THE STUDY ON LIVER FUNCTION DERANGEMENTS IN ASSOCIATION WITH SEVERITY AND BLEEDING OUTCOME IN DENGUE FEVER

by

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LIST OF ABBREVIATIONS

DF	Dengue fever
DHF	Dengue haemorrhagic fever
DSS	Dengue shock syndrome
WHO	World Health Organisation
AST	Aspartate Transaminases
ALT	Alanine Transaminases
ALP	Alkaline Phospatase
IgG	Immunoglobulin G
IgM	Immunoglobulin M
PCR	Polymerase chain reaction
ELISA	Enzyme linked immunosorbent assay
IL	Interleukin
TNF	Tumour Necrosis Factor
CD	Cluster of differentiation antigen
IFN	Interferon
NSI	Non structural protein
DEN	Dengue Serotype
HS	Heparin Sulphate
ICAM	Intercellular Adhesion Molecule
GRP78	Glucose regulated protein 78
RANTES	Regulated upon activation, normal T cell expressed and secreted

ABSTRAK

Latar Belakang

Demam denggi merupakan sejenis penyakit berjangkit yang semakin meningkat semula di Malaysia. Kerosakan pada sel hati telah dikaitkan dengan demam denggi, tetapi hubungannya dalam kejadian pendarahan belum dibuktikan secara konsisten dalam kajian-kajian yang lalu.

Objektif

Objektif kajian ini ialah untuk menyiasat gangguan pada fungsi hati dan hubungannya dengan demam denggi serius dan pendarahan.

Metod

Ini adalah kajian keratan rentas melibatkan 144 pesakit di Hospital Tengku Ampuan Afzan Kuantan yang dijangkiti demam denggi (mengikut criteria WHO) dari bulan Oktober 2005 sehingga Mac 2006. Pesakit dikelaskan kepada demam denggi biasa dan demam denggi berdarah. Pesakit juga dibahagikan kepada 4 kumpulan berdasarkan tahap keseriusan pendarahan. Kajian analisa univariat di antara gangguan fungsi hati dan demam denggi berdarah dilakukan menggunakan "independent t test" manakala kajian multivariat menggunakan "multiple logistic regression". Kajian diantara gangguan fungsi hati dengan tahap pendarahan dianalisa menggunakan ujian "Kruskal Wallis" dan "Mann Whitney".

Keputusan

Purata umur pesakit ialah 31 $\pm$ 12 tahun. Terdapat 85 (59.7%) pesakit mengidap demam denggi berdarah. Seramai 31 (21.5%) pesakit tiada mengalami pendarahan, 39 (27.1%) mendapat "petechiae", 53(36.8%) mendapat pendarahan minor dan 21 (14.6%) mengalami pendarahan major. Purata nilai AST dalam demam denggi berdarah ialah 253.5 $\pm$ 205.5 IU/L ($p<0.001$). Purata nilai ALT dalam demam denggi berdarah ialah 171.1 $\pm$ 185.2 IU/L ($p<0.001$). Purata nilai albumin dalam demam denggi berdarah ialah 32.0 $\pm$ 5.7 IU/L ($p < 0.001$). Purata nilai ALP dalam denggi berdarah ialah 97.6 $\pm$ 48.9 μmol/L ($p = 0.027$). Purata nilai bilirubin konjugasi dalam denggi berdarah ialah 6.2 $\pm$ 5.4 ($p<0.001$). Analisa multivariat menunjukkan AST (OR 1.025, $p < 0.001$, 95% C.I. 1.014 – 1.036) dan ketirisan plasma dikaitkan dengan demam denggi berdarah. Keseriusan pendarahan juga dikaitkan dengan nilai AST, ALT, albumin ($p<0.001$) dan juga bilirubin konjugasi ($p = 0.038$) tetapi bukannya nilai ALP ($p=0.585$) dan INR ($p=0.593$).

