AN INVESTIGATION ON THE SERODETECTION OF DENGUE VIRUS IN SABAH, MALAYSIAN BORNEO

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ABSTRACT

Background: Dengue is re-emerging mosquito-borne infection and one of significant public health concern and has been expanding its distribution in a various part of the world. Dengue cases were more common in tropical and subtropical regions of the world. Dengue infection is endemic disease and can be transmitted by blood transfusion. Malaysia is one of the severe dengue-endemic country in Southeast Asia with the presence of all four serotypes (DENV1, DENV2, DENV3, DENV3, DENV4). Secondary infection of DENV infection by different DENV serotype from primary infection increase the risk for DHF and DSS and mostly related to antibody dependent enhancement (ADE) and transfusion of blood or blood product is one of the dengue treatments increase the blood transfusion safety measured. Periodically seroprevalence study can be used to understand the real burden of dengue in Malaysia population since many cases of dengue is unreported and the total cases of dengue don't reflect the actual dengue situation burden in the population and also marking the readiness of Sabah population for dengue vaccine implementation according to WHO recommendation. The objective of the study is to determine the rate of dengue seroprevalence among healthy adult's population from Sabah, Malaysia. **Methods**: A prospective cross-sectional study was conducted on blood donor samples from December 2016 to August 2017. This research was approved by the Medical Research & Ethics Committee (MREC), Ministry of Health, Malaysia and Research and Ethical Committee University Malaysia Sabah. Serum samples from 364 eligible blood donors were collected from Sabah Women and Children Hospital (SWACH) age 18-65 years and examined for the presence of dengue-specific immunoglobulins G (IgG) using enzyme-linked immunosorbent assay (ELISA) according to manufacturer's protocol. The ABO group of the blood donor were identified by agglutination test according to manufacturer protocol. Data were entered and statistically analyzed by SPSS, Version 24 (IBM) for gender, age, ethnicity, localities area, blood grouping and the results of dengue IgG. **Results**: Majority of the resident having previous dengue infection belong to the age group of 18 to 65 years was 36.5% (N=133, 95% Confidence interval [CI] = 0.059-0.117%). Dengue antibodies IgG prevalence increased with age with the lowest in 18-26 years age group (30.5%) and the highest in 56-65 years age group (73.3%). There are no significant differences for dengue seroprevalence seen when compare according to gender, localities, ethnicity and blood group. **Conclusions**: This result shows the high prevalence of dengue antibodies among Sabah blood donor. High-prevalence of Dengue IgG seropositivity reflects the high endemicity of dengue disease in this region of Malaysia. This finding also alerts the authorities to reconsider on adding the dengue screening in the blood screening panel for blood product to reduce the ADE risk. DENV seroprevalence among Sabah population were rather low for dengue vaccine implementation according to WHO recommendation in 2017.

