

RESEARCH ARTICLE

The Association of Lipoprotein Changes and the Development of Plasma Leakage in Dengue Infection

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Abstract

There's interrelationship between infection and lipoprotein. This is a cohort prospective study which conducted November 2010 – February 2011. This study aimed to assess the changes of HDL-C, LDL-C, total cholesterol (TC), triglyceride (TG), in acute and critical phase of dengue infection and its association with plasma leakage. Subjects who had fever 48 hours or less and Dengue NS1 antigen test positive were admitted to Cipto Mangunkusumo Hospital Jakarta. We examined clinical and CBC daily; level of albumin, HDL-C, LDL-C, TC, TG; ultrasound to find ascites and pleural effusion. Among 51 subjects, 21 subjects (41%) had plasma leakage. There were significant lower of HDL-C, LDL-C, and higher TG in critical phase than acute phase. In critical phase, subjects with plasma leakage had a significant lower HDL-C level [26.3 (8.2) vs 33.1 (12.1) mg/dL, $p=0.029$] but not for LDL-C, TC, and TG. They also had a significantly higher reduction in HDL-C [19.6 (9.1) vs 11.5 (5.8) mg/dL, $p<0.0001$] and TC [25.1 (20.0) vs 15.2 (14.5) mg/dL, 0.045] over the course of acute to critical phase. Lipoprotein changes during dengue infection were more pronounced among subjects who developed plasma leakage. The higher reduction in HDL-C is associated with the development of plasma leakage.

Keywords: dengue infection, plasma leakage, cholesterol, lipoprotein, HDL

Hubungan antara Penurunan Kadar Kolesterol dengan Kejadian Kebocoran Plasma pada Infeksi Dengue

Abstrak

Terdapat hubungan timbal balik antara infeksi dengan lipoprotein. Penelitian ini adalah penelitian kohort prospektif yang dilakukan November 2010 – Februari. Penelitian ini bertujuan untuk mengetahui perubahan kolesterol HDL, LDL, kolesterol total, dan trigliserid (TG) pada fase akut dan kritis infeksi dengue serta hubungannya dengan kebocoran plasma. Subyek dengan demam 48 jam atau kurang dan uji Dengue NS1 antigen positif dirawat di RSUPN Cipto Mangunkusumo Jakarta. Dilakukan pemeriksaan klinis dan darah perifer lengkap setiap hari; pemeriksaan albumin, HDL, LDL, kolesterol total, TG, ultrasonografi untuk mendeteksi asites dan efusi pleura. Dari 51 subyek, 21 subyek (41%) mengalami kebocoran plasma. Kadar HDL, LDL, kolesterol total lebih rendah secara bermakna pada fase kritis dibanding fase akut (TG lebih tinggi). Pada fase akut, HDL subyek dengan kebocoran plasma lebih rendah secara signifikan dibandingkan tanpa kebocoran plasma [26.3 (8.2) vs 33.1 (12.1) mg/dL, $p=0.029$] tetapi tidak untuk LDL, kolesterol total, TG. Subyek dengan kebocoran plasma mengalami penurunan HDL [19.6 (9.1) vs 11.5 (5.8) mg/dL, $p<0.0001$] dan kolesterol total [25.1 (20.0) vs 15.2 (14.5) mg/dL, 0.045] yang lebih tinggi dari fase akut ke fase kritis. Terdapat perubahan signifikan kadar lipoprotein pada subyek dengan kebocoran plasma selama infeksi dengue. Penurunan kadar HDL yang lebih tinggi berhubungan dengan kebocoran plasma.