Rumusan

Kajian ini mununjukkan dengan jelas kaitan di antara gangguan fungsi hati terutamanya AST dengan demam denggi berdarah dan keseriusan pendarahan. AST boleh dijadikan sebagai petanda awal dalam mengenalpasti penyakit denggi yang serius.

ABSTRACT

Background

Dengue fever is an endemic infective disease which has seen a significant re-emergence in Malaysia. Liver function derangements have been described in dengue infection but its association with bleeding occurrence has not been consistently shown in previous studies.

Objectives

The main objective was to study the association between liver function derangements and the severity of dengue virus infection and bleeding outcomes.

Methodology

This is a cross-sectional study involving 144 patients with dengue virus infection (World Health Organisation criteria) recruited from October 2005 until March 2006 in Hospital Tengku Ampuan Afzan, Kuantan. Severity of dengue infection was classified into uncomplicated dengue fever (DF) and dengue haemorrhagic fever (DHF) with or without Dengue Shock Syndrome (DSS). The bleeding outcomes were grouped into "no bleeding", "petechiae", "minor bleeding" and "major bleeding". The association between liver derangement and DHF was analysed using independent t-test and multiple logistic regression analysis. The association between liver derangement and bleeding outcome was analysed using Kruskal-Wallis and Mann-Whitney tests.

Results

The mean patient's age was 31 $\pm$ 12 years old. There were 85 (59.7%) patients with DHF. There were 31 (21.5%) patients who had no bleeding, 39 (27.1%) patients had petechiae, 53 (36.8%) patients had minor bleeding and 21 (14.6%) patients had major bleeding. The mean AST level in DHF was 253.5 $\pm$ 205.5 IU/L ($p<0.001$), mean ALT level in DHF was 171.1 $\pm$ 185.2 IU/L ($p < 0.001$), mean albumin level in DHF was 32.0 $\pm$ 5.7 IU/L ($p < 0.001$), mean ALP level in DHF was 97.6 $\pm$ 48.9 µmol/L ($p = 0.027$) and mean direct bilirubin level in DHF was 6.2 $\pm$ 5.4 ($p < 0.001$). Multiple logistic regression analysis showed that AST (OR 1.025, $p < 0.001$, 95% C.I. 1.014 – 1.036), as well as abdominal pain and plasma leakage were significantly associated with DHF. Bleeding outcome was associated with the derangement of AST, ALT, albumin ($p<0.001$) and direct bilirubin ($p = 0.038$) but not with ALP ($p=0.585$) and INR ($p=0.593$).

Conclusion

The study showed that a significant association existed between liver function derangements, predominantly AST with DHF and bleeding outcome. AST can be a useful surrogate marker to predict disease severity and bleeding outcome in dengue infection.

CHAPTER ONE

INTRODUCTION

1. INTRODUCTION

1.1 Background

Dengue is the most prevalent mosquito-borne viral disease in people, especially in South East Asia. Dengue virus consisted of four serotypes DEN-1, DEN-2, DEN-3, and DEN-4, of the genus *Flavivirus*, and family *Flaviviridae* which contains approximately 70 viruses. Dengue virus is transmitted to the humans by the vector *Aedes aegypti* mosquitoes. The flaviviruses are relatively small (40-50 mm) and spherical with a lipid envelope. All flaviviruses have common group epitopes on the envelope protein that result in extensive cross-reactions in serologic tests (Gubler, 1998). These render unequivocal serologic diagnosis of flaviviruses difficult. This is particularly true among the four dengue viruses serotypes. Infection with one dengue serotype confers lifelong immunity to that virus, but there is no cross-protective immunity to other serotypes. Therefore, a person can be infected with probably four dengue serotypes during their lifetime.

Dengue virus infection had a significant re emergence in many parts of the tropics. Dengue infection resulted in serious morbidity and mortality in most tropical and subtropical areas of the world; mainly Southeast and South Asia, Central and South America and the Caribbean (Gubler, 1998). There are approximately 2.5 billion people at risk in the world for infection with dengue viruses. Over 500 000 cases of dengue haemorrhagic fever were reported to the World Health Organisation (WHO) annually, with a mortality rate of 1 – 5% among patients with shock (Kurane, 2007).

