Changes in the Plasma Lipid Profile as a Potential Predictor of Clinical Outcome in Dengue Hemorrhagic Fever

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In 50 consecutive children admitted to the intensive care unit with the clinical diagnosis of dengue hemorrhagic fever (DHF)/dengue shock syndrome (grade III or IV), 20 patients with mild DHF (grade I or II), and 20 healthy control patients, the plasma lipid profile was measured. Levels of total plasma cholesterol, high-density lipoprotein, and low-density lipoprotein were significantly decreased in patients with the severest cases, compared with patients with mild DHF and healthy controls. Changes in the plasma lipid profile differentiate between patients with different stages of DHF disease severity and could be used as a potential predictor for clinical outcome.

Lipoproteins are thought to play a pathophysiological role in the host's immune response during severe infection [1]. Changes in the lipoprotein profile during infection probably are primarily cytokine induced [2]. In patients with bacterial infection, lipoproteins, including very low–density lipoprotein VLDL, bind endotoxin and thereby neutralize the toxic effects of endotoxin [3, 4]. Interactions between microorganisms and lipoproteins also occur in viral infections. Certain viruses use low-density lipoprotein (LDL) receptors to enter the cell, which implies that LDL may compete with viruses for these cellular receptors [5]. Therefore, high LDL levels may be beneficial

because they decrease virus uptake by cells. Lipoproteins also bind viruses and neutralize their toxic effects [6, 7].

In the present prospective study, we investigated whether changes in the serum lipid levels are found in patients with dengue hemorrhagic fever (DHF) and whether such changes may be relevant to clinical outcome. The study was performed in Semarang, Central Java, Indonesia, at the Dr. Kariadi University Hospital of the University of Diponegoro. Dengue is endemic in Java and there are yearly outbreaks of infection. Consecutive patients (children) with a clinical diagnosis of severe DHF (i.e., grade III or IV), as determined by the criteria of the World Health Organization (WHO), who were admitted to the pediatric intensive care unit during July–October 1996 were included [8]. In addition, 20 age-related patients with mild DHF (i.e., grade I and II) admitted to the pediatric ward and 20 age-related, nonfebrile, healthy children were included. The study protocol received approval from the Institutional Review Board of the University Hospital of Diponegoro University in Semarang, and informed consent was obtained from children's parents or guardians prior to study inclusion.

The clinical diagnosis of grade I and II DHF was made on the basis of abrupt onset of fever, thrombocytopenia ($<100 \times 10^9$ platelets/L), evidence of plasma leakage (manifested by hemoconcentration or signs of serous effusion), and a hemorrhagic tendency. A positive tourniquet test result and/or easy bruising in the absence of spontaneous bleeding differentiates DHF I from DHF II. A diagnosis of DHF III was made if there was additional circulatory failure manifested by a rapid and weak pulse and narrowing pulse pressure (<20 mm Hg) or hypotension in the presence of a cold clammy skin and restlessness. Patients with undetectable blood pressure or pulse were given a diagnosis of DHF IV [8].

In all subjects, the plasma lipid profile (i.e., cholesterol, high-density lipoprotein [HDL], LDL, and triglyceride levels) was determined. The presence of dengue virus was objectively confirmed by serologic assays. A capture and indirect ELISA detected dengue-specific IgM and IgG antibodies in serum samples, in accordance with a procedure described elsewhere [9]. Blood samples were obtained from all patients for culture to exclude bacterial infections. Blood samples for analysis of the serum lipid profile and cytokines were obtained on the day of admission. Venous blood samples were drawn into vacutainer tubes that contained 0.105 mL of sodium citrate (1 v; Becton Dickinson). All blood samples were immediately immersed in melting ice and subsequently centrifuged at 4°C for 20 min at 1600*g*. Plasma samples were stored at −70°C until assayed.
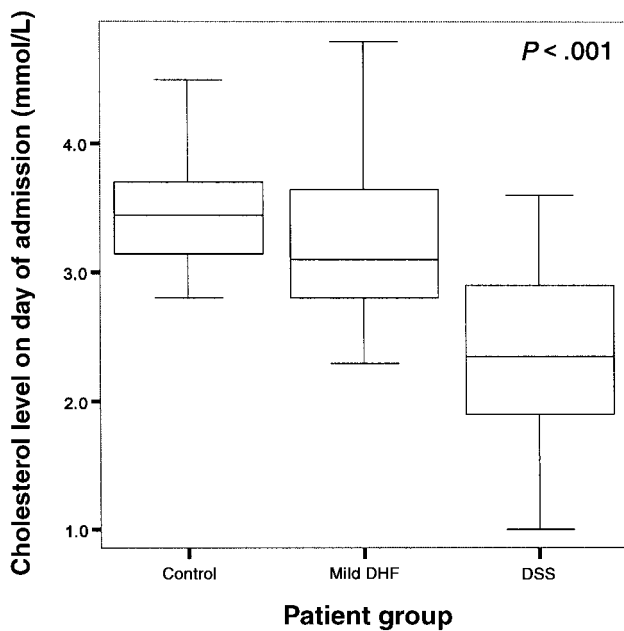
Figure 1. Box plots of serum cholesterol levels in control subjects and patients with dengue hemorrhagic fever (DHF) on day of admission to the hospital. Box plots show the median *(center horizontal line),* interquartile range (the 25th to the 75th percentile [*box*]), and the 5th and 95th percentiles *(whiskers). P* values were determined by the Kruskal-Wallis test for comparison of 3 groups. The median cholesterol level in the control group was 3.45 mmol/L (interquartile range, 3.08–3.70 mmol/L); in the mild DHF group, it was 3.10 mmol/L (interquartile range, 2.80–3.73 mmol/L); and in the dengue shock syndrome (DSS) group, it was 2.35 mmol/L (interquartile range, 1.85–2.90 mmol/L).