Kata kunci: infeksi dengue, kebocoran plasma, kolesterol, lipoprotein, HDL

Introduction

Dengue infection is a mosquito-borne viral disease that has rapidly spread in all regions of WHO in recent decades. About half of the world's population is now at risk.¹ Southeast Asia is one of the endemic areas of dengue wherein Indonesia is the largest contributor with 57% of dengue cases and 70% of mortality cases.² Climate changes, a very large people mobilization, and lifestyle changes make the transmission area and dengue problem expanded. Dengue cases in Indonesia can be found throughout the year and usually associated with the rainy season. The peak incidence of dengue cases occur at the end of the season.³

Clinical manifestations of dengue infection are varying from asymptomatic, shock or death. The course of dengue fever is divided into three phases: acute phase (1st-3rd days of fever), critical phase (4th-6th days of fever), and recovery phase.⁴ During acute phase, the proinflammatory cytokines usually produced excessively; most of them reached the peak at the end of the acute phase or before entering the critical phase. During critical phase, plasma leakage may occur. Severity of dengue infection symptoms associated with severity of plasma leakage. The mortality of dengue infection primarily occurs due to hypovolemic shock due to leakage of plasma from the intravascular to extravascular arising from endothelial dysfunction.^{5,6}

Endothelial plays important roles in pathogenesis of plasma leakage.^{5,7} Proinflammatory cytokines produced by the infection will activate endothelium, enlarge gap between the endothelium, and cause plasma leakage. The increasing of endothelial activation marker such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule (VCAM), E-selectin associated with the occurrence of plasma leakage.⁸⁻¹⁰ It has been widely investigated that cholesterol and lipoproteins, especially high-density lipoprotein cholesterol (HDL-C), play important roles in endothelial function. HDL-C can reduce the expression of endothelial activation marker but its role in plasma leakage in dengue infection need further research.¹¹

Regardless the roles of lipoprotein in endothelial function, lipid metabolism has changed during acute phase and these changes mostly induced by the changes of cytokine levels.¹²⁻¹⁴ Cholesterol and lipoprotein levels changes are associated with the severity of some conditions such as sepsis, trauma, post-surgery, respiratory tract infection, acute renal failure, and heart failure.¹⁵⁻²⁰ Lipid profile in dengue

infection has not been widely studied and the existing studies were conducted in children and used cross sectional design.²¹⁻²³ This study aimed to evaluate the changes of lipid and lipoprotein levels in the natural course of adult dengue infection from acute phase up to critical phase and to explore their relationship with the incidence of plasma leakage.

Methods

This study is a cohort prospective study which conducted November 2010 – February 2011 at community in East Jakarta Indonesia. The aiming of this study is to compare the changes mean levels of HDL-C, low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and triglyceride (TG) between acute phase and critical phase of dengue infection and its association with the event of plasma leakage.

Subjects are patients aged 14 years old or older with history of sudden fever 48 hours or less, diagnosed as dengue fever according to WHO criteria year 1997 and had positive result on Dengue NS1 antigen test. Patients with mixed infection, pregnancy, comorbidities such as renal failure, congestive heart failure, cirrhosis, and familial hypercholesterolemia were excluded. All patients who fulfilled the inclusion and exclusion criteria were admitted to Cipto Mangunkusumo Hospital to receive standard treatment of dengue infection. Written informed consents were obtained from all subjects and study protocols were approved by ethics committee of Cipto Mangunkusumo Hospital.

Anamnesis, physical examination and laboratorium examination were conducted at the beginning of admission. Subjects were performed routine blood examination every 24 hours at the third days until seventh day. Profile lipid examination, such as TC, HDL-C, LDL-C, and TG, were performed in the morning of the third days and fifth day (subjects had fasting 12 hours before the examination). Data collected include: 1. Patients' characteristics: gender, age, duration of the fever, hemorrhagic manifestations 2. Daily level of hemoglobin, hematocrit, leukocytes and platelets 3. Level of TC, HDL-C, LDL-C and TG on the third and fifth day 4. Dengue serology on the seventh day 5. Level of blood albumin on the third and fifth day. Levels of HDL-C, LDL-C, TC and TG were measured by enzymatic method, using C501 Cobas Roche/Hitachi. All laboratory tests were conducted at the Department of Clinical Pathology, Faculty of Medicine Universitas Indonesia.

