Lipid Profile Changes as a Potential Prognostic Marker for the Prediction of Dengue Fever Severity in Pediatric Patients

Alia Chuck
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Abstract

Background: Dengue fever (DF) remains the most rapidly spreading mosquito-borne viral disease worldwide, and in recent decades a re-emergence of the virus and its severe forms has been observed. DF can present in a wide spectrum of manifestations ranging from self-limited DF, to severe forms such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Certain elements of the lipid profile may aid early prognostication in patients at risk for severe types of dengue. The primary goal of this literature review is to address whether lipid profile changes can be utilized to predict development of dengue fever severity in pediatric patients.

Methods: An exhaustive search of available literature was conducted using MEDLINE-Ovid, Web of Science, CINAHL, and Google Scholar using the keywords: lipoprotein, lipid profile, and dengue fever. Studies were included if the primary data evaluated lipid profile changes in the pediatric population and their correlation with severe outcomes of DF. Bibliographies were also searched for relevant studies. Articles were excluded if studies were conducted on adult patients. Relevant articles were assessed for quality using the GRADE.

Results: Three studies fit the inclusion criteria and were included in this systematic review. All studies were observational prospective studies looking at the relationship between lipid profile alterations with severe forms of dengue infection. Decreases in total cholesterol (TC) and LDL levels were statistically significant. Other lipid profile components, such as HDL, VLDL and triglycerides, did not have a significant change with increasing dengue severity.

Conclusion: Studies demonstrated that specific changes in the lipid profile can anticipate development of severe forms of dengue fever in pediatric patients. Total cholesterol and LDL measurements were consistently lower in association with DHF and DSS. However, TC and LDL's sole use as prognostic biomarkers for increased dengue severity is premature and inconclusive. While a great starting point for more research, more studies involving time-ordered analysis and uniform measurements are needed to effectively and accurately predict progression to severe forms of dengue.

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Degree Name
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Keywords
dengue fever, severity, lipid profile, lipoprotein, pediatric

Subject Categories
Medicine and Health Sciences

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Alia N. Chuck

A Clinical Graduate Project Submitted to the Faculty of the School of Physician Assistant Studies Pacific University Hillsboro, OR

For the Masters of Science Degree, August 12, 2017

Faculty Advisor: Annjanette Sommers, PA-C, MS

Clinical Graduate Project Coordinator: Annjanette Sommers, PA-C, MS
Biography

[Redacted for privacy]
Abstract

**Background:** Dengue fever (DF) remains the most rapidly spreading mosquito-borne viral disease worldwide, and in recent decades a re-emergence of the virus and its severe forms has been observed. DF can present in a wide spectrum of manifestations ranging from self-limited DF, to severe forms such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Certain elements of the lipid profile may aid early prognostication in patients at risk for severe types of dengue. The primary goal of this literature review is to address whether lipid profile changes can be utilized to predict development of dengue fever severity in pediatric patients.

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**Keywords:** Dengue fever, lipid profile, lipoprotein, pediatric
Acknowledgements

[Redacted for privacy]
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List of Abbreviations

DENV  Dengue virus
DF  Dengue Fever
DHF  Dengue Hemorrhagic Fever
DSS  Dengue Shock Syndrome
HDL  High-Density Lipid
LDL  Low-Density Lipid
LPS  Lipopolysaccharide
OFI  Other Febrile Illness
TC  Total Cholesterol
TG  Triglyceride
VLDL  Very-Low Density Lipid
WHO  World Health Organization
Lipid Profile Changes as a Potential Prognostic Marker for the Prediction of Dengue Fever Severity in Pediatric Patients

BACKGROUND

Dengue fever (DF) remains the most rapidly spreading mosquito-borne viral disease worldwide,¹ and in recent decades a re-emergence of the virus and its severe forms has been observed. Found in the tropical and sub-tropical regions of the world, over 2.5 billion people live in dengue endemic countries, with approximately 50 million dengue infections diagnosed annually.² Of those diagnosed, an estimated 500 000 people, the majority being children, develop severe forms of dengue and require hospitalization annually. About 2.5% of those affected do not survive.¹ In recent years, increases in incidence have partly been attributed to urbanization and air travel,³ and infections are only expected to rise.

