C-REACTIVE PROTEIN : DETERMINANT VALUE PREDICTING LENGTH OF STAY IN DENGUE PATIENT ATTENDING EMERGENCY DEPARTMENT HUSM KUBANG KERIAN

BY

DR. BADRUL HISHAM B MD SUPIAN

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LIST OF ABBREVIATION

APEX	Accelerated Programme for Excellent
ASA	American Association of Anesthesiologists
CCU	Coronary Care Unit
CRP	C-reactive Protein
CT	Computed Tomography
DBP	Diastolic Blood Pressure
DF	Dengue Fever
DHF	Dengue Haemorrhagic Fever
DSS	Dengue Shock Syndrome
ED	Emergency Department
HDU	High Dependency Unit
ICU	Intensive Care Unit
MAP	Mean Arterial Pressure
MODS	Multiple Organ Dysfunction Score
SBP	Systolic Blood Pressure
SIRS	Systemic Inflammatory Response Syndrome
SOFA	Sequential Organ Failure Assessment
SPSS	Statistical Packages for Social Science
USM	Universiti Sains Malaysia
WHO	World Health Organisation

ABSTRAK

Tajuk : Protein C-reakrif : Hasil nilai untuk menjangkakan jangkamasa pesakit denggi berada di wad untuk pesakit yang hadir ke Jabatan Kecemasan Hospital Universiti Sains Malaysia.

Latarbelakang : Protein C-reaktif ialah parameter makmal yang mudah dan murah yang boleh digunakan di Jabatan Kecemasan untuk menjangkakan jangkamasa berada di wad dan keseriusan sesuatu penyakit.

Objektif: Menentukan perhubungan di antara Protein C-reaktif kepada jangkamasa di wad, keseriusan penyakit dan mortaliti pesakit dengue di Jabatan Kecemasan.

Kaedah : Kajian ini merupakan satu kajian keratan rentas yang melibatkan pesakit denggi yang berumur di antara umur 18 hingga 70 tahun yang hadir di Jabatan Kecemasan Hospital Universiti Sains Malaysia dan seterusnya masuk ke wad perubatan. Jangkamasa di wad, keseriusan penyakit dan kadar kematian diambilkira.

Keputusan : Seramai 50 orang pesakit telah terlibat di dalam tempoh 6 bulan kajian dijalankan. Daripada kajian ini, nilai protein c-reaktif adalah tinggi bagi pesakit yang tinggal lama di wad dan pesakit yang mengalami denggi yang serius. Kajian ini juga menunjukkan purata menetap di wad ialah 4 hari bagi pesakit yang mempunyai nilai protein c-reaktif yang tinggi. Walaupun, daripada ujian Mann Whitney U, ia menunjukkan tidak signifikan dengan nilai padalah 0.28. Daripada ujian Chi-square

pula, menunjukkan tidak signifikan antara nilai protein c-reaktif dan pesakit denggi yang serius.

Kesimpulan : Secara kesimpulannya, disebalik beberapa limitasi kajian kami, bacaan CRP tidak menunjukkan sebarang kolerasi antara purata menetap di wad, keseriusan tahap denggi dan mortaliti dalam kalangan pesakit denggi.

ABSTRACT

Title: C -reactive Protein: Determinant value predicting length of stay in dengue patient attending Emergency Department HUSM Kubang Kerian, Kelantan.

Background: C-reactive protein is simple and cheap laboratory parameters that can be use in Emergency Department or inpatient ward to predict hospital stay and severity of disease.

Objectives: To determine association between C-reactive protein between length of stay, severity of disease and survival rate of dengue patient in Emergency Department.

Methodology: A cross-sectional study of adult confirmed dengue patients (age between 18-70 years) presented to ED Hospital Universiti Sains Malaysia then subsequently admitted to the medical ward. Length of stay, severity and survival rate of dengue patient was assessed in relation to C-reactive protein level.

Results: Fifty patients were included during study period of 6 months. From this study, it showed that there was significant increase level of C-reactive protein with longer hospital stay and in severe dengue patient. For this study, mean length of stay was 4 days for patient with high level of c-reactive protein. However, from Mann Whitney U test, it was shown that there was no significant correlation between CRP level and length of stay with p-value of 0.28. There was also, higher level of C-reactive protein

in patient who developed severe dengue. But from Chi-square test, was no significant correlation between CRP level with risk of develop into severe dengue.

Conclusion: In conclusion, despite of few limitations in our study has shown no correlation between CRP level and length of stay, severity of clinical course or mortality in dengue patient .