Key Words: Dengue fever, seroprevalence, blood donor, Sabah

ABSTRAK

KAJIAN SEROLOGI BAGI MENGESAN KEHADIRAN VIRUS DENGGI DI SABAH, MALAYSIA BORNEO

Latar belakang: *Demam denggi adalah penyakit bawaan nyamuk yang kembali menyerang kebanyakan negara di seluruh dunia dan merupakan penyakit berjangkit yang memberi impak negatif terhadap kesihatan sejagat. Kes demam denggi biasanya dilaporkan di kawasan tropika dan subtropika di dunia. Jangkitan denggi adalah penyakit endemik dan boleh disebarkan melalui pemindahan darah. Malaysia adalah salah satu daripada negara-negara endemik denggi di Asia Tenggara dengan kesemua serotype virus denggi dikesan ada di Malaysia. Pesakit yang mempunyai antibodi terhadap virus denggi hasil daripada jangkitan pertama boleh menyumbang kepada peningkatan risiko kematian dalam jangkitan sekunder demam denggi dengan serotype berlainan. Kajian serologi bagi mengesan antibodi terhadap virus denggi secara berkala dapat digunakan untuk memahami beban sebenar penyakit denggi dikalangan penduduk di Malaysia kerana kebanyakan kes denggi tidak dilaporkan dan jumlah kes denggi yang dilaporkan tidak mencerminkan beban penyakit denggi sebenar dalam populasi di Malaysia. Objektif kajian ini adalah untuk mengkaji sejauh mana magnitud jangkitan virus denggi di kalangan penduduk dewasa yang sihat di Sabah.* ***Metodologi:*** *Kajian ini dijalankan ke atas sampel penderma darah di Hospital Wanita & Kanak-Kanak Sabah selama dua bulan dari bulan Disember 2016 hingga Ogos 2017. Kajian ini telah diluluskan oleh Jawatankuasa Penyelidikan & Etika Perubatan (MREC), Kementerian Kesihatan Malaysia dan Jawatankuasa Penyelidikan dan Etika Universiti Malaysia Sabah. Sampel serum dari 364 penderma darah berusia diantara 18 hingga 65 tahun yang layak dikumpulkan dan disaring bagi mengesan kehadiran imunoglobulin G (IgG) yang khusus terhadap virus denggi menggunakan enzim berkaitan imuno-serapan asai (ELISA) mengikut protokol yang ditetapkan oleh pihak pengeluar. Statistik bagi umur, jantina dan kelaziman antibodi terhadap virus denggi dianalisa menggunakan perisian SPSS, Versi 24 (IBM) untuk umur, jantina dan keputusan IgG denggi.* ***Keputusan:*** *Majoriti penduduk yang pernah dijangkiti demam denggi yang berumur 18 hingga 65 tahun adalah 36.5% (N = 133, 95% selang Keyakinan [CI] = 0.059-0.117%). Antibodi IgG terhadap virus denggi meningkat selari dengan kenaikan umur penduduk dimana peratusan terendah dipantau dalam kumpulan umur 18-25 tahun (30.5%) dan tertinggi adalah dikalangan penduduk yang berumur 56-65 tahun (73.3%).* ***Kesimpulan:*** *Hasil kajian ini menunjukkan bahawa kelaziman antibodi virus denggi yang tinggi di kalangan penderma darah Sabah. Peratusan yang tinggi terhadap kelaziman antibodi terhadap virus denggi adalah seiring dengan laporan kes demam denggi yang tinggi di rantau ini di Malaysia. Tahap pendedahan kepada jangkitan demam denggi di kalangan generasi muda dilihat masih rendah walaupun terdapat episod wabak denggi yang dilaporkan di Sabah. Penduduk Sabah terutamanya generasi muda adalah berisiko mengalami wabak denggi yang magnitude yang lebih tinggi berbanding wabak sebelumnya.*

Kata kunci: *demam denggi, kelaziman antibodi virus denggi, penderma darah, Sabah.*

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LIST OF ABBREVIATION

WHO	-	World Health Organization
NTDs	-	Neglected Tropical Disease Strategy
RNA	-	Ribonucleic Acid
DENV	-	Dengue virus
DENV1	-	Dengue serotype 1
DENV2	-	Dengue serotype 2
DENV3	-	Dengue serotype 3
DENV4	-	Dengue serotype 4
MOH	-	Ministry of Health
CPG	-	Clinical Practice Guideline
WSN	-	West Nile Virus
YV	-	Yellow Fever Virus
CHKV	-	Chikungunya Virus
RVF	-	Rift Valley Fever
JE	-	Japanese encephalitis
CDC	-	Clinical Diagnosis Centre
ZIKV	-	Zika Virus
DF	-	Dengue Fever
DHF	-	Dengue Hemorrhagic Fever
DSS	-	Dengue Shock Syndrome
SPWD	-	Dengue Epidemic Management System
DENV5	-	Dengue serotype 5
nm	-	Nano meter
kDa	-	Kilo Dalton
E	-	Envelope
C	-	Capsid
prM	-	Pre-Membrane
NS1	-	Non-Structural Protein 1
IFN	-	Interferon
ER	-	Endoplasmic Reticulum
TGN	-	Trans-Golgi Network
CD8	-	Cluster of Differentiation

HIV	-	Human Immunodeficiency Virus
HCV	-	Hepatitis C Virus
IgG	-	Immunoglobulin G
Anti-DENV	-	Dengue antibody
GIS	-	Geographic Information System
STI	-	Sterile insert technique
IGRS	-	Insect Growth Regulators
ADE	-	Antibody Dependent Enhancement
CYD-TDV	-	Dengue Vaccine Candidate
SAGE	-	Strategic Advisory Groups of Expert
Fcy	-	Receptor protein on White Blood Cells
ELISA	-	Enzymatic Line
RT-PCR	-	Real Time Polymerase Chain Reaction
sNS1	-	Secreted NS1
SWACH	-	Sabah Women and Children Hospital
IgM	-	Immunoglobulin M
HRP	-	Horseradish peroxidase
TMB	-	Tetramethylbenzidine
OD	-	Optical Density
HI	-	Hemagglutination Inhibition Test
NMRR	-	National Medical Research Register
'N	-	North
'E	-	East
°C	-	Degree Celsius
HBV	-	Hepatitis B Virus
G6PD	-	Glucose-6 Phosphate Dehydrogenase
n	-	Number
Za	-	Standard Normal Deviate
P	-	Prevalence Rate
ml	-	Milliliter
N	-	Negative Control
R	-	Reactive Control
CAL	-	Calibrator
uL	-	Microliter