Plasma levels of cholesterol and triglycerides were determined by enzymatic methods that used commercially available reagents (CHOD-PAP reagent; Roche) by means of a Hitachi 747 automatic analyzer. Plasma HDL-cholesterol concentrations were determined after precipitation of LDL, VLDL, and chylomicrons by use of phosphotingsta-Mg^{2+} [10]. LDL-cholesterol concentrations were calculated according to the Friedewald formula [11].

The plasma levels of the analytes measured are presented as median values with their corresponding interquartile ranges (IQRs) and 95% CIs. The Mann-Whitney *U* test was used to compare the respective plasma levels among the different groups: healthy control subjects, patients with mild DHF, and patients with severe DHF. The Kruskal-Wallis test was used for comparison of the 3 groups. Two-tailed *P* values of <.05 were considered to indicate statistical significance. Analyses were performed by use of the statistical software SPSS, version 9.0 (SPSS).

From July 1996 through October 1996, a total of 50 consecutive children (mean age ± SD, 6.5 ± 2.8 years) with a clinical diagnosis of DHF III and IV were enrolled in the study. During follow-up, 13 patients (26%) in the dengue shock syn-

drome group (i.e., those with DHF grade III or IV) died of shock or bleeding complications in the intensive care unit. The baseline characteristics of patients in the severe DHF group who survived were comparable to those of patients who did not survive with regard to age, sex, disease severity at admission, and day of admission to the hospital (mean ± SD, 4.3 ± 0.9 vs. 4.1 ± 1.4) [12]. The mild DHF group also did not differ from the severe DHF group with regard to age, sex, and admission day, although the patients in the latter group already had evidence of severe infection at admission. The 20 children in the healthy control group were similar to the other groups in age and sex.

The clinical diagnosis of DHF was confirmed by serologic assay in all patients, either by an IgM response or a 4-fold increase in IgG titers. Antibody profiles were typical for secondary dengue infection. All patients were of Javanese origin, excluding racial differences. In all patients, blood cultures revealed no bacterial growth. Patients who had severe DHF were compared with patients who had mild DHF and with healthy control subjects, which revealed that groups differed significantly with regard to serum cholesterol, HDL, and LDL levels;
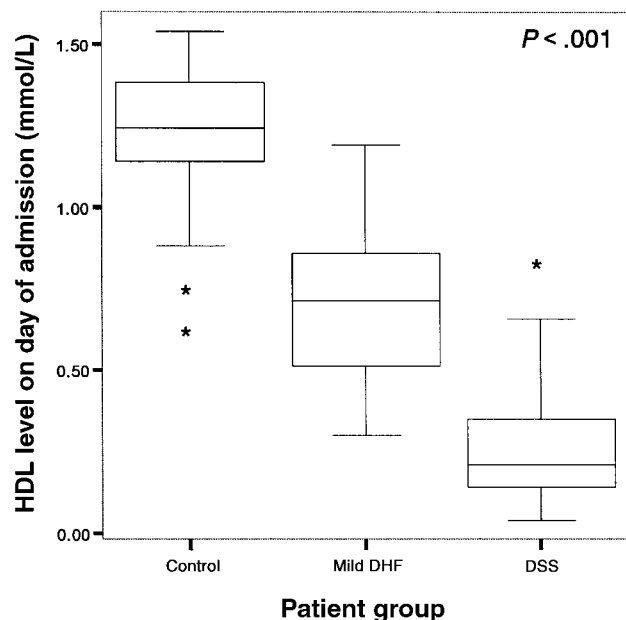