1. INTRODUCTION

Dengue fever is a febrile disease caused by a virus in genus Flavivirus, family Flaviridae that transmitted by Aedes mosquitoes in tropical areas. Dengue virus divided in four serotypes DEN-1 to DEN-4, (Shekhar & Huat et al, 1992;Suwandono A et al, 2006). Dengue virus infection may evolve from asymptomatic, mild and benign illness called dengue fever and severe form like Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) which is life threatening condition. Infection with one dengue serotypes provides lifelong immunity but only partial or transient immunity against other serotypes. The pathogenesis of dengue infection towards severe form partially understood, it related to enhancement of antibody-dependant viral infection which leads to plasma leakage and bleeding manifestations.

The *Aedes aegypti* and *Aedes albopictus* are the incriminated dengue vectors in Malaysia (Li et al, 1985; Yap et al, 1984 ; Lee HL et al, 1987; Rosen et al, 1987; Chan et al, 1971; Lum, 1993; Lee H et al,1990). Experimental results in studies on the possibility of transovarian transmission of dengue virus in *Ae. Aegypti* and *Ae.albopictus*has been reported by Lee *et al.* (1991). Rohani *et al.* (2011) has isolated and detected dengue virus from mosquitoes larvae collected from dengue high risk areas. Thus confirmed the maintance of the virus in the larval stage through transovarian transmission.Most of the dengue cases reported was from urban areas (70 – 80%) where there is a high density of its population and rapid development activities factors which favour dengue transmission.

The number of reported dengue fever (DF) and dengue haemorrhagic fever (DHF) cases in Malaysia shows an up going trend (Figure 1). The incidence rate also shows an upward trend from 44.3 cases/100,000 population in 1999 to 181 cases/100,000 population in 2007 (Figure 2). Latest update from Ministry of Health Malaysia , date from 25 may 2014 to 31 May 2014(22nd week) there is 1755 cases compare on 21st week 2014 , there is up going trends of cases. From January till end of May 2014 , there is 36,825 cases of dengue reported compared to last years 10, 401 cases at same period last year, there was markly increased to 254%. Mortality also showed increment up to 279% this year from January till 31 May 2014 from same period last years. This exceeds the national target for the incidence rate of DF and DHF which is less than 50 cases/100,000 population. The incidence rate is higher in the age group of 15 years and above (Figure2). The highest incidence rate is among the working and school-going age groups (Media release from Director General of Health Malaysia, 2014).

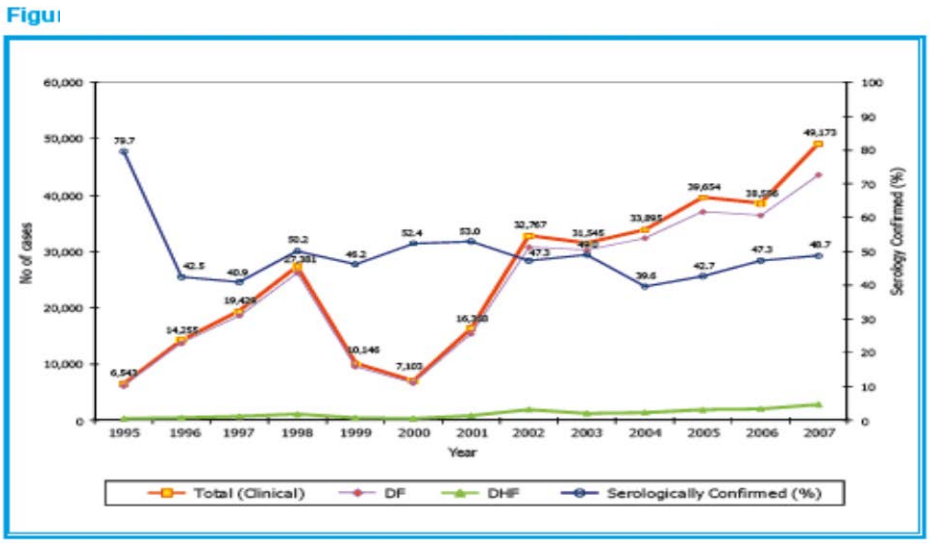

Figure 1: Number of Dengue Cases, Malaysia 1995- 1997

Dengue fever accounts for almost 95% of all reported cases. The serologically confirmed cases are approximately 40-50% of these cases at the time of notification. This relatively low percentage of seropositivity is due to lack of convalescent samples (second blood specimen) being sent for confirmation.