There are 4 serotypes of the dengue Flavivirus that can present in a wide spectrum of manifestations. Presentations range from self-limited DF, causing arthralgia, myalgia, and headache, to the more severe forms, such as dengue hemorrhagic fever (DHF), presenting with thrombocytopenia, hemorrhagic manifestations and increased vascular permeability, and dengue shock syndrome (DSS), which can be fatal if left untreated.³⁴ The World Health Organization (WHO) developed criteria in 1997 and 2009 for dengue fever severity classification.²⁵ See Figure 1 and 2.

Treatment for DF has always been supportive, while severe forms of the disease require critical medical management of the patient’s body fluid volume.¹ The ability to make early predictions of severe dengue in patients who display no warning signs and are at risk of
developing severe DHF and DSS is critical in choosing appropriate intensive supportive therapy and improving survival since the newly developed vaccine is not widely accessible.³

Early detection of individuals at risk of developing severe forms of dengue could be possible with the identification of an ideal biomarker, which has been the focus of several research studies. While the mechanism behind individuals who contract DF and progress to severe dengue remains unclear; research speculates that the host immune response is a significant factor in dengue pathogenesis and suggest severe forms are most likely associated with immunopathology.³ This has led to the examination of immune response components, endothelial activation markers, and other biochemical and genetic markers as potential biomarkers.³,⁶

It has been established that hypolipidemia occurs in critically ill patients and is an independent predictor of clinical outcomes.⁷,⁸ Theories postulate that the immunopathogenesis of dengue infections demonstrate the possibility of lipoprotein’s ability to modify inflammatory immune function and host immune response during infections.⁹ Research also suggests that during viral infections, lipoproteins bind to viruses and neutralize their negative effects, while certain viruses use LDL receptors for entrance into cells.¹⁰ Similarly, HDL and LDL have been shown to modulate hepatitis C virus infectivity, promoting virus-cell interaction by facilitating virus-cell entry.¹¹⁻¹⁵ Research also demonstrated that using markers to indicate thrombocytopenia and hemorrhagic manifestations have been associated with severe forms of dengue.¹⁰ Despite several studies looking at possible biomarkers to indicate severe forms of dengue, there have been few studies that address and elucidate a correlation between dengue fever severity and
changes in the lipid profile. Can lipid profile changes predict dengue fever severity in the pediatric patient?

METHODS

An exhaustive search of available literature was conducted using MEDLINE-Ovid, Web of Science, CINAHL, and Google Scholar using the keywords: lipoprotein, lipid profile, and dengue fever. Bibliographies were also searched for other relevant studies. Studies were included if the primary data evaluated lipid profile changes in the pediatric population and their correlation with severe outcomes of dengue fever. Further search refinement included data collected using human trials only and articles published in the English language. Articles were excluded if studies were conducted on adult patients. Relevant articles were assessed for quality using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE). According to the National Institute of Health (NIH) clinical trials inquiry, there are no current trials in any phase examining the lipid profile or its components as a prognostic biomarker in patients with dengue fever.

RESULTS

The initial search yielded 48 articles for review. Following elimination of duplicates and screening of relevant articles for primary data and human studies published in English, a total of three articles met eligibility criteria. All articles selected were prospective observational studies. See Table 1. Another prospective observational study was considered, but fell under exclusion criteria because it evaluated adults.
Biswas et al

Biswas et al\textsuperscript{17} conducted a prospective study between 2005-2013 to understand the correlation between cholesterol levels during a dengue infection, understand how cholesterol levels change over time among patients with severe forms of dengue, and to assess the relationship of cholesterol level at presentation with development of severe dengue. The study was conducted at the Infectious Disease Ward of the Hospital Infantil Manuel de Jesus Rivera in Managua, Nicaragua, which is the National Pediatric Reference Hospital that manages the vast majority of children in need of tertiary care across the country.\textsuperscript{17}