Southeast Asia had seen large epidemics of dengue fever in recent years with mortality from dengue haemorrhagic fever and dengue shock syndrome (Ong, 2007). In Malaysia, the total dengue cases from 2004 until 2005 (until 24th September 2005) were reported to be 49, 355 cases with 138 deaths (Ministry of Health Malaysia, 2005). Meanwhile, in the state of Pahang, there was a rise from 412 cases in 2004 to 1,159 cases in 2005 (until 24th September 2005) (Ministry of Health Malaysia, 2005).

1.2 Definition of Dengue Fever (DF) and Dengue Haemorrhagic Fever (DHF)

1.2.1 Dengue Fever (WHO, 2002)

Diagnosis of dengue fever needs to include laboratory tests. A confirmed dengue infection is defined as an acute febrile illness with two or more of the following manifestations; headache, retro-orbital pain, myalgia, arthralgia, rash, haemorrhagic manifestations or leucopenia plus supportive serology or occurrence of the same location and time as other confirmed cases of dengue fever. Laboratory criteria for confirmation of dengue fever include the following; isolation of the dengue virus from serum or autopsy samples, demonstration of a fourfold or greater change in reciprocal IgG or IgM antibody titres to one or more dengue virus antigens in paired serum samples, demonstration of dengue virus antigen in autopsy tissue, serum or cerebrospinal fluid samples by immunohistochemistry, immunoflourescence or ELISA, or detection of dengue virus genomic sequences in autopsy tissue, serum or cerebrospinal fluid samples by polymerase chain reaction (PCR).

1.2.2 Dengue Haemorrhagic Fever (DHF) (WHO, 2002)

Dengue haemorrhagic fever is defined once all of the following are present; history of acute fever lasting 2-7 days, haemorrhagic tendencies (either a positive torniquet test, petechiae, ecchymoses, purpura or bleeding from any location), thrombocytopenia (100 $x10^3/mm3$ or less) and evidence of plasma leakage due to increased vascular permeability. Plasma leakage includes haemoconcentration (haematocrit > 47% for male, > 40% for female, a drop in haematocrit following volume replacement equal or greater than 20% of haematocrit at presentation and signs of plasma leakage evidenced by pleural effusion, ascites and hypoalbuminaemia (albumin < 35 g/L)

The severity of DHF was classified as:

Grade 1: In the presence of haemoconcentration, fever and non-specific constitutional symptoms, a positive torniquet test is the only haemorrhagic manifestation

Grade II: Spontaneous bleeding in addition to the manifestation from Grade I

Grade III: Circulatory failure, pulse pressure less than 20 mmHg but systolic pressure is still within normal.

Grade IV: Profound shock, hypotension or unrecordable blood pressure.

1.2.3 Dengue Shock Syndrome (DSS) (WHO, 2002)

Grade III and Grade IV of DHF constitutes DSS.

1.3 Liver Funtion Tests

Liver function tests represented a broad range of tests for normal functions performed by the liver. The cellular function is mainly reflected by the level of alaninine aminotransferases (ALT) and aspartate aminotransferase (AST). The excretory function is reflected by the level of direct bilirubin and alkaline phosphatase (ALP). Meanwhile the biosynthetic function is mainly reflected by the level of albumin, globulin and coagulation profile (prothrombin time or INR level) (Edmundowicz, 2002).

1.3.1 Alanine Aminotransferase (ALT)

This enzyme is the most sensitive marker for hepatocellular damage. It is produced within the cells of the liver. As the cells are damaged, the ALT leaks into the bloodstream leading to a rise in the serum levels. Therefore, it is currently considered that serum ALT correlates with the degree of histological inflammation and necrosis of the liver cell. Furthermore, the higher the ALT level, the more rapid the development of cirrhosis and hepatocellular carcinoma.