Figur

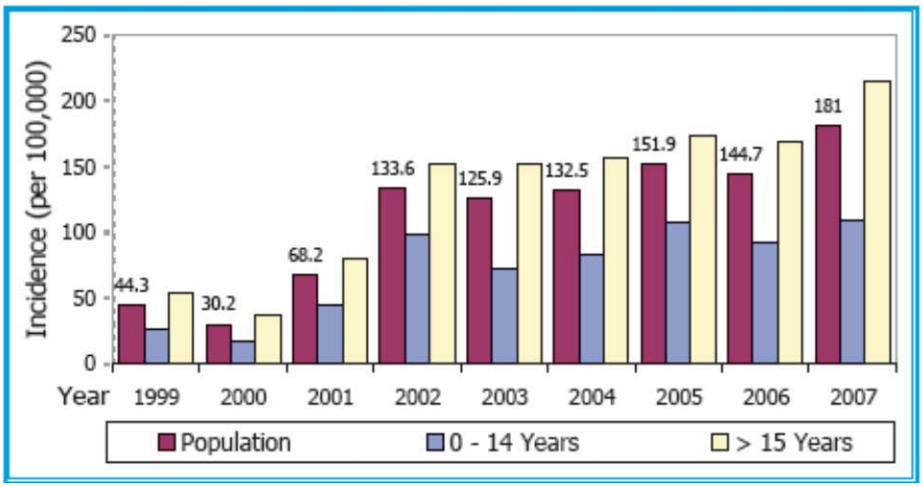

Figure 2: Dengue Incidence Rate by Age Group in Malaysia, 1999- 2007

Currently there is no specific test to measure progress of disease and outcome of dengue patient. In 2008, Restrepo, et al., evaluation of TNFα, IL-6,IL8, nitric oxide(NO), CRP, C3 and C4 are involved in evolving of dengue fever toward severe manifestation of dengue like dengue haemorrhagic and dengue shock syndrome. In 2009, Levy et al described the levels of TNF alpha, IL-6, IL-1 beta, nitric oxide (NO), CRP, C3 and apoptosis in 36 patients with dengue fever (DF), 34 patients with dengue haemorrhagic fever (DHF) and in virus-infected monocyte cultures. IL-6, TNF alpha, NO (nitrites) and CRP levels were increased and C3 diminished in patients with DF and DHF.

2. LITERATURE REVIEW

2.1 General

Dengue infection is a dynamic disease and it course changes as the disease progresses. Once the incubation period ends around 4-7days, the illness begins abruptly and will be followed by 3 phases: febrile, critical and recovery phase (Figure **3**)(Clinical Practice Guidelines in Adult Dengue, Revised 2010).

1. **Febrile phase**- during this phase patient start to develop high grade fever and accompanied by facial flushing, skin erythema, generalised body ache, myalgia, arthralgia and headache. This phase usually last around 2-7 days.

2. **Critical phase**-occurs towards the late febrile phase (usually after 3rd dayof fever) or around defervescence (often between 3rd to 5th day of illness but may go up to 7th day) when a rapid drop in temperature associated with an increase in capillary permeability in some patients. This phase ends after 24-48 hours.

3. **Recovery phase**- later then 24 to 48hours of afebrile episodes. General condition and haemodynamics of patient start to improve. Full blood picture start to recover by increase in platelets count and total white.

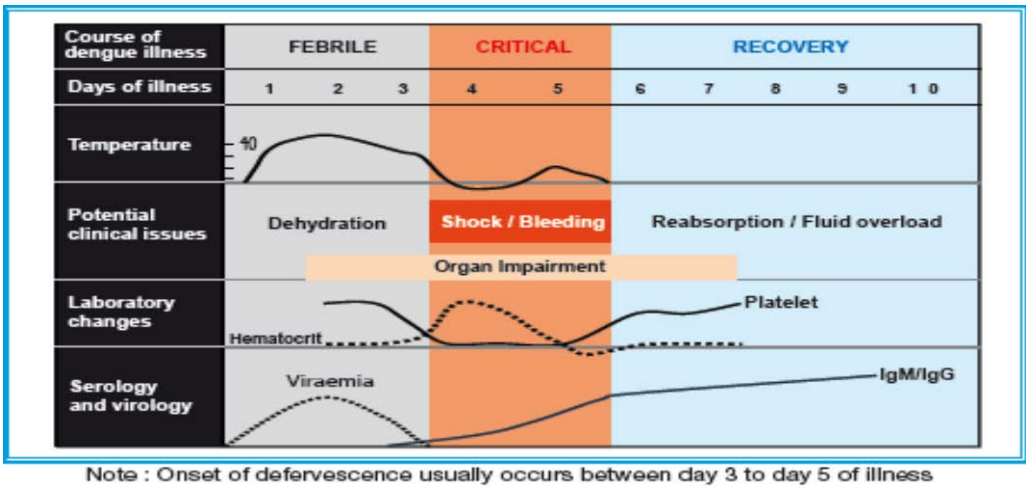

Figure 3: Clinical course of Dengue Fever

Clinical symptoms and signs of dengue fever are rather vague and non-specific compare to other viral infection, can easily misinterpreted. High index of suspicion and good history taking, appropriate physical examination and laboratory investigation are needed to rule this infection.(Wichmann, et al., 2006 ; Ying RS et al, 2007)

The main concern is the development of dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) which occur in up to 1% of cases. The mortality rates of DHF and DSS can be up to 10–20% and 40% respectively. Dengue haemorrhagic fever is often a poorly understood term because it implies that haemorrhage is the major feature. However, many patients with uncomplicated dengue fever have haemorrhagic manifestations, such as epistaxis, petechiae and gum bleeding. The new suggestion of dengue classification and levels of severity by Word Health Organisation (WHO) (Table 2.1). Deterioration during DHF tends to occur around the time the fever subsides. Dengue shock syndrome is a severe form of DHF. Clinical indicators of impending DSS include severe abdominal pain, change from fever to hypothermia, restlessness, sweating, prostration and tender hepatomegaly (Australian Family Physician, August 2006; Malavige, et al (2004)).