Plasma leakage was also evaluated using abdominal ultrasound in addition to the value of hematocrit and blood albumin. The examination were conducted on the fifth day because of the incidence of plasma leakage occurred mostly at the fifth day of dengue infection.

The acute phase is the initial phase in dengue infection, characterized by fever. Critical phase is a phase of declining body temperature and increasing capillary permeability which accompanied by an increased in hematocrit. The significant of plasma leakage usually lasts 24-48 hours. Plasma leakage is defined as the presence of plasma into the third room, which is characterized by at least one sign as followed: 1. Increased hematocrit > 20% compared to standard, according to age and gender 2. Decreased in hematocrit > 20% after receiving fluid therapy compared to the previous hematocrit values 3. Presence of plasma leakage such as: pleural effusion, ascites, or gallbladder wall thickening on the ultrasound; hypoproteinemia (albumin <3 mg/dL).

This study used size sample formula for the mean differences between two independent groups hypothesis test. Type I errors set at 5%, one-way hypothesis, so $Z\alpha = 1.64$. A type II error set at 20%, so $Z\beta = 0.84$. In the preliminary study ($n=10$), standard deviation of changes levels of HDL-C in dengue infection was 8 and clinical differences were considered significant at 6, then size samples required for each group were 21.

Numerical data presented in mean and standard deviations, whereas the nominal data presented in proportion. Independent t-test was used to analyze the association of lipoprotein changes between group with plasma leakage and without plasma leakage. Paired t-test was used to analyze the association of lipoprotein changes

between acute phase and critical phase. Statistical analysis was performed using SPSS version 20 software.

Results

During this study, November 2010 – February 2011, we have collected 51 dengue infected patients whom fulfill research criteria. The age of subjects was ranging 14-51 years old with mean was 24.3 (SD 9.4) years old (Table 1). Plasma leakage was observed in 41% of the subjects: 67% was diagnosed using ultrasound, 57% was evaluated from hematocrit, and 5% were from hypoalbumin. Plasma leakage was more frequently observed in subjects with secondary infection (43% vs 40% in primary infection).

Table 1. General Characteristics of Dengue Infected Patient

Characteristics	n (%)
Male	24 (47)
Female	27 (53)
Primary infection	30 (58.8)
Secondary infection	21 (41.2)
Dengue infection without plasma leakage	30 (58.8)
Dengue infection with plasma leakage	21 (41.2)

There were significant lower levels of HDL-C, LDL-C, and TC in critical phase than acute phase on dengue infection; while level of TG was significantly higher (Tabel 2). There were no significant differences levels of lipoprotein in acute phase between dengue with plasma leakage and without plasma leakage. The level of HDL-C was significantly lower on dengue with plasma leakage in critical phase (Table 3). We observed significant higher reduction levels of HDL-C and TC between acute and critical phase in dengue with plasma leakage (Tabel 4).

Table 2. Levels of HDL-C, LDL-C, TC and TG in Acute and Critical Phase of Dengue Infection

Lipid Profile	Acute Phase (n=51)	Critical Phase (n=51)	p value
HDL-C	45.2 (SD 11.3)	29.8 (SD 11.2)	<0.0001
LDL-C	74.9 (SD 24.2)	62.5 (SD 24.1)	<0.0001
TC	134.2 (SD 27.5)	115.2 (SD 28.6)	<0.0001
TG	70.6 (SD 36.9)	109.1 (SD 34.5)	<0.0001

Table 3. The Differences Levels of HDL-C, LDL-C, TC, and TG in Acute and Critical Phase between Dengue with and without Plasma Leakage