Eligibility criteria included infants and children between the ages of 6 months and 14 years old presenting with a fever or history of fever for less than 7 days and one or more of the following signs and symptoms: headache, arthralgias, myalgias, retro-orbital pain, positive tourniquet test, petechiae, or signs of bleeding. Annually, both inpatients and outpatients were enrolled during the peak of dengue season (August 1 to January 31) and were followed clinically through the acute phase of illness. For each enrolled participant, a medical history and complete physical exam was performed. Vital signs, signs and symptoms, and fluid balance and treatment were documented 2 times a day, and a blood sample was drawn daily for 3 days to include: complete blood counts, platelets, blood chemistry, and serological, virological, and molecular biological tests for dengue infection. Additionally, a convalescent serum sample (14-21 days post-onset of illness) was drawn for paired serological testing. Information was collected every 12 hours for inpatients and every 24 hours for outpatients who were asked to return daily. For inpatients, a non-fasting blood sample was taken each morning to measure serum lipids, while non-fasting blood sample was obtained at each follow-up visit for outpatients. A case was considered laboratory-confirmed dengue when acute infection was demonstrated by: detection of
DENV RNA by RT-PCR; isolation of DENV; seroconversion of DENV-specific IgM antibodies observed by MAC-ELISA in paired acute and convalescent phase samples; and/or a > 4-fold increase in anti-DENV antibody titer measured using Inhibition ELISA in paired acute and convalescent phase samples. Patients who tested negative for DENV infection were classified as patients with other febrile illness (OFI). The laboratory-confirmed cases were classified by severity using 1997 and 2009 WHO classification criteria (See Figure 1 and 2), and 3 standardized clinical intervention levels that were established in the DENCO study sponsored by the WHO. To evaluate the effect of cholesterol at presentation on risk of development of severe dengue, relative risks and 95% confidence intervals were calculated using multi-variable modified Poisson models.

Following exclusions, 1236 patients were suitable for analysis, including 789 (64%) laboratory-confirmed as DENV positive and 447 (36%) tested negative and classified as OFI. From the participants, it was observed that children aged 9 to 12 were more likely to be classified as having more severe forms of dengue, most patients (82%) were hospitalized and patients generally presented on day 4-5 of illness. As the first study to analyze changes in cholesterol levels by day of illness in dengue patients, with use of time-ordered analysis, it was found that total serum cholesterol (TC), was significantly lower in dengue-positive patients than in dengue-negative patients on day 3-8 of illness (p<0.05), which is illustrated in Figure 3. Trajectories for LDL levels were similar to those of TC on day 2 (p<0.05) and days 4-8 of illness (p<0.001). In contrast, HDL levels were significantly decreased only on days 5-7 of illness (p<0.001). Moreover, results showed that LDL levels had the greatest decrease, which suggests its effect on lowering total cholesterol. When examining the trajectories of cholesterol by severity during the course of illness regardless of severity classification, TC was significantly lower in patients who
developed severe dengue compared to patients with mild dengue on days 5-7 of illness using the WHO 1997 classification (p<0.001), on days 4-8 of illness using the WHO 2009 classification (p<0.05), and on days 5-8 of illness using standardized intervention categories (p<0.05). See Figure 4. Finally, using the WHO 1997 disease severity classification showed that for each 10 mg/dl decrease in TC and LDL-C at presentation, the risk of development of DHF/DSS increased by 9% (95% CI: 0-19%) and 12% (95% CI: 0-26%), respectively, suggesting its potential use as a prognostic marker for severe forms of the disease.\textsuperscript{17} See Table 2.