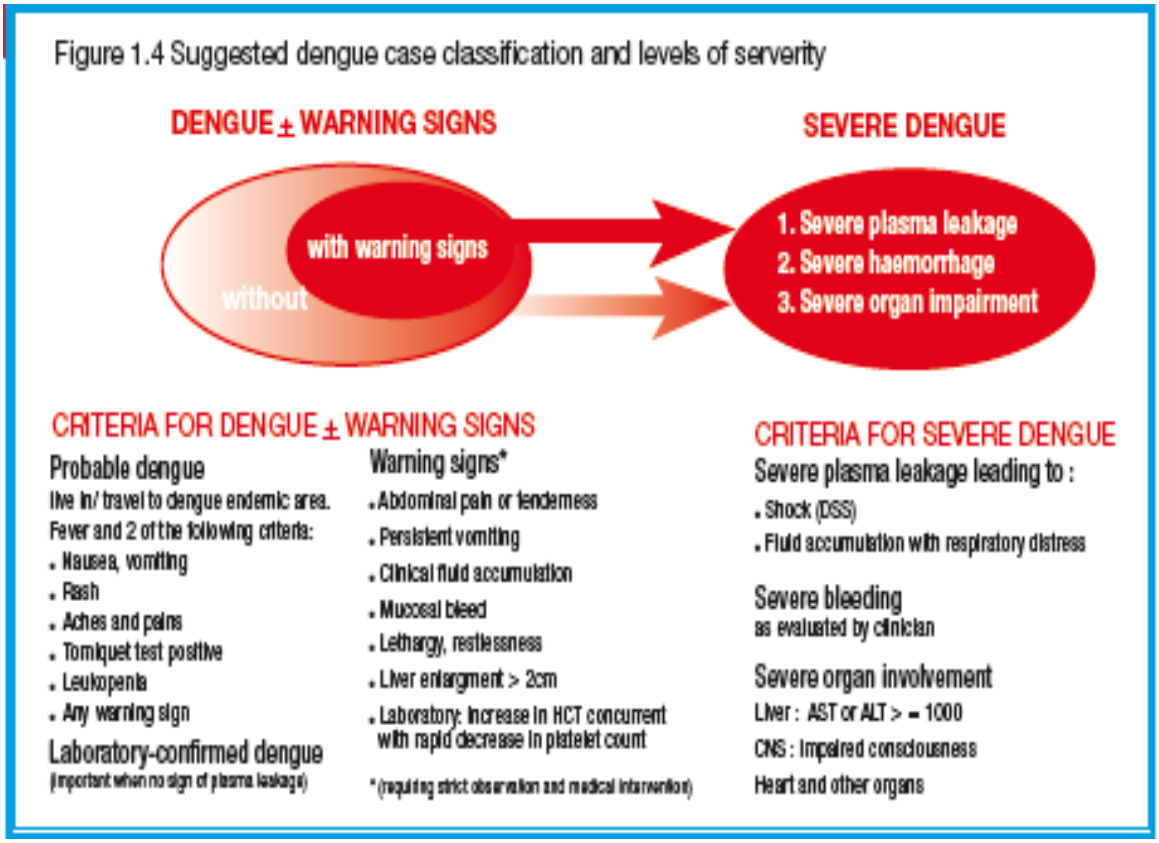

Figure 4: Suggested dengue case classification and levels of severity

2.2 Uptrend dengue case

In the year of 2003/2004, there were 8,548 reported dengue cases, with 24 deaths in the nation. Selangor state had highest with 3,132 cases (2,978 DF and 154 DHF cases). The cases increased by 287.3% in the 1st quarter of 2005 in comparison to the corresponding period in the previous year (Vector Control Unit of Selangor, Malaysia, 2004, unpublished data). Between 1st January and 9th April 2005, 12,813 people were hospitalized with DF and DHF. The disease claimed 41 lives. Statistics for the first 14 weeks of this year showed that Selangor state recorded the highest number of DF and DHF cases, 5,028 cases with 17 deaths (New Sunday Times, 24 April 2005).

Dengue cases were increasing in trend from January to July 2014, total dengue case had been reported were 49,948 cases which 247% (35,542 case increase) going up compare to same period on 2013. While there were 94 dengue death cases, rises 236% than, 28 dengue death last year. Latest update from Ministry of Health Malaysia on June 2014, there was 2,021 dengue case reported whole Malaysia, 12.5% going up case compare to 1,797 case week before.

