Gallbladder Wall Thickening for Early Detection of Plasma Leakage in Dengue Infected Adult Patients

Leonard Nainggolan, Candra Wiguna, Irsan Hasan, Esthika Dewiasty

Departement of Internal Medicine, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

Corresponding Author:
Leonard Nainggolan, MD, PhD. Division of Tropical and Infectious Disease, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital. Jl. Diponegoro no. 71, Jakarta, 10430, Indonesia. email: leonard.nainggolan@ui.ac.id, leonard.golan@yahoo.com.

ABSTRAK

Latar belakang: *kebocoran plasma didefinisikan sebagai peningkatan hematokrit ≥20% dari baseline atau penurunan pemulihan atau bukti kebocoran plasma seperti efusi pleura, asites atau hypoproteinaemia/ hypoalbuminaemia. Tanda-tanda kebocoran plasma ini, pada fase awal, biasanya sulit untuk dipastikan dengan pemeriksaan fisik dan tes laboratorium di mana pasien hanya mencerminkan tingkat ringan kebocoran plasma. Penelitian ini bertujuan menentukan peran penebalan dinding kandung empedu dalam mendeteksi kebocoran plasma pada fase awal infeksi dengue.* ***Metode:*** *penelitian ini adalah suatu studi diagnostik yang dilakukan pada pasien dengue yang mengalami demam kurang dari tiga hari dengan hasil uji non-structural protein 1 antigen dengue dan RT-PCR positif. Pemeriksaan laboratorium dan USG toraks dan abdomen dilakukan setiap hari mulai hari ke-3 hingga hari ke-7 demam untuk melihat adanya penebalan dinding kandung empedu dan kebocoran plasma berdasarkan kriteria WHO 1997 selama perawatan.* ***Hasil:*** *dari 69 subyek penelitian yang didapat, 52,2% adalah laki-laki, rerata usia 24,2 tahun, dan 46 pasien (66,7%) mengalami infeksi dengue sekunder. Pada hari ketiga demam, terdapat 37 pasien dengan penebalan dinding kandung empedu dan 30 di antaranya terbukti mengalami kebocoran plasma selama perawatan. Dari 46 pasien yang mengalami kebocoran plasma, 12 di antaranya sudah menunjukkan kebocoran plasma sejak hari ketiga demam. Penebalan dinding kandung empedu pada demam hari ketiga memiliki nilai sensitivitas dan spesifisitas sebesar 65% (IK 95%: 0,51-0,79) dan 70% (IK 95%: 0,51-0,88); nilai duga positif dan nilai duga negatif sebesar 81% (IK 95%: 0,68-0,94) dan 50% (IK 95%: 0,33-0,67); rasio kemungkinan positif dan negatif sebesar 2,14 (IK 95%: 1,12-4,12) dan 0,5 (IK 95%: 0,31-0,81).* ***Kesimpulan:*** *penebalan dinding kandung empedu dapat dipergunakan untuk mendeteksi adanya kebocoran plasma pada fase awal infeksi dengue.*

Kata kunci: *kebocoran plasma, penebalan dinding kandung empedu, ultrasonografi, kriteria WHO 1997, dengue.*

ABSTRACT

Background: *plasma leakage is defined as ≥20% elevation of hematocrit from baseline or decrease in convalescence or evidence of plasma leakage such as pleural effusion, ascites or hypoproteinaemia/ hypoalbuminaemia. These signs of plasma leakage, in the early phase, are usually difficult to ascertain by physical examination and laboratory tests where the patient is only reflecting a mild degree of plasma leakage. This study aimed to investigate whether gallbladder wall thickening (GBWT) in the early phase of the disease can be used to detect the occurrence of plasma leakage in dengue patients.* ***Methods:*** *a diagnostic study was conducted among dengue patients. Patients with fever less than 3 days, positive results of non-structural*

protein 1 antigen dengue and RT-PCR examination were included consecutively. Laboratory tests and chest and abdominal ultrasonography examination were also performed daily from day-3 to day-7 of fever to confirm the occurrence of plasma leakage using WHO 1997 criteria during treatment. ***Results:*** *there were 69 patients included in this study. Male patients were found more frequently (52.2%), average age was 24.2 years, and 46 patients (66.7%) presented with secondary dengue infection. On the third day of fever, 37 patients presented with GBWT, 30 of which showed plasma leakage during treatment. Out of 46 patients found to have plasma leakage during treatment, 12 patients had presented with plasma leakage on the third day of fever. Sensitivity and specificity of GBWT on the third day of fever were 65% (95% CI: 0,51-0,79) and 70% (95% CI: 0.51-0.88); PPV and NPV were 81% (95% CI: 0.68-0.94) and 50% (95% CI: 0.33-0.67); LR (+) and LR (-) were 2.14 (95% CI: 1.12-4.12) and 0.5 (95% CI: 0.31-0.81), respectively.* ***Conclusion:*** *gallbladder wall thickening in the early phase of the disease can be used to detect the occurrence of plasma leakage in adult dengue infected patients.*