**Suvarna and Rane**

During a 2006 dengue outbreak in Mumbai, India, this prospective study\textsuperscript{18} was conducted over 18 months at a tertiary care hospital on dengue patients to determine the relationship between lipoproteins and dengue severity, as well as other outcomes, such as patient survival, bleeding severity, capillary leakage, supportive therapy requirement, and duration of hospital stay.\textsuperscript{18}

Included in this study were 50 children (1 month to 18 years old) with dengue fever (DF) and 50 age- and sex-matched non-febrile children, considered controls. After participants were classified and diagnosed using standard WHO criteria, 18 (36%) patients were DF, 19 (38%) were DHF and 13 (26%) were DSS. Age distribution demonstrated that severe dengue was more frequent in the 0 to 3-year age group vs. the 12 to 18-year age group. The opposite was shown for mild dengue (DF), where it was more common at age 12-18 years than at 0-3 years. The study compared lipid profile of dengue cases and controls, between lipid profile in survived and expired patients and between lipid profile and disease severity. Excluded from this study were cases or controls with nephrotic syndrome, obesity (BMI $\geq$30), or parental history of diabetes mellitus, obesity, lipid disorder, or coronary artery diseases at age $< 55$.\textsuperscript{18}
When comparing lipid profile of cases and controls, mean triglycerides (TG) and VLDL levels were higher in cases than controls (P value < 0.05), while mean HDL and LDL levels were lower in cases than in controls (P values = 0 and 0.001, respectively). The study also showed that mean cholesterol level was lower in DSS than in controls (P = 0.005), but not in those with DHF and DF (P values 0.724 and 0.108, respectively). Cholesterol and VLDL were different among the disease severity groups, whereas TG, HDL and LDL did not differ. Lower levels were seen in DSS and higher levels in DF. Data showed that mean cholesterol level was higher in DF than in DHF, DSS (P-value 0.01, 0.001, respectively) but did not differ between DHF and DSS. The study also demonstrated that the odds of having DSS are four times more with a TG <150, than at a TG>150. Otherwise, none of the lipids significantly predicted survival or death.\(^{18}\)

**Van Gorp et al**

This prospective study\(^ {19}\) focused on significant changes in lipid profile in patients with DHF, and whether these changes are relevant to clinical outcomes.

With yearly outbreaks of dengue fever, the study was conducted at the Dr. Kariadi University Hospital of the University of Diponegoro in Semarang, Central Java, Indonesia. From July-October 1996, enrolled in the study were 50 consecutive children (mean age +/- SD, 6.5 +/- 2.8 years) clinically diagnosed using WHO criteria with severe DHF (DHF grade III and IV) who were admitted to the pediatric intensive care unit, 20 age-related patients with mild DHF (DHF grade I and II) admitted to the pediatric ward and 20 age-related, non-febrile healthy children. Clinical diagnosis of DHF grade I-IV was determined by 1997 WHO criteria.\(^ {4}\) For all participants, blood samples for analysis of serum lipid profile (TC, HDL, LDL, and triglyceride) and cytokines were collected on day of admission. Dengue virus infection was objectively confirmed using serologic assays. Additionally, blood samples were taken for culture to exclude
bacterial infections. In follow-up, 13 patients (26%) with DHF III and IV did not survive due to shock or bleeding complications.19

Results demonstrated significant difference in serum cholesterol, HDL and LDL levels when comparing patients with mild DHF, severe DHF, and healthy control subjects, as well as with the patients who did not survive. The lowest levels of TC, HDL and LDL were shown in patients with severest disease, and in the sub-analysis of patients with severe DHF who survived and who did not survive, the lowest lipid levels were seen in the non-survivor group. In contrast, triglyceride levels were the opposite. The highest levels were seen in patients with the most severe cases, but values were not significantly different among the 3 groups. Similarly, the highest triglyceride levels were shown in the patients who died.19 See Figure 5.