Total 453 dengue locality detected around the country involved 14 states. From that total, 75 hotspots locality reported involved 64 localities in Selangor, 7 in Negeri Sembilan, 3 in Kelantan and 1 in Sarawak.

Table 2.1: Reported dengue case week 25th of 2014 in Malaysia

KES DENGGI DILAPORKAN PADA MINGGU 25/2014
(15 hingga 21 Jun 2014)

Bil.	Negeri	Bilangan Kes dan Kematian di laporkan		Jumlah Terkumpul kes denggi sehingga minggu 25/2014	Jumlah Terkumpul kes denggi sehingga minggu 25/2013
		Minggu 25 (15 – 21 Jun 2014)	Minggu 24 (8 – 14 Jun 2014)		
1	Perlis	9	6	91	101
2	Kedah	60	33	504 (4)	380 (1)
3	P. Pinang	43	28	878 (3)	322 (1)
4	Perak	112	68	2,666 (7)	898
5	Selangor	1,038 (2)	1,099 (2)	24,197 (32)	5,292 (6)
6	WPKL & Putrajaya	179	147 (1)	3,628 (7)	999 (6)
7	N. Sembilan	132	107	2,134 (3)	280
8	Melaka	49	42	1,190 (4)	220 (1)
9	Johor	110 (2)	88	2,390 (14)	1,537 (7)
10	Pahang	31	18	566	259
11	Terengganu	19	16	373 (1)	137
12	Kelantan	167	88	2,381 (4)	621
13	Sarawak	41	36	631 (2)	766 (2)
14	Sabah	31	21	589 (1)	328 (1)
15	WP Labuan	0	0	11	3
	MALAYSIA	2,021 (4)	1,797 (3)	42,229 (82)	12,143 (25)

Source: National Dengue Control Unit

Dengue fever (DF) is usually a benign illness with significant morbidity but low mortality (World Health Organization, 1997). However, dengue hemorrhagic fever (DHF) has greater risk of complications including death from dengue shock syndrome (DSS). DHF and DSS was associated with mortality of 0.2% with fluid resuscitation (Wills et al., 2005) but mortality could be higher (16.6%) (Shah et al., 2004). Dengue is a major public health concern globally (Guzman MG et al, 2002). Study by Edelman R et al (2007), some 1.8 billion of the population at risk for dengue worldwide live inmember states of the WHO South-East Asia Region and Western Pacific Region, which bear nearly 75% of the current global disease burden due to dengue. To aid clinicians in determining need for admission, we described the features of dengue infections and determined clinical and laboratory predictors of DHF upon first presentation to hospital.

2.3 Test for dengue case

Early recognition of dengue is challenging because the initial symptoms are often non-specific, viraemia may be below detectable levels and serological tests confirm dengue late in the course of illness (Ramos MM et at,2009). Study by Ligon BL et al (2005), prompt diagnosis during the febrile stage is essential for adjusting appropriate management.

Definitive test for dengue infection only can be confirmed in laboratory. Common laboratory test been use was Haemagglutination Inhibition Test, Dengue IgM test and Indirect IgG ELISA test (Wichmann, et al., 2006). In study by Kao CL et al (2005) and Shu PY et al (2004), dengue diagnostic methods are based on virus isolation,RNA and antigen detection, and serology. Haemagglutination Inhibition (HI) test has been the gold standard for serological diagnosis but because it is labour intensive and requires paired samples for

interpretation. Hence, this test now only being used mainly for research purpose (Clinical Practice Guidelines in Adult Dengue, Revised 2010).

Virus isolation is definitive test but it takes at least 2 weeks to complete and the cost is expensive. It can only be performed in the lab equipped with tissue culture and other virus isolation facilities. It is useful only at the early phase of the illness. However it only can be performed before days 5 of illness. Hence, the test take up to two weeks to complete and expensive (Clinical Practice Guidelines in Adult Dengue, Revised 2010). Polymerase chain reaction (PCR) also not widely used as certain center only available. This reverse transcriptase – polymerase chain reaction (RT- PCR) test are useful for the diagnosis of dengue infection in the early phase (< 5 days of illness) and shown to have a sensitivity of 100% in the first 5 days of disease, but reduced to about 70% by day 6 (Kong YY et, 2006)(Yong YK et al, 2007).