Key words: *plasma leakage, gallbladder wall thickening, ultrasound, WHO 1997 criteria, dengue.*

INTRODUCTION

Dengue infection is an arbovirus disease (arthropod-borne virus) that has become a major public health concern across the world. Most of these infections are asymptomatic, but infections can also show clinical manifestations ranging from flu-like illness, mild fever, myalgia, arthralgia accompanied by signs of leucopenia, rash, lymphadenopathy, thrombocytopenia and diathesis, to fatal manifestations that are characterized by hypovolemic, shock, and death.[1]

Several studies have suggested that plasma leakage is the main hallmark in dengue pathogenesis. A complex interaction between virus, host immune response and endothelial cells likely impacts the barrier integrity and functions of endothelial cells leading to plasma leakage.[2] It is important to be able to demonstrate signs of plasma leakage because this condition can lead to the loss of intravascular volume and circulatory insufficiency, shock, and death.[3] Early detection of plasma leakage is essential in order to improve management and decrease mortality rate in dengue infection.

According to World Health Organization (WHO) 1997 criteria, plasma leakage is defined as elevation of hematocrit by ≥20% from baseline or decrease in convalescence or evidence of plasma leakage such as pleural effusion, ascites or hypoproteinaemia/hypoalbuminaemia.[1] These signs of plasma leakage, in the early phase, are usually difficult to ascertain by physical examination and laboratory tests where the patient is only reflecting a mild degree of plasma leakage. Several studies have demonstrated that ultrasonography is useful to detect plasma leakage signs such as gallbladder wall thickening (GBWT), ascites, and pleural effusion but unfortunately, as of now, GBWT has not been included in the WHO criteria. A few other studies suggest that the underlying mechanism of gallbladder wall thickening is gallbladder subserosal edema and have been said to be the most common initial ultrasound finding compared to ascites and pleural effusion.[4-5] Therefore the purpose of this study is to investigate whether gallbladder wall thickening in the early phase of the disease can be used to detect the occurrence of plasma leakage in adult dengue infected patients.

METHODS

This was a diagnostic study comparing the accuracy of an index test of plasma leakage in the form of gallbladder wall thickening with a reference test in the form of WHO 1997 criteria. This research was performed in the internal medicine ward of Cipto Mangunkusumo Hospital from July 2011 until October 2012. This study had been approved by the Ethical Committee on Health Research, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital with reference number 063/PT2.FK/ETIK/2010.

Selection of Research Subjects

The sample selection was done by consecutive sampling; inclusion and exclusion criteria were set prior to selection of trials. The inclusion

criteria in this study were dengue patients with a history of fever of less than 3 days, positive result of non-structural protein 1 antigen dengue (NS1) examination, which was later confirmed by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), and willing to be included in the study. Exclusion criteria in this study were patients who experienced other conditions that can cause gallbladder thickening, such as patients who suffers from gallstones, gallbladder malignancy, cirrhosis, right-sided heart failure, renal failure, and pancreatitis.

Sample Analysis

Variables obtained and examined during this study including laboratory tests and ultrasonography examination were performed daily from day-3 to day-7 of fever to confirm the occurrence of GBWT and plasma leakage using WHO 1997 criteria during treatment. Signs of plasma leakage according to WHO 1997 criteria include ≥20% elevation of hematocrit from baseline or decrease in convalescence or evidence of plasma leakage such as pleural effusion, ascites or hypoproteinaemia/ hypoalbuminaemia.[1] Assessment of hemoglobin, hematocrit, leucocyte, and platelet levels were performed using the Sysmex KX-21 Hemocytometer (Medical Electronics, Japan). NS1 dengue and dengue serological test were conducted using the SD Bioline Dengue Duo (Dengue NS1 Antigen and Antibody Combo). RT-PCR was performed using Light Cycler 2.0 and its reagents. Abdominal and thoracic ultrasound examination was performed using USG Aloka SSD 3500 series using convex probe with the frequency of 2.5-5 MHz. This examination was performed to detect plasma leakage in the form of gallbladder thickening, ascites and pleural effusion.