SPSS	-	SPSS
CI	-	Confident Interval
%	-	Percentage
RBC	-	Red Blood Cell
>	-	More than

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CHAPTER 1

INTRODUCTION

1.1 Background of the study

Dengue fever is a major public health concern with more and more cases reported worldwide. Dengue and severe dengue are listed as one of the 17 diseases in the World Health Organization (WHO) "Accelerating Work to Overcomes the Global Impact of Neglected Tropical Diseases (NTDs)" launched in 2012. The aimed to overcome the global impact of the neglected tropical disease (NTDs) so the diseases can be controlled more effectively and in many cases the disease can be eliminated and the ultimate goal to eradicated the disease with nearly half of the worldwide population living in risk area (WHO, 2012). There are five strategies suggested by WHO to achieved the goal against these 17 diseases worldwide. For Dengue and severe dengue diseases, WHO as put a target to focus on sustained the dengue vector by established the vector control interventions in 10 priorities country by 2015. Furthermore, WHO aim to established the dengue control and surveillance system in all region by 2020. The Dengue cases was targeted to be reduce more than 25% compare to 2009-2010 dengue cases (base line) and the mortality cases reduce at least 50% by 2020 (WHO, 2012).

The Dengue virus is a single-stranded positive RNA virus with lipid-envelope, small and under the genus *Flavivirus*, family *Flaviviridae*. Sequence variation of 30-35% allows DENV to be grouped into four known serotypes (DENV1, DENV2, DENV3, and DENV4) and each is capable of causing the entire range of dengue-related disease symptoms (de-Azeredo et al.,2015, Velasco-Salas *et al.*, 2014). Fifth dengue serotype has been found in Sarawak in 2013 although this claim is still controversial (Dejnirattisai *et al.*, 2010, Mustafa *et al.*, 2015). Dengue infection is reported to thrive in poor urban area in the tropic and subtropical region.

The worldwide map of dengue distribution worldwide was seen dramatic changes when Singapore and Japan reported the sudden increase in dengue cases after a decade of silence (Pang *et al.*, 2016). Many studies have concluded that dengue infection has been invaded in a variable region involve various geographic covering urban or rural area. The abilities of mosquitos *Aedes aegypti* and *Aedes albopictus* well-known as most prolific dengue vector strive that adapted well in a rapidly changing related to urban environment. These special abilities can be one of the factors that contribute dengue cases remain increasing trends throughout the years (de-Azeredo *et al.*, 2015).

Dengue Cases has dramatically increased worldwide including Malaysia from 2012 (21,900) until 2015 (120,836) up to 551%-fold (Tauqeer *et al.*, 2017). WHO reported the current worldwide Dengue burden is 2.5 billion infected people in more than 125 countries with approximately 20,000 mortality cases per year (Ayukekbong *et al.*, 2014, Mahmood *et al.*, 2013). Increasing urbanization in various part of the world, a leak of personal protection again mosquito, increasing factor that promoting the mosquitoes breading site and the changes of meteorological with high rainfall, humidity, and temperature related to global warming (Pang *et al.*, 2016). Another factor that contributes to increasing of dengue infection is the potential role of the foreign visitor who arrives in this region from Asian countries with known dengue endemic (Ashshi *et* al., 2017).

The sudden increase in dengue cases can also be attributed to the east access online data collection and reporting. However, dengue cases are generally considered as under-reported, misdiagnosed as there are many patients who do not seek treatment in the government facilities such as hospital or primary care outpatient clinic (Higa *et al.*, 2011). Dengue symptoms can be similar to other viruses (Mahmood *et al.*, 2013). There are also possibilities may be other possibilities that patients may seek medical attention from private health facilities and hence the cases ware not captured and included in WHO dengue statistic (Ng *et al.*, 2015). WHO reported in 2015, 70% of the world population are exposed to the risk of dengue infection live in the Asia Pacific regions (Bhatt *et al.*, 2013, WHO, 2017).

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