The IgM capture enzyme-linked immunosorbent assay (ELISA) is the most widely used serological test and this antibody titre is significantly higher (100%) in primary infections after 7 days. However in secondary infections, it was detected only 78% of patients after day 7. Once the IgM is detectable, it rises quickly and peaks at about 2 weeks after the onset ofsymptoms, and it vanishes slowly to undetectable levels within 1 months. Establishing a negative IgM early in the illness, and then demonstrate a positive serology later will be essential to exclude false negative results (Clinical Practice Guidelines in Adult Dengue, Revised 2010). In study by Schilling Set al 2004, IgM was detected in only 55% of patients with primary dengue infections between day 4-7 onset of fever, and positive in 100%of the patients after day 7. Furthermore, in secondary dengue infections, IgM was detected in only 78% of patients after day 7. This test also advantages in detecting dengue serotypes (Kumaria R et al, 2005). Study by Dongmei Hu et al (2011), anti-dengue IgM

antibody was detectable on the third day of onset with the positive rate of 42.9%, and rapidlyincreasing to 100% by day 8 of illness.

Indirect IgG ELISA test detected in 100% of patients after day 7 of onset of fever and recommended if dengue IgM is still negative after day 7 with the negative IgG in the initial test sample (Chanama S et al, 2004).

More recently, dengue virus non-structural protein 1 (NS1) antigen capture ELISAs have been reported as being a promising tool for the diagnosis of acute dengue infections (Wang SM et, 2010)(Bessoff K et al, 2008)(Hang VT et al, 2009) .This test widely available in Malaysia and now widely use in private medical facilities. Dengue Non-structural Protein 1 (NS1) antigen is an early antigen presenting in sera of dengue patients and involves in the pathogenesis of dengue infection (Lee HY et al, 2001)(Hsieh CJ et at, 2009). Study by Chaiyaratana W et al(2009), NS1 antigen strip has also been suggested as a rapid, easy-to-perform, sensitive, and specific test for the early diagnosis of dengue infection after the onset of fever.The detection rate is much better in acute sera of primary infection (75%-97.3%) while in acute sera of secondary infection (60% -70%). Meanwhile, sensitivity of NS1 antigen detection drops from day 4-5 of illness onwards and usually becomes undetectable in the convalescence phase (Clinical Practice Guidelines in Adult Dengue, Revised 2010). The levels of NS1 antigen might reflect the viral load during the course of disease as demonstrated by other test (Qiu LW et al, 2009; Huhtamo et al, 2010; Xu H et al, 2006). The NS1 circulating in a patient's blood is longer periods than does viral RNA (Singh MP et al, 2010). In study by Dongmei Huet et al, (2011), showed sensitivity of NS1 detection was ranged from 81.8% to 91.1% with samples taken during the first 7 days. Combining the results of NS1 and IgM antibody detection allowed positive diagnosis in 96.9% -100% for samples taken after day 3 of onset.

2.4 Uses of C-reactive protein detecting infection

C-reactive protein (CRP) is part of the non specific acute phase response to most forms of inflammation, infection and tissue damage. Plasma CRP is produced only by hepatocytes and half-life is about 19 hours (Vigushin et al, 1993). It production and synthesis is constant under all conditions of health and disease, sole determinant of circulating CRP concentration thus directly reflects the intensity of pathological processes. When the stimulus for increased production completely ceases, the CRP level will completely falls rapidly. In young adult, median concentration of CRP is 0.8 mg/l, the 90th centile is 3.0 mg/l, and the 99th centile is 10 mg/l (Shine B et al,(1981). But following acute-phase stimulus increase from 0.5 mg/l to 50mg/l, that is 10,000 fold (Mark B et al, 2003). In study of M. Juffrie et al, 2001, it believed that there is association between CRP production and IL1 and IL-6. The circulating value of CRP reflects ongoing inflammation and/or tissue damage much more accurately than others laboratory parameters of acute-phase protein (Kuse ER e al, 2000). The CRP concentration is very useful nonspecific biochemical marker of inflammation. It important for:-

1) screening for organic disease

2) monitoring of response to treatment of inflammation and infection

3) detection of intercurrent infection in immunocompromised individuals and few specific disease (Table 2.2)(Pepys & Hirschfield et al, 2003).

Table 2.2: Routine clinical uses of CRP measurement

Screening test for organic disease
Assessment of disease activity in inflammatory conditions
Juvenile chronic (rheumatoid) arthritis
Rheumatoid arthritis
Ankylosing spondylitis
Reiter disease
Psoriatic arthropathy
Vasculitides Behçet syndrome
Wegener granulomatosis
Polyarteritisnodosa
Polymyalgia rheumatica
Rheumatic fever
Familial fevers including familial Mediterranean fever
Acute pancreatitis
Diagnosis and management of infection
Bacterial endocarditis
Neonatal septicemia and meningitis
Intercurrent infection in systemic lupus erythematosus
Intercurrent infection in leukemia and its treatment
Postoperative complications including infection
and thromboembolism
Differential diagnosis/classification of inflammatory disease
Systemic lupus erythematosus vs. rheumatoid arthritis
Crohn disease vs. ulcerative colitis