Findings from this study suggest that demonstrated alterations in the lipid profile could be used as prognostic markers to predict clinical outcome in dengue infected patients. The authors also consider these differences to represent surrogate markers for severe infection as they could denote actual markers for risk for severe infection leading to negative clinical outcomes. From prior studies, researchers believe these results are due to the link between lipid metabolism and cytokine production. The authors recommend further, prospective, follow up to determine if these measurements can be used in clinical practice.19

**DISCUSSION**

With the advent of climate change, continued globalization, and viral evolution, dengue infections along with other mosquito-borne illnesses are expected to rise. The recent emergence and global impact of the Zika virus has underlined the need for additional studies and research to improve mosquito-borne disease detection and effective treatment. Determining the relationship between DF and specific biomarkers could potentially pave the way for earlier detection and
thus, improved outcomes for other mosquito borne illnesses as well. Rising numbers of dengue infections has made it critical to determine a reliable prognostic marker to predict dengue severity and ensure timely and proper therapeutics.\textsuperscript{20}

This review aimed on evaluating whether changes in the lipid profile can predict development of severe forms of dengue. Although evidence is variable and limited, results of the studies presented suggest that alterations of certain components of the lipid profile may play a vital role in predicting the development of more severe forms of DF. In all studies reviewed,\textsuperscript{17,18,19} results showed decreasing serum cholesterol levels correlated with increasing dengue severity, suggesting the utility of the lipid profile as a surrogate biomarker and indicator of a poor clinical outcome.

In addition to observing lower levels of total cholesterol with severe forms of DF, studies\textsuperscript{17,19} also saw decreases in LDL with increasing dengue severity. In Biswas et al,\textsuperscript{17} greater decreases in LDL were evident in DHF and DSS compared to mild dengue, which was theorized to drive the reduction in total cholesterol. Duran et al\textsuperscript{10} explains this as a result of LDL’s ability to facilitate entry of the dengue virus into the cell via its receptors. Moreover, this study\textsuperscript{10} noted a positive correlation between lipid values and platelet count. Lower LDL values in severe dengue were linked with low platelet counts, which aligns with hemorrhagic status in severe forms of dengue. The study by Suvarna and Rane\textsuperscript{18} also found LDL levels associated with an increased likelihood of DHF, but statistical tests were not significant between lower TC and severe dengue.

Other components of the lipid profile were inconclusive as the studies reviewed demonstrated inconsistent results. Shown in Figure 5, the van Gorp et al study\textsuperscript{19} found a significant decrease in total cholesterol, LDL and HDL among patients with severe DHF in
comparison to those classified with mild DF or healthy subjects, suggesting their use as prognostic markers to predict clinical outcome. In contrast, both studies\textsuperscript{17,18} determined that HDL results were not statistically significant. Similarly, while VLDL was looked at in two studies,\textsuperscript{18,19} its role was not conclusively defined, warranting future studies in the pathogenesis of the viral infection and its underlying mechanism.

Additionally, the study conducted by Biswas et al\textsuperscript{17} solely accounts for time ordered analysis of cholesterol levels in relation to dengue severity outcome, highlighting the need for more time ordering studies. This way, results will determine whether cholesterol levels affect development of severe dengue or if it is a result of developing severe dengue. Anticipation and early recognition of DHF and DSS will not only guide therapy, but prompt timely and appropriate medical management to improve patient outcomes.

Despite these findings, significant limitations should be addressed regarding small sample size, variability in the use of appropriate group comparisons, inability to generalize evidence to general population, use of PCR in diagnosing dengue objectively, failure to evaluate the lipid profile in its entirety, and measuring lipoproteins over time. Two studies\textsuperscript{17,18} were of small sample size, likely causing imprecision in results. In comparison to Biswas et al,\textsuperscript{17} which analyzed 1236 who fit inclusion criteria over the course of 9 years, sample size and study period were considerably small. Suvarna and Rane\textsuperscript{18} studied 100 subjects total over an 18-month period, while van Gorp et al\textsuperscript{19} evaluated 90 subjects over four months.