1.3.2 Aspartate Aminotransferase (AST)

Elevation of AST also reflects damage to hepatic cells, but it is less specific for liver diseases. It maybe elevated in other conditions such as myocardial infarction and muscular damage. However, if the elevations are more than four times of the normal upper limit, it

is a significant indicator for infiltrative liver disease especially in the presence of elevated gamma-glutamyltransferase levels. Although AST is not as specific for liver diseases as the ALT, the ratio between ALT and AST is beneficial to physicians in assessing the etiology of liver enzyme abnormalities.

1.3.3 Coagulation Profile

The blood clotting factors are synthesized exclusively in the hepatocytes, except for factor VIII. Serum prothrombin time collectively measures factors II, V, VII and X. which are associated with the incorporation of vitamin K metabolites into a protein and allows normal coagulation (clotting of blood). Therefore, prothrombin time is useful to measure hepatic bio-synthetic function and is helpful in both the diagnosis and assessing the prognosis of liver disease. However, a prolonged PT is not a specific test for liver disease, therefore confirmation of other abnormal liver tests is essential. Diseases such as malnutrition, in which a decreased vitamin K ingestion is present, may result in a prolonged PT time. An indirect test of hepatic synthetic function includes the administration of vitamin K (10mg) subcutaneously over three days. Several days later, the prothrombin time can be measured. If the prothrombin time normalized, then hepatic synthetic function is considered intact. This test does not indicate that there is no liver disease, but is suggestive that malnutrition may coexist with (or without) liver disease.

1.3.4 Serum Albumin

Albumin is the major protein present within the blood. Serum albumin is synthesised exclusively by hepatocytes and therefore is a marker for the ability of the liver to synthesize proteins. It has a long half-life, approximately 15 to 20 days with 4% degradation per day. Therefore it can be used as the chronicity markers of liver disease. In hepatitis, if the albumin levels fall below 3 g/dL, it should raise the possibility of chronic liver disease. Since it is easy to measure, it represents a reliable and inexpensive laboratory test for physicians to assess the degree of liver damage present in any particular patient. Malnutrition and to a lesser extent severe infection can also cause low albumin (hypoalbuminemia) with no associated liver disease.

1.3.5 Serum Alkaline Phospatase (ALP)

ALP is an enzyme associated with the biliary tract. However, it is not specific to the biliary tract as it is also found in the bone and the placenta. Renal and intestinal damage may also result in elevated levels of ALP. If the ALP is elevated, biliary tract damage and inflammation should be considered. Isoenzyme determination or characterization can be utilized to determine the causes for raised ALP. Another method to assess the etiology of the elevated ALP is to determine whether the GGT is elevated as well, or whether other tests are abnormal (such as bilirubin).

1.3.6 Serum Bilirubin

Bilirubin is a major breakdown product of hemoglobin The elevation of bilirubin can be due to elevation in indirect or direct bilirubin. The direct bilirubin fraction is that portion of bilirubin that has undergone metabolism by the liver. When this fraction is elevated, the cause of elevated bilirubin (hyperbilirubinemia) is usually outside the liver, predominantly obstruction to biliary tract. If the direct bilirubin is low, while the total bilirubin is high, this reflected liver cell damage or bile duct damage within the liver itself.

1.4 Pathogenesis of dengue haemorrhagic fever

In its most severe form, DHF was associated with haemorrhagic complications, plasma leakage, shock and disseminated intravascular coagulopathy. Often considered more common in children, DHF was increasingly diagnosed in adults as a consequence of shifting patterns of infection and immunity.

