 0.6% in year 2000 to 0.2% in year 2014, down to the national target rate. Most of the dengue death was observed to be higher in the age group

of 15 years and above, corresponding to the high incidence in this age group, and the highest rate was observed in 2004 (MOH, 2015a).

In Malaysia, exposure to dengue virus is high. It is demonstrated by a study conducted in West Malaysia involving 1000 subjects found that the rate of Immunoglobulin G (IgG) seropositivity is >90% (Azami *et al.*, 2011). In this study, they also found out that age was a significant risk factor in dengue, in which the seroprevalence was increased with every 10 year increment. However seroprevalence of dengue was similar among ethnic groups and between genders. There was also not much different among urban and rural residents, suggesting that dengue was widespread across the country (Azami *et al.*, 2011). In another study conducted in Barbados, a country where dengue is also an endemic, similar findings were observed. The findings showed that by the age of 18, over 80% of the studied population had seroconverted and by 25 years, more than 90% of the study population was positive for the IgG antibody to one or more dengue serotypes. Other than that, this study also found out that dengue was three to four times more likely to be a secondary than a primary infection in country where dengue was an endemic. These findings indicate that this population was immune to only some of the dengue serotypes. Therefore, further dengue infection with different serotypes in partially immune individuals would potentially increase the risk of more severe forms of dengue such as DHF in the future (Kumar and Nielsen, 2015).

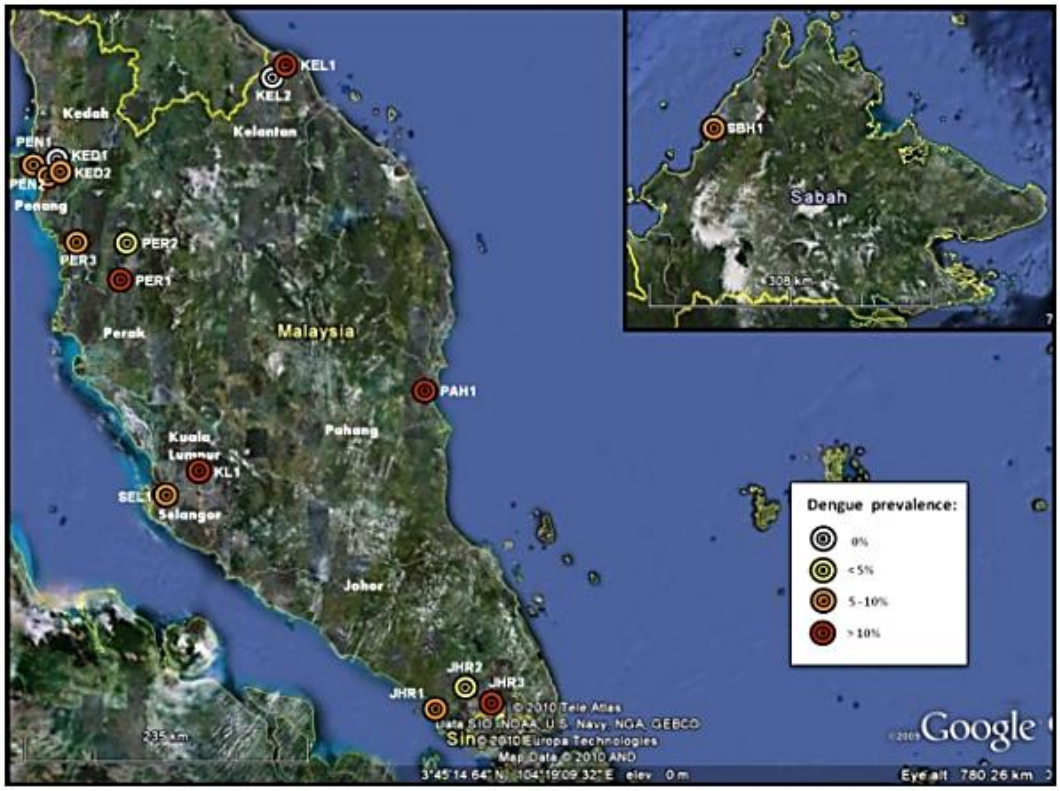

Figure 2.5. Seroprevalence of dengue at the different study sites in Malaysia. The percentage of dengue prevalence at each site is presented as different coloured concentric circles. (Adapted from (Tiong *et al.*, 2015)).