Gallbladder wall thickness was measured by calipers between two layers of the anterior wall and detected when the thickness of the posterior wall was perpendicular to the axis and the short axis was more than 3 mm and was seen as an anechoic zone visible as a double-layered wall. The examination was done after the patient underwent fasting for 6 hours. We examined the hepatorenal pouch and retrovesicular area in a supine position for ascites. Fluid present in the hepatorenal pouch was recorded as present or absent. To detect pleural effusion, longitudinal scans of the right hemithorax at the midclavicular and the midaxillary line and a transverse scan of the right upper abdominal quadrant were performed in the supine position. A longitudinal scan at the right midaxillary line in an upright position was performed after subjects were in an upright position for at least 3 minutes. The vertical dimensions of the fluid collection were determined by measuring the distance between the top of the dome of the diaphragm and the base of the lung.[6]

The ultrasound examination was performed by an experienced sonographer who did not know the clinical and laboratory data of the subjects examined. Inspection results will be printed, recorded, and reinterpreted by another experienced sonographer. Interoperator suitability was assessed with Kappa >0.9.

Statistical Analysis

Statistical analysis was carried out with SPSS Statistics software version 17. Univariate analysis are shown in the form of subject characteristic table. Categorical variables are presented in numbers and percentages, while numerical variables are shown in the mean and standard distribution. 2x2 crosstab is then created to get a positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio.

RESULTS

From July 2011 to October 2012, there were 69 patients out of 72 patients with dengue infection treated at the Internal Medicine ward of Cipto Mangunkusumo Hospital, who were included in the study. Three patients were excluded due to a negative RT-PCR result. A total of 36 patients were men. The mean age of the subjects was 24.2 years. The majority of patients were admitted on the second day of fever. A total of 46 patients or 66.7% had secondary infections. Demographic and clinical characteristics of the subjects can be seen in **Table 1**.

Table 1. Characteristics of study subjects

Variables	3rd day of fever	During treatment
Male gender, n (%)	36 (52.2)	
Age (years), mean (SD)	24.2 (10)	
2nd day of fever on admission, n (%)	50 (72.5)	
Secondary infection, n (%)	46 (66.7)	
Platelet count (/µL), mean (SD)	145.130 (48.430)	
Gallbladder wall thickness (mm), mean (SD)	3.30 (1.10)	
Gallbladder wall thickness, n (%)	37 (53.6)	44 (63.7)
Hemoconcentration, n (%)	0 (0)	15 (21.7)
Hypoalbuminemia, n (%)	0 (0)	25 (36.2)
Ascites, n (%)	10 (14.5)	19 (27.5)
Pleural effusion, n (%)	2 (2.9)	10 (14.5)
Plasma leakage according to the WHO 1997 criteria, n (%)	12 (17.4)	46 (66.7)

Thickening of the Gallbladder Wall and Plasma Leakage

On the third day of fever, there were 37 patients presented with GBWT with a mean thickness of 3.30 mm. Among these 37 patients, 30 were proved to have plasma leakage during treatment. There were 16 patients who did not experience GBWT but were proved to have plasma leakage during treatment. By using the WHO 1997 criteria on the third day of fever, plasma leakage was only detected in 12 patients out of the 46 patients with plasma leakage during treatment. In the following days of treatment, there were 7 additional patients with GBWT which made it 44 patients out of 69 total.

Table 2. Crosstab table of gallbladder wall thickening on the third day of fever and plasma leakage based on WHO 1997 criteria during treatment

	Plasma leakage during treatment		Total
	Yes	No	
GBWT on the third day of fever			
- Yes	30	7	37
- No	16	16	32
- Total	46	23	69

From **Table 2**, it can be calculated that the sensitivity of thickening of gallbladder wall on the third day of fever was 30/46 or equal to 65% (95% CI: 0.51-0.79), whereas its specificity was 16/23 or 70% (95% CI: 0.51-0.88) The positive predictive value was 30/37 or 81% (95% CI: 0.68-0.94), while the negative predictive value was 16/32 or of 50% (95% CI: 0.33-0.67). Its positive likelihood ratio was (30/46): (7/23) or equal to 2.14 (95% CI: 1.12-4.12) while its negative likelihood ratio was (16/46): (16/23) or equal to 0.5 (95% CI: 0.31-0.81).