Study by Pepys et al (2003) and Eberhard OK et al (1997), first acute-phase protein to be described and is an exquisitely sensitive systemic marker of inflammationand tissue damage. In addition of CRP been reported to stimulate formation of foam cells, which are a typical feature of atherosclerotic plaques (Zwaka T.P et al, 2001). Though, study by Pepys et al (2003), speculated that CRP may have significant proinflammatory effects, and that, by binding to ligands exposed on cells or other autologous structures as a result of infection, inflammation, ischemia and other pathologies, and triggering complement activation, leading to exacerbate tissue damage, leading to more severe disease. Study by Laura M et al., (2013) and Hack CE et al., (1997), confirms the reliability of WBC count, CRP and PCT as diagnostic and prognostic biomarkers of infection and sepsis in a wide range of ages, but particularly in aged patients who are the most frequent infected/septic patients hospitalized, and for this reason these biomarkers could be particularly useful and suitable in ED. The routine use of CRP is motivated more by low cost, easy availability and historical practice (Muller B Et al, 2002). Various studies report higher PCT and CRP values in patients with bacterial infection as compared with those with viral infection, autoimmune disorders, or other nonbacterial infection-related inflammatory disease (Brunkhorst FM et al 1998, Grendel et al, 1997, Hammer et al, 1997) and in patients with sepsis, severe sepsis or septic shock with documented infections (Muller ET al, 2000).

2.5 C-creative protein in dengue

There are only limited data on the importance of CRP in dengue-infected patients. These data are somewhat conflicting (Bethell et al 1998, Pinto et al., 1999). The IL-6, TNF, IL-1, nitric oxide (NO), CRP, C3 and apoptosis are known to be involved in the pathogenesis of dengue (Chatuverdi UC et al, 2000). But there is no specific test to diagnose progression of dengue fever to DHF/ DSS. Evaluation of TNFα, IL-6,IL8, nitric oxide(NO), CRP, C3 and C4 are involved in evolving of dengue fever toward severe manifestation like DHF/DSS(Levy et al,2009; Restrepo et al, 2008; Pinto LM et al, 1999). Study of Levy et al., (2008) , levels of TNF alpha, IL-6, IL-1 beta, nitric oxide (NO), CRP, C3 and apoptosis in 36 patients with dengue fever (DF), 34 patients with dengue haemorrhagic fever (DHF) and in virus-infected monocyte cultures. IL-6, TNF alpha, NO (nitrites) and CRP levels were increased and C3 diminished in patients with DF and DHF.

The positive predictive value for laboratory-confirmed dengue infection with combination of leukopenia (< 4000/cm), thrombocytopenia (< 150 × 10^3/cm), prolonged aPTT (> 38sec), elevated aminotransferase (AST/ALT > 1.5) and low CRP (< 20 mg/L) is 93.1%. These clinical and laboratory findings may serve as predictive markers to promote early diagnosis of dengue infection in Taiwan (Tzong-Shiann Ho et al, 2013). Levy A et al., (2009) and Chatuverdi UC et al., (2000), demonstrated that IL-6, TNF, NO, CRP and complement are involved in dengue virus infection. IL-6, CRP and decreased values of C3 appear to be good markers for severe infection. Study by M. Juffrie et al., (2001), showed that in dengue patients, 13% had an elevated IL-6 level on admission and 32.4% also had elevated CRP levels.

C-reactive protein was chose to be a marker or clinical parameters to predict course of dengue fever due to low cost laboratory handling and easily available compared to others immunology markers. There was no specific study to analyse or elaborate between CRP level and clinical course of dengue fever such as length of stay, severity of dengue and survival rate.

In summary, C-reactive protein (CRP) can be sensitive to predict the severity of the dengue fever. It also helps to determine changes of laboratory finding in dengue those presented to ED.

3. RESEARCH HYPOTHESES AND OBJECTIVES

3.1 Research Hypotheses

Null hypothesis:

There is no association of CRP level with length of stay, severity and survival rate in dengue patient.

3.2 General research objective

To analyze the association of C-reactive Protein (CRP) level in dengue patient presented to Emergency Department(ED) with length of stay, severity and survival rate.

3.3 Specific objective

a. To identify the association between CRP level and length of stay in ward.
b. To identify the association between of CRP level and severity of dengue infection.
c. To identify the association between CRP level and survival rate of dengue.

4. METHODOLOGY

4.1 Study Design and Duration

This is a cross-sectional study. This study was carried out for the period of 6 months from 1st January 2014 until 30th June 2014.

4.2 Study Setting and Population

This study will involve 50 subjects and, who ranged in age between 18 and 70 years old and will be selected among the patient attended to Emergency Department HUSM.