Another weakness involved the variability across studies. An important variable to account for is the differences or non-specifications in control groups or lack of control groups. Two studies\textsuperscript{17,18} provided control groups defined as non-febrile or healthy controls. Comparing
these studies, age distribution was not specified in one study\textsuperscript{17}, but summarized as “age-related”, which is potentially an important flaw considering that the burden of disease and severe dengue was found predominantly in infants aged 4-9 months and in children 5-9 years old.\textsuperscript{4} Providing an age distribution would have accentuated the results, identifying what pediatric population was more at risk of developing severe dengue. Additionally, one study\textsuperscript{17} did not provide a control group defined as healthy subjects, but instead classified a study group as OFI or not having DF. Nonetheless, results from these prospective studies are consistent in demonstrating that total cholesterol and LDL were consistently higher in controls and OFI, and lower in severer forms of dengue.

However, the importance of including healthy controls is further emphasized by the fact that lipid profiles are non-specific and can also change in patients with severe infections or illness. The Wilson et al 2005 study\textsuperscript{20} demonstrated low cholesterol levels in sepsis or critically ill or injured patients, and is consistent with a study\textsuperscript{7} identifying hypolipidemia as an independent predictor of clinical outcome in critically ill patients. Generalizing the results of the reviewed studies\textsuperscript{17,18,19} is limited by the lack of specificity of using lipid profiles for prognosis in any patient with fever, but altering lipoprotein levels are useful in patients presenting where dengue fever is suspected.

Limitations were observed in the methods sections regarding clinically and objectively diagnosing DF and severity. Across studies, either or both 1997 or 2009 WHO criteria were used to clinically diagnose dengue severity, while various methods were used to objectively diagnose DF in study subjects. Two studies\textsuperscript{17,19} utilized dengue PCR for diagnosis, but due to financial and infrastructure constraints, the Denguecheck-WB test was performed for diagnosis in the Suvarna
The Denguecheck-WB test, which studies have shown to have high specificity but low sensitivity, and poor predictive capacity to differentiate between primary and secondary dengue infections.\textsuperscript{18}

Another evident variability between studies are the lipid profile components. Only one study\textsuperscript{18} examined the entire lipid profile, including TC, HDL, LDL VLDL and triglycerides, against changes in dengue severity, which affects accurate comparisons. Specifically, in Biswas et al,\textsuperscript{17} the LDL was not measured for the first two years of study, which affected measurements for analysis, while triglycerides were not measured at all. Consistently looking at the entire lipid profile would facilitate researchers in determining which components are negatively or positively correlated with dengue severity. While LDL has been emphasized as a probable determinant of severe dengue outcome, ascertaining consistent trends in lipid components would help researchers understand whether LDL alone was driving reduction in total cholesterol, or if other components are contributing. This would make the evidence more promising.

Finally, a limitation can also be considered in examining changes in cholesterol levels throughout the course of dengue infection. While Biswas et al\textsuperscript{17} study design did account for time-ordering analyses, which ensured exposure of the virus preceding the outcome, obtaining baseline lipid profile values or convalescent measurements from subjects would have been interesting and informative for researchers. This finding may suggest whether high cholesterol and lipoproteins have protective and beneficial effects on the immune system in the immunopathogenesis of dengue fever. Interestingly in a study by Iribarren et al,\textsuperscript{21} it was observed that individuals with low cholesterol at the start of the study endured more hospital admissions for infectious diseases in the next 15 years.
Despite this review demonstrating an apparent correlation between dengue severity and lipid profile, the evidence is of very low quality based on the current research available. Currently, the use of the lipid profile as a surrogate biomarker to predict dengue severity is non-specific, thus underlining the need for further research to validate its role in a clinical setting. To be utilized as an actual biomarker, there is a definite need for more time-ordered studies that will help clarify if lipid profile, or certain components of the lipid profile impact development of severe dengue or if it is a result of developing severe dengue. Regardless, elucidating these findings for clinical practice would be vital.