2.3.3 Clinical features

As agreed by expert consensus groups of WHO, dengue is described as "one disease entity with different clinical presentations and often with unpredictable clinical evolution and outcome" (WHO, 2009). Clinically, dengue infection may result in wide spectrum of illness, ranging from asymptomatic, mild fever to severe and potentially fatal disease in small proportion of patients (Chawla *et al.*, 2014). Most of the dengue infections are asymptomatic. In symptomatic patient, majority will present with mild febrile illness, but small proportion will developed severe dengue with severe bleeding manifestation, organ impairment, and may be complicated with hypovolemic shock due to systemic plasma leakage (WHO, 2009; Chawla *et al.*, 2014).

Previously, the dengue syndrome can be categorized into undifferentiated fever, classic dengue fever, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) (Chawla *et al.*, 2014; Pawar and Patravale, 2015). However, due to its practical limitations in triaging the dengue patients, a revised dengue classification which was classified by an expert group was developed (WHO, 2009; Guzman *et al.*, 2010). Figure 2.6 showed the revised dengue classification and its criteria.

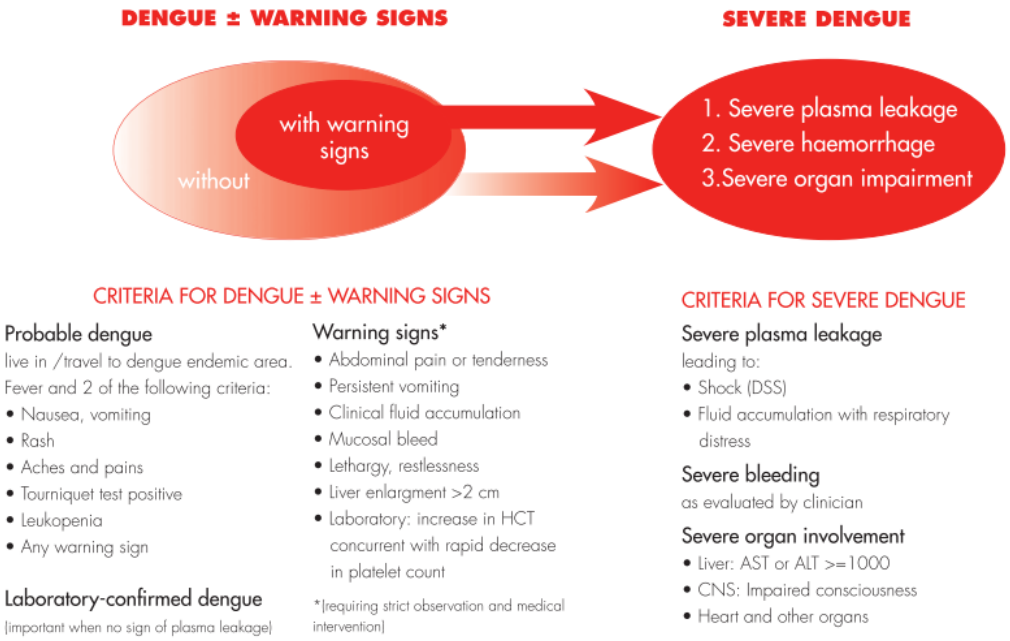

Figure 2.6 Suggested dengue case classification and levels of severity (adapted from(WHO, 2009)).

In general, it can be divided into 3 phases; febrile, critical and recovery/ resorption phase (Figure 2.7) (WHO, 2009; Chawla *et al.*, 2014). In febrile phase, it is commonly manifested as fever associated with rash, headache, ocular pain, arthralgia, myalgia, with or without mild upper respiratory tract and gastrointestinal symptoms (Guzman *et al.*, 2010; Liew *et al.*, 2016). However, these symptoms are overlapping with other tropical diseases such as measles, typhoid, leptospirosis and influenza. Therefore in this phase, it is difficult to differentiate between them. Positive tourniquet test make dengue diagnosis more favourable. Mild bleeding manifestations like petechiae and mucosal bleeding (e.g. epistaxis or gum