DISCUSSION

Dengue fever (DF) is typically a self-limiting illness with a mortality rate of <1%. Contrastingly, dengue hemorrhagic fever (DHF) has a mortality rate of 2-5% with treatment but without treatment mortality rate is as high as 50%.[7] The course of dengue infection is divided into three phases: febrile, critical, and recovery phases. Febrile phase is characterized by rapid onset of high-grade fever associated with muscle pain, vomiting, epigastric discomfort, and headache which lasts for up to about three days. In the critical phase, usually on day 3–7 of illness, there is a significant increase of capillary permeability which leads to plasma leakage and laboratory changes. The convalescent phase which occurs after critical phase is characterized by the stop of plasma leak and concomitant reabsorption of extravasated plasma and fluids occurs. Even in those with complications, if managed successfully, full recovery often occur without sequelae and develop a life-long immunity against the particular virus serotype.[1,7]

Increased capillary permeability is the main hallmark in dengue pathogenesis that leads to plasma leakage, hemoconcentration,

hypovolemic, shock and death, which generally occurs in critical phase.[1,2,8] Serial hematocrit measurements to detect hemoconcentration, as well as serum albumin levels, evidence of pleural effusions and ascites by physical examination are frequently used to identify plasma leakage but often hard to be detected. A number of factors may affect hematocrit findings, such as individual variations (e.g. age, gender, and race), absence of baseline hematocrit levels, intravenous fluid administration, and bleeding.[1] The albumin level cut-off used in this study was <3.5 g/dL, while the increase of vascular permeability up to a certain point would not change the level of serum albumin and studies showed that in severe cases, level of albumin was no longer decreased.[9] Determining ascites and pleural effusion by clinical and physical examination are generally difficult when only small amount of fluid is present.

Ultrasonography (USG) is a cheap, safe, rapid and widely available non-invasive imaging modality. Furthermore, ultrasonography is also a portable imaging method that can be done at patient's bedside.[11] The main advantage of ultrasonography is its high sensitivity to detect even smaller amounts (minimal) of pleural effusion, ascites, and the possibility to visualize the gallbladder wall thickening.[4] The rapid resolution of gallbladder wall thickening and ascites suggests that the underlying mechanism was a transient increase in permeability rather than inflammation. Gallbladder wall thickening was also seen to significantly occur during 3rd to 5th day of fever, which proves that GBWT has started to occur from the febrile phase up until the critical phase of illness.[6,11] GBWT resolves fast, there are higher chance of obtaining positive findings of GBWT when ultrasound examination is performed within 5 days from the onset of fever. Gallbladder wall thickening is correlated with the degree of hemoconcentration, plasma albumin, and AST levels.[6] So far, GBWT has not been included in WHO plasma leakage criteria, therefore GBWT ultrasonography examination in the febrile phase (early phase) may be needed to detect the presence of plasma leakage, and thus, the severity of the disease before they become clinically apparent could be detected.

The aim of this study was to investigate whether gallbladder wall thickening can be used to detect the occurrence of plasma leakage in the early phase of the disease. The subjects included in this study were patients admitted to the Internal Medicine Ward of Cipto Mangunkusumo Hospital with onset of fever less than 3 days. Evidence of dengue infection was based on a positive NS1 examination which later confirmed by RT-PCR. Onset of fever less than 3 days was one of the inclusion criteria and was intended to enable ultrasound examination on the third day of fever in order to have early detection of plasma leakage.

In this study, male gender was slightly higher (52.2%). This gender ratio was similar with studies conducted by Vedaraju et al[7] with 54 males out of a total 102 patients (52.9%), while Hegde et al[12] reported 68 male patients versus 32 female patients (68%). In general, it is found that in dengue infection the male: female ratio is 1:1. Most studies on dengue infection was investigated in children while our study was conducted in adults with the mean age of 24.2 years. This is similar with studies conducted by Vedaraju et al.,[7] with the most common age group reported was 20-39 years[7] and Nagolu et al.[13] who performed a study in dengue infection with an average age of 48.9 years. In endemic areas such as Jakarta, secondary infections are usually more prevalent. In this study, 46 patients (66.7%) were admitted with secondary dengue infection. This figure was greater than Zulkarnain's study of 47%, but similar to the data from Jakarta (67%).[13,15]