Figure 2. Box plots of serum high-density lipoprotein (HDL) levels in control subjects and patients with dengue hemorrhagic fever (DHF) on day of admission to the hospital. Box plots show the median *(center horizontal line),* interquartile range (the 25th to the 75th percentile [*box*]), and the 5th and 95th percentiles *(whiskers). P* values were determined by the Kruskal-Wallis test for comparison of 3 groups. The median HDL level in the control group was 1.25 mmol/L (interquartile range, 1.13–1.39 mmol/L); in the mild DHF group, it was 0.72 mmol/L (interquartile range, 0.5–0.87 mmol/L); and in the dengue shock syndrome (DSS) group, it was 0.21 mmol/L (interquartile range, 0.14–0.37 mmol/L). Outliers are presented as asterisks.
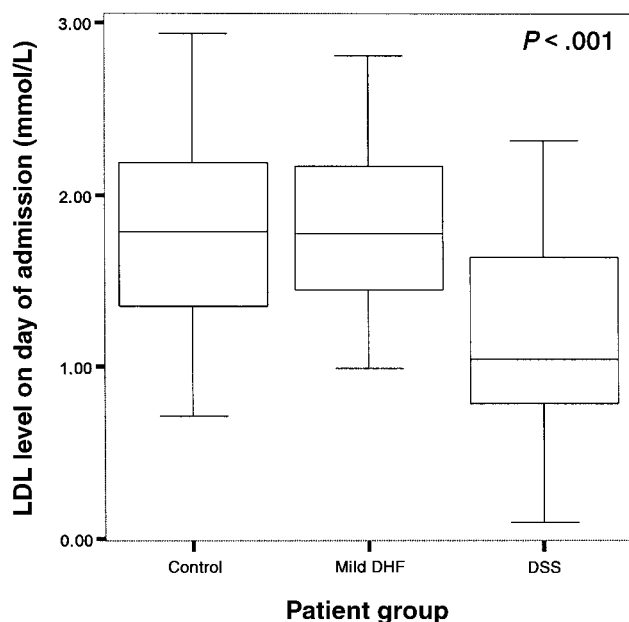
Figure 3. Box plots of low-density lipoprotein (LDL) levels in control subjects and patients with dengue hemorrhagic fever (DHF) on day of admission to the hospital. Box plots show the median *(center horizontal line),* interquartile range (the 25th to the 75th percentile [*box*]), and the 5th and 95th percentiles *(whiskers). P* values were determined by the Kruskal-Wallis test for comparison of 3 groups. The median LDL level in the control group was 1.79 mmol/L (interquartile range, 1.30–2.26 mmol/L); in the mild DHF group, it was 1.77 mmol/L (interquartile range, 1.42–2.18 mmol/L); and in the dengue shock syndrome (DSS) group, it was 1.04 mmol/L (interquartile range, 0.78–1.67 mmol/L).

the lowest levels occurred in patients with severest disease, according to the Kruskal-Wallis test for comparison of total cholesterol, HDL, and LDL levels ($P < .001$; figures 1–3). In a subanalysis of the patients with severe DHF (i.e., grade III and IV) who survived ($n = 37$) and who did not survive ($n = 13$), the lowest serum cholesterol, HDL, and LDL levels were noted in the nonsurvivor group. Patients who survived were compared with those who did not survive with regard to median values of plasma lipid profile with 25th and 75th IQRs; the Mann-Whitney *U* test was used for comparison. The plasma cholesterol levels were 2.70 versus 2.05 mmol/L (IQR, 2.15–3.10 vs. 1.28–2.28 mmol/L; $P < .03$), the plasma HDL levels were 0.25 versus 0.14 mmol/L (IQR, 0.17–0.42 vs. 0.13–0.24 mmol/L; $P < .03$), and the plasma LDL levels were 1.32 versus 0.83 mmol/L (IQR, 0.83–1.76 vs. 0.61–1.11 mmol/L; $P < .04$), respectively. The contrary was demonstrated for the triglyceride levels, for which the highest levels measured were noted in patients with the most severe cases, although values were not significantly different among the 3 groups (figure 4). In the subanalyses of patients who survived versus those who did not survive, the highest triglyceride levels were also noted in the

patients who did not survive (2.26 mmol/L [IQR, 1.50–3.29 mmol/L] vs. 2.34 mmol/L [IQR, 1.76–3.10 mmol/L]; $P = $ NS).