4.3 Study Approval

This study was undertaken as a dissertation study for the Degree of Master of Medicine (Emergency Medicine) under the USM and approved by the department board review and hospital ethics committee on the 24th June 2013 **(reference USMKK/PPP/JEPeM [264.3.(7)])**

4.4 Selection of Subjects

Patients who attended and diagnosed as dengue at Emergency Department in HUSM.

i. Inclusion criteria:

a. Patient diagnosed with dengue in the Emergency Department at the time of study. Must fulfill all the criteria below:-

- Clinical features of dengue infection
- Full blood count shows suggestive dengue infection.
- Dengue patient positive by laboratory confirmatory test.

b. Consented

c. Age more than 18 years old.

d. Malaysian.

ii. Exclusion criteria:

a. Patient with :-

I. inflammatory disease

II. trauma

III. immunocompromised patient

b. Children less than 18 years old.

4.5 Mode of Data Collection

Convenience sampling method was used. There was no specific time dedicated for sampling and it depend on the primary investigator availability. 50 patients were selected as decided by sample size calculation. Equally number of subjects were selected because to avoid bias in analysis. After the total number of subjects completed for each group, the enrolment of subject was stopped. All eligible patients who fulfilled the inclusion and exclusion criteria were selected during admission to ED HUSM. Written and informed consent were taken from the patients or from the caretakers. Demographic and physiological details were collected and recorded on the data collection form by the primary investigator at enrolment. The primary investigator who collected physiological information had received formal training to do so. The physiological details were obtained on the admission to ED and also before transfer to the ward. The outcomes of the patients (date of discharged, date of death and whether patients were transferred to HDU/ICU or not) were obtained from the patient or caretaker via daily follow up or via phone call to the respective ward.

The following information was collected:

a. Date of admission
b. Register number
c. Age (years)
d. Gender
e. Ethnic
f. Presenting complaint

g. Past Medical Illness

h. Any previous dengue infection

i. Blood pressure (mmHg)

j. Heart rate (bpm)

k. Respiratory rate (bpm)

l. Temperature (°C)

m. Mean arterial pressure(MAP)

n. Shock index(HR/SBP)

o. Hest test

p. General condition

q. Systemic examination

r. Status of Dengue Haemorrhagic fever or Dengue shock syndrome

s. Any admission to ICU

t. Length of stay in ward

u. Full blood count

v. CRP level

w. Outcome of patient(in 30days): death or alive

4.6 Plans for minimizing result error

The following steps were taken to reduce the error while conducting the study:

I. Subjects were selected strictly according to the inclusion and exclusion criteria for the study.
II. Subjects were not selected if they already had been selected once during the study period.
III. The subjects or caretaker were called by phone to get a complete data if there were inadequate information available.

4.7 Sampling method and Sample Size

4.7.1 Sampling Method

The sampling method in this study is random sampling.

4.7.2 Sample Size

This study will be involved 50 subjects and, who ranged in age between 18 and 60 years old and will be selected among the patient attended to Emergency Department HUSM.

This sample size is determined by using the formula by Kish and Leslie from Survey Sampling 1965

$S = Z \times Z [P (1-P)] / (D \times D)$

S is the sample size for a very large population

D is one half the width of the desired sample confidence interval

Z is percentile of the standard normal distribution determined by the specified confidence level

The final estimated sample size is obtained by implementing a finite population correction factor

Sample size = S/ [1+ (S/ population)

$S = Z \times Z [P (1-P)] / (D \times D)$

$= 1.96^2 (0.85)(0.15) / (0.1 \times 0.1)^2$

$= 46.98$

The sample size calculated for each group was 48 patients. 10% of drop out were added in anticipation of incomplete data. Therefore, the sample size required was 46 + 4 = 50 patients.

4.8 Data Analysis

Statistical analysis was performed with SPSS version 21.0.Continuous variables are expressed as mean (standard deviation) and median (interquartile range), and categorical variables are expressed as frequency. Descriptive &univariate analysis had been used. For descriptive analysis, numerical data were described in mean (standard deviation) or median (interquartile range) for non-normally distributed data whilst categorical data were described in median (interquartile range) . While for univariate analysis, independent t-test had been used for normally distributed data or Mann Whitney U test for non-normally distributed data. The homogeneity of variance was checked with Levene's test. Pearson's chi square test had been used to determine the association between categorical variables. The significance level was set at $p < 0.05$.

4.8.1 For the first specific objective, A Mann- Whitney U test had been used to determine the correlation of C- reactive protein (CRP) with length of stay in dengue patient.

4.8.2 Specific objective no 2, Chi Square test had been used to determine the association between CRP level and severity of dengue status (DHF/DSS).

4.8.3 For third specific objective, Chi Square test had been applied to determine the association between CRP level and survival rate.

4.9 Data Dictionary

4.9.1 C-reactive protein level (CRP)

Normal CRP level was 0-5mg/l. In this study, high level of CRP was defined as more than 8mg/l. (Mark B et al, 2003)

4.9.2 Shock Index

Shock Index formulation was derived by dividing pulse rate and Systolic blood pressure. Normal value for shock index was lee than 1.If the value more than 1, it shown there is shock in progress.