By using the WHO 1997 criteria, on the third day of fever plasma leakage was obtained in 12 patients (17.4%), ascites in 10 patients and pleural effusion in 2 patients. Among these 12 patients, 9 patients had gallbladder wall thickening on the third day of fever, and 2 patients on the fifth day of fever. Only 1 patient until the end of treatment did not have gallbladder wall thickening. There was no hemoconcentration and hypoalbuminemia on the third day of fever. Those findings could be explained by the fact that signs of plasma leakage by USG such as pleural and pericardial effusion, ascites, and gallbladder wall thickening

were detectable before changes in hematocrit level and furthermore hypoalbuminemia and hemoconcentration generally occur on day 4 to day 7 of fever.[9]

During treatment, plasma leakage was found in 46 patients (66.7%) using the WHO 1997 criteria. Hemoconcentration was seen in 15 patients (21.7%) while it was seen in 21 patients (31.8%) in a study conducted by Michels et al.[7] The difference of these findings was due to the fact that the patients in this study had been given fluid therapy shortly after the patients were admitted to the hospital. Hypoproteinemia was seen in 25 patients (36.2%) and this hypoproteinemia was similar with previous findings which occur on the fourth and fifth day of fever.[9]

Ascites was seen in 19 patients (27.5%). In other studies, ascites was found in 78.4% of patients by Oliveira et al.[5] and 55% of patients by Hegde et al.[12] We found pleural effusion to be the least common USG finding in this study, which was found in 10 patients (14.5%). Oliveira et al[5] and Hedge et al[12] reported that pleural effusion was present in 70.3% and 47% out of all their subjects, respectively. The lower number of patients found with ascites and pleural effusion in this study might be related to the fact that the subjects in this study were adult. Some researchers suspected that vascular permeability in children is more common than in adults.[16] The subjects of the other studies mentioned above were children.

In our study, gallbladder wall thickening on the third day of fever was found in 37 patients (53.6%) and was the most common USG finding. To the best of our knowledge, there has been no study that shows the prevalence of gallbladder thickening on the third day of fever, however, the findings of our study are in accordance with previous studies by Oliveira et al[12] (89.2%) and Vedaraju et al[7] (83.3%), which also showed GBWT as the most common USG finding. Lower number of patients found with GBWT in our study was due to the fact that the ultrasound examination in this study were done at an early phase of fever that was within 3 days of the onset of the fever (febrile phase) while the ultrasound examination on the studies mentioned above were mainly done after 3 days of the onset of the fever (critical phase) where plasma leakage has occurred. The difference in antigenic strains and patient susceptibility might also have contributed for the difference in the number of incidence of GBWT findings. Furthermore, the presence of gallbladder wall thickening was associated with severity of the disease.

The mean thickness of the gallbladder wall in this study was 3.30 mm (1.68 to 7.35 mm). Kim et al[17] reported mean gallbladder wall thickness of 6.1 mm (4.0 - 15 mm), but the study was conducted in dengue patients with impaired renal function. Other studies that examined gallbladder wall thickening in patients with dengue infection did not mention the average thickness of the gallbladder wall.

This study showed that on the third day of fever, the WHO 1997 criteria was only able to detect 12 patients with plasma leakage. WHO criteria is indeed often unable to detect plasma leakage that occurs in the early phase of dengue infection. In this study, it has been demonstrated that the sensitivity and specificity of thickening of gallbladder wall on the third day of fever were 65% and 70% respectively. Whereas the positive predictive value and negative predictive value were 81% and 50% respectively. Its positive likelihood ratio was 2.14, while its negative likelihood ratio was 0.5. Based on these findings GBWT that appears at the beginning of dengue infection was actually a good indicator of plasma leakage.

The presence of gallbladder wall thickening on the third day of fever was evidence that plasma leakage occurs on the third day of fever when physical and laboratory examination had not been able to detect plasma leakage. Gallbladder wall thickening in dengue often precedes other plasma leakage parameters and these findings revealed that ultrasound examination of the gallbladder wall on the third day of fever has an important role in detecting plasma leakage in the early phase of dengue infection. Gallbladder wall thickening in the early phase of the disease can be used to detect the occurrence of plasma leakage in adult dengue-infected patients. The early detection of plasma leakage is expected to be able to reduce mortality rate.

CONCLUSION

We have conducted a study in adult patients with dengue infection and obtained a good positive predictive value for the early detection of plasma leakage by finding GBWT using ultrasonographic examination. Ultrasonographic measurement of gallbladder wall thickening is a cheap, safe, rapid and non-invasive imaging method that can be useful to detect the occurrence of plasma leakage in the early phase of dengue infection. We believe that GBWT can be relied upon as an additional criterion to support the clinical diagnosis of dengue with plasma leakage (DHF).

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