Lipid Profile	Plasma Leakage		p value
	Negative (n=30)	Positive (n=21)	
Acute Phase			
HDL-C	44.7 (SD 12.0)	45.9 (SD 10.5)	0.715
LDL-C	76.7 (SD 26.8)	72.5 (SD 20.4)	0.553
TC	136.2 (SD 28.8)	121.4 (SD 26.0)	0.546
TG	74.2 (SD 36.5)	65.6 (SD 37.9)	0.419
Critical Phase			
HDL-C	33.1 (SD 12.1)	26.3 (SD 8.2)	0.029
LDL-C	65.0 (SD 25.3)	59.7 (SD 22.1)	0.442
TC	121.0 (SD 30.7)	106.3 (SD 25.7)	0.070
TG	112.8 (SD 29.9)	101.6 (SD 41.5)	0.267

Table 4. The Changes Levels of HDL-C, LDL-C, TC, and TG between Acute and Critical Phase in Dengue with Plasma Leakage and without Plasma Leakage

ΔLipid Profile	Plasma Leakage		p value
	Negative (n=30)	Positive (n=21)	
Δ HDL-C	- 11.5 (SD 5.8)	- 19.6 (SD 9.1)	<0.0001
Δ LDL-C	- 11.7 (SD 14.1)	- 12.9 (SD 16.4)	0.789
Δ TC	- 15.2 (SD 14.5)	- 25.1 (SD 20.0)	0.045
Δ TG	+ 38.7 (SD 39.2)	+ 36.1 (SD 27.6)	0.780

Discussion

Studies about the interrelationship between infection and lipoprotein have been widely published. Infection can alter lipoprotein profiles and vice versa lipoproteins play a role in pathophysiology of immune responses. Changes of HDL-C metabolism in the process of infection is closely associated with the production of cytokines such as tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6) and interferon- α (IFN- α). TNF- α will reduce levels of HDL-C by affecting the enzyme HMG-CoA reductase and lowering of lecithin-cholesterol acyltransferase (LCAT) activity (these two enzymes play a role in the esterification of free cholesterol in HDL-C).^{16,17,24} Moreover, production of cytokines may increase fatty acid and triglycerides in the blood by increasing fat tissue lipolysis, fatty acid synthesis in liver, lipogenesis, and suppression of lipoprotein lipase activity which decreased triglyceride clearance.^{16,17}

In non-dengue infection, Dunham, et al²⁵ reported that cholesterol levels were decreased in 90% of post-operative patients who experienced with infections. Bonville et al²⁶ reported that on the same population there were significant

changes levels of HDL-C, LDL-C, TC and TG on the second days of infection and continued till the seventh days. Reguero et al²⁷ reported on a cohort study in patients with pneumonia community that cholesterol level decreased in acute infection and returned to normal within 15 days.

This study showed that there were changes of lipoprotein levels from acute phase into critical phase: HDL-C, LDL-C, and TC levels were decreased while TG levels was increased. The changes of the significant reduction HDL-C and TC levels were more pronounced in the group with plasma leakage. This study has confirmed previous researches about the changing of lipoprotein levels in acute infection.

This study is the first study which evaluated the lipoprotein changes during acute phase and critical phase of dengue infection; there were significant reduction levels of HDL-C and TC in dengue with plasma leakage. The elevated levels of proinflammatory cytokines at the end of the acute phase of dengue infection, such as TNF- α , IFN- α , IL-6 and IL-10, can affect lipid metabolism. The higher levels of cytokines equal to the changes of lipid metabolism. Braga et al²⁸ reported that there was increased level of TNF-a in dengue infection; the cytokine was higher in dengue hemorrhagic fever (DHF) patient compared to dengue fever (DF) patient. Lee et al²⁹ reported that TNF-a levels were significantly higher on dengue shock syndrome (DSS) patient compared to DHF and DF patient and higher on DHF patient than DF patient. Hober et al³⁰ reported that levels of TNF-a and IL-6 were increased in dengue infection. Level of TNF- α increased before the sixth day of disease and stopped down till day tenth. On the other side

the decreased levels of HDL-C might be related to the high concentration of phospholipase A2.³¹ Juffrie et al³² reported that levels of phospholipase A2 were higher on DSS patient. Considering that the increasing of proinflammatory cytokines and phospholipase A2 were higher in dengue infection with plasma leakage make sense that the levels of HDL-C and TC were lower in the group with plasma leakage compared to patients without plasma leakage.