In the present study, we found a significant difference in the plasma cholesterol, HDL, and LDL levels among patients with mild DHF (i.e., grade I or II), severe DHF (i.e., grade III or IV), and a control group of healthy subjects, as well as between patients who did and patients who did not survive in the severe DHF group. The observed differences could be considered to represent surrogate markers for severe infection, because they could represent actual markers of risk for severe infection and poor clinical outcome. In particular, the findings in the severe DHF group suggested that cholesterol, HDL, and LDL levels can be used as prognostic markers to predict clinical outcome. A major question is, what mechanism is behind these lipoprotein changes? From data largely derived from experimental studies involving humans and animals, we know that lipid metabolism and cytokine production are linked. The interaction between cytokines and lipoproteins is bidirectional. Lipids are involved in the regulation of cytokine levels and thereby modify the host immune response [2]. On the other hand, cytokines are known to have the ability to modify lipid metabolism. TNF-
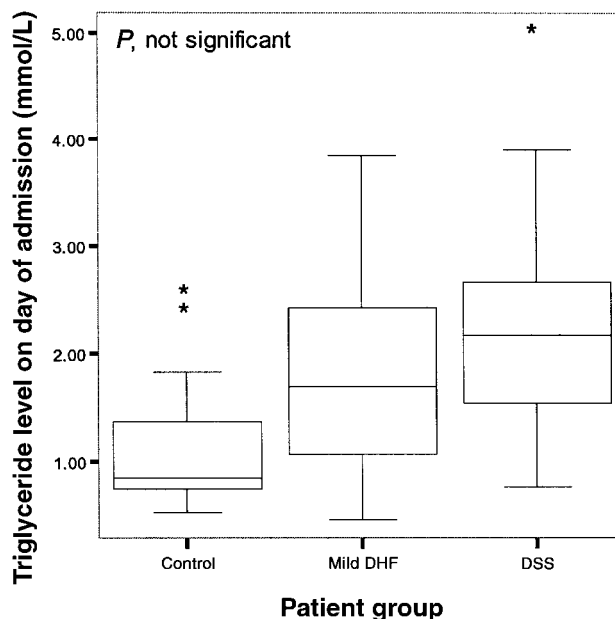

Figure 4. Box plots of triglyceride levels in control subjects and patients with dengue hemorrhagic fever (DHF) on day of admission to the hospital. Box plots show the median *(center horizontal line),* interquartile range (the 25th to the 75th percentile [*box*]), and the 5th and 95th percentiles *(whiskers). P* values were determined by the Kruskal-Wallis test for comparison of 3 groups. The median triglyceride level in the control group was 0.83 mmol/L (interquartile range, 0.74–1.44 mmol/L); in the mild DHF group, it was 1.69 mmol/L (interquartile range, 1.05–2.48 mmol/L); and in the dengue shock syndrome (DSS) group, it was 2.17 mmol/L (interquartile range, 1.54–2.67 mmol/L). Outliers are presented as asterisks.

α and IL-1 decrease serum cholesterol levels, probably by influencing the enzyme hydroxymethylglutaryl (HMG) coenzyme A (CoA) reductase. Also, the decrease in HDL levels observed during infection is probably enzyme mediated. TNF-α may decrease the plasma activity of lecithin cholesterol acyl transferase (LCAT), the enzyme responsible for esterifying free cholesterol in HDL [13]. The increase in triglyceride levels observed during infection may be the result of an increase in lipolysis [14] and de novo fatty acid synthesis in the liver [14, 15]. This process is also enzyme mediated by increasing the activity of the CoA carboxylase enzyme. In accordance with these findings, we and others [16] have found elevated levels of TNF-α and IL-1 in patients with DHF.

Our findings are in line with the results of studies of patients with illnesses comparable to severe DHF [17, 18] and of a study of dengue virus–infected patients [19] published elsewhere. Ray et al. [19] reported differences in cholesterol and triglyceride levels in dengue virus–infected patients with different degrees of severity. However, no HDL or LDL concentrations were measured, and details on cholesterol and triglyceride levels in association with clinical outcome were missing. We demonstrated that the plasma lipid profile differs according to the stage of DHF disease severity and that cholesterol, HDL, and LDL levels could be used as potential predictors of clinical outcome. The question of whether we can use this in clinical practice has to be answered in a future, prospective, follow-up study.

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