1.4.1 Role of antibody-dependent enhancement (ADE)

The role of host immune responses had been suggested by epidemiologic data. In dengue epidemic of dengue serotype 2 in Cuba in 1981, most DHF cases were those who had acquired dengue antibodies in the previous epidemics by dengue serotype 1 in 1997 and 1978 (Guzman, 1990). Meanwhile, studies in Thailand demonstrated that nearly 90% of DHF cases were those with secondary infection with a serotype of dengue virus different

from that which caused the primary infection (Halstead, 1977a, Gubler, 1998). It was suggested that the pre existing non-neutralizing antibodies may form complexes with the virus and enhanced its uptake and replication in the macrophages (Halstead, 1977b, Halstead, 1977a)

1.4.2 Role of Cytokines

A series of studies had suggested that plasma leakage in DHF was caused by malfunction of vascular endothelial cells induced by cytokines or chemical mediators rather than by destruction of the small vessels (Green, 2006). Plasma levels of various cytokines including tumour necrosis factor-alpha (TNF-α), interleukin-2 (IL-2), IL-6, IL-8, IL-10 and IL-12 were significantly elevated in DHF than in DF. Both dengue virus infected monocytes or mast cell / basophile line and activated specific T lymphocytes were believed to be responsible for this increased level of cytokines (Kurane, 2007). It was also suggested that on secondary exposure to a different viral serotype, most serotype-cross-reactive CD4+ and CD8+ T cells were able to augment infection by producing various cytokines (Kurane, 2007).

A recent study in Taiwan comparing immune mediators among patients with DHF and DF had demonstrated that patients with DHF had a higher rate of secondary dengue infection as well as higher IL-10, higher soluble vascular cell adhesion molecule level 1 (svCAM) levels than DF patients (Chen, 2007). However, they had a lower interferon-gamma (IFN-

y) level. Thus it had been suggested that predominant Th2 responses (IL-2, IL-10) occur in DHF while Th1 (IFN-y) responses seem to protect against infections.

1.4.3 Role of Complement Activation

It was reported that the levels of complement 3a (C3a) and complement 5a (C5a) were correlated with the severity of DHF, and the levels reached the peak at the time of defervescence when the plasma leakage became more obvious. It had been assumed that complement was activated by various mechanism in DHF including immune complexes and high levels of secreted non-structural protein 1 (NSI) and pre-existing cross-reactive antibody (Aviruthan, 2006).

1.4.4 Virulence of viruses

There were multiple genotypes in each of the four dengue viruses serotypes. There were some cases in which primary dengue infection resulted in DHF, and this suggested that virulent dengue virus strains cause DHF. The introduction of Southeast Asian genotype coincided with the appearance of DHF in different countries in the America continent, while the original American genotype was only correlated with DF, not DHF (Rico-Hesse, 1997b, Rico-Hesse, 1997a). The molecular determinants of dengue virus virulence were still not exactly determined, and attempts had been made by many groups to determine it. One group researched that the determinants for virulence resided at the amino acid 390 of

the E protein, in the 5' non-translated region and in the upstream 200 nucleotides of the 3' non-translated region (Leitmeyer, 1999). Further studies were required to define further the molecular bases underlying different dengue virulence.

1.4.5 Liver involvement in the pathogenesis of dengue demorrhagic fever

Dengue virus can infect many cell types and resulted in diverse clinical and pathological effects. Its main effects were on the vascular and hematological systems. However, both clinical and experimental observations suggested that there was liver involvement during dengue infection, especially a more severe and complicated form of dengue infection. Although the liver was not a major target organ, the involvement of the liver in the pathogenesis of dengue virus infection, in particular concerning the development of DHF was demonstrated by the abnormal liver function tests, tissue injury, presence of viral antigens, and RNA in human liver tissue.

1.5 Histological changes in the liver and detection of dengue virus

Most reports of histological changes in the liver of dengue were based on small numbers of samples obtained from fatal cases. The changes include hepatocellular necrosis, microvesicular steatosis, Kuppfer cell hyperplasia and destruction, Councilman bodies and cellular infiltrates at the portal tract (Seneviratne, 2006). Hepatocellular necrosis in dengue generally occurred in the midzonal area and sometimes the centrilobular area. It was believed that the hepatocytes in this area were more prone to hypoxia or the products of an