There are limited studies have discussed the changes levels of lipoprotein in dengue infection. However, all of the studies were limited on cross sectional design. Suvarna et al²¹ and Van Gorp et al²² observed the changes of lipoprotein levels in dengue patients compared to healthy subjects whereas Villar Centeno et al²³ compared it with other infection. Suvarna et al studied on 50 children with dengue infection and reported that the subjects with dengue infection had lower HDL-C, LDL-C, and TC levels, and higher TG levels. Van Gorp et al studied on 70 children with DSS and DHF also reported the similar findings. Meanwhile, Villar Centeno et al reported the different finding; DHF patient had lower levels of TC and TG. These different might be caused by different sampling time.

Van Gorp et al²² did sampling at the time of patients admitted to intensive care unit and it might be at the critical phase. Suvarna et al²¹ did sampling at the first day of admission and it might be at the critical phase. Villar Centeno et al²³ did sampling at 48-96 hours from the onset of fever and it might be at the acute phase of dengue infection. Those findings were analogous to this study which showed that there were no significant differences of cholesterol level in acute phase but HDL-C level was significantly lower during critical phase in patients with plasma leakage. Similar tendency was also possible on TC levels.

The important finding of this study was a lower HDL-C level at critical phase in patients with plasma leakage (DHF). The mechanism of this finding remained unclear. As was mentioned above that the level of cytokines in dengue infection with plasma leakage were higher compared to dengue without plasma leakage (DF).^{5,6} Cytokine levels inversely with levels of HDL-C: the higher levels of cytokines, the lower levels of HDL-C.¹⁷ It was also suggested the other possible mechanisms of decreased HDL-C levels during critical phase. Diameter of HDL-C is 5-10nm, the smallest among lipoprotein and similar to diameter of albumin (3.6-6nm).³³ There is widening of endothelial gap during

plasma leakage and may permit HDL-C throughout the vascular and caused decreasing level of HDL-C.

The impact of decreased HDL-C level during critical phase to endothelial function and its association in plasma leakage is appealing to be known. Many studies had shown that HDL-C play an important role in endothelial function. It is reported that HDL-C decreased the expression of adhesion molecules such as ICAM-1, VCAM, and E-selectin. Activation of endothelial cell in dengue infection will be followed by the changes of vascular permeability and plasma leakage. The administration of recombinant HDL (rHDL) may modulated the expression of adhesion molecules.¹¹ Cockeril et al³⁴ reported that administration of rHDL would decreased expressions of VCAM, E-selectin, and ICAM-1 which was induced by TNF- α .³⁵

HDL-C contained paraoxonase enzyme (PON1) and platelet-activating factor-acetylhydrolase enzyme (PAF-AH). PON1 protected lipoprotein from oxidation and limited the endothelial activation. Active form of platelet activating factor (PAF) activate leucocytes and increase vascular permeability but PAF-AH deactivated it.^{11,35,36} Based on this study and other studies we suggested that decreased HDL-C level in dengue infection play a role in endothelial activation and plasma leakage.

Some limitations in this study: we didn't measure the level of cytokines which can affect lipid metabolism and this condition make us difficult to explain the association between levels of cytokines kinetics and the changes of lipid levels. There is also no measurement of endothelial activation markers which led to difficulties in understanding the association between low HDL-C levels with plasma leakage.

Conclusion

The changes of HDL-C and TC level during critical phase in dengue infection are higher in group with plasma leakage. A study that explains the mechanism is needed. For clinical practice, serial examination of HDL-C level in dengue infection may become potential markers to predict the plasma leakage or as a warning sign of plasma leakage.

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