CONCLUSION

In conclusion, these studies demonstrate that specific changes in the lipid profile can anticipate development of severe forms of dengue fever in pediatric patients. Total cholesterol (TC) and LDL measurements were consistently lower in association with DHF and DSS. However, TC’s and LDL’s sole use as prognostic biomarkers for increased dengue severity is premature and inconclusive. These studies are a great starting point for more research, but additional studies involving time-ordered analysis and uniform measurements are needed to effectively and accurately predict progression to severe forms of dengue. Furthermore, the ability to explore and identify trends in all aspects of the lipid profile, as well as other routine laboratory markers, have potential in creating a prognostic biomarker panel to identify risk of developing severe forms of dengue fever in susceptible populations. With the effects of climate change, globalization and viral evolution, it is in the interest of global medicine that future research discern at risk groups for severe infections and reliable prognostic markers not only for dengue fever, but for its fellow mosquito-borne illnesses, such as chikungunya, Japanese encephalitis and Zika virus, that are of concern for widespread outbreak.
References


Table 1: Quality Assessment of Reviewed Articles

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Downgrade Criteria</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biswas et al&lt;sup&gt;16&lt;/sup&gt;</td>
<td>Prospective Observational</td>
<td>Serious&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Not serious Not serious Not serious No bias likely Very low</td>
</tr>
<tr>
<td>Suvarna and Rane&lt;sup&gt;17&lt;/sup&gt;</td>
<td>Prospective Observational</td>
<td>Serious&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Not serious Not serious Serious&lt;sup&gt;c&lt;/sup&gt; Likely&lt;sup&gt;d&lt;/sup&gt; Very low</td>
</tr>
<tr>
<td>VanGorp et al&lt;sup&gt;18&lt;/sup&gt;</td>
<td>Prospective Observational</td>
<td>Not serious</td>
<td>Not serious Not serious Serious&lt;sup&gt;e&lt;/sup&gt; No bias likely Very low</td>
</tr>
</tbody>
</table>

<sup>a</sup> LDL-C not measured in first two of nine years and triglycerides were not measured
<sup>b</sup> Due to financial and infrastructure constraints, dengue PCR was not performed for diagnosis, instead the Denguecheck-WB test was performed.
<sup>c</sup> The sample size is small n=100
<sup>d</sup> The high disease severity in this cohort due to KEM Hospital being the premier tertiary care centres with referrals from all over the state of Maharashtra, India
<sup>e</sup> The sample size is small n=90

Table 2. Effect of cholesterol level at presentation on development of severe dengue outcome using the WHO 1997 disease severity classification<sup>16</sup>

<table>
<thead>
<tr>
<th>Cholesterol type</th>
<th>Total serum cholesterol RR (95% CI)</th>
<th>LDL-C RR (95% CI)</th>
<th>HDL-C RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol per-10 mg/dl</td>
<td>1.09 (1.00-1.19)</td>
<td>1.12 (1.00-1.26)</td>
<td>1.18 (0.98-1.42)</td>
</tr>
</tbody>
</table>
**Figure 1** - WHO guidelines (1997) for the treatment of dengue fever/dengue hemorrhagic fever\(^5\)

<table>
<thead>
<tr>
<th>DF/DHF</th>
<th>Grade*</th>
<th>Symptoms</th>
<th>Laboratory picture</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF</td>
<td></td>
<td>Fever with two or more of the following signs: Headache, retro-orbital pain, myalgia, arthralgia</td>
<td>Leukopenia occasionally. Thrombocytopenia may be present. No evidence of plasma loss</td>
</tr>
<tr>
<td>DHF</td>
<td>I</td>
<td>Above signs + positive tourniquet test</td>
<td>Thrombocytopenia positive tourniquet &lt; 100,000, Hct rise ≥20%</td>
</tr>
<tr>
<td>DHF</td>
<td>II</td>
<td>Above signs + spontaneous bleeding</td>
<td>Thrombocytopenia &lt; 100,000, Hct rise ≥20%</td>
</tr>
<tr>
<td>DHF</td>
<td>III</td>
<td>Above signs + circulatory failure (weak pulse, hypotension, restlessness)</td>
<td>Thrombocytopenia &lt; 100,000, Hct rise &gt;20%</td>
</tr>
<tr>
<td>DHF</td>
<td>IV</td>
<td>Profound shock with undetectable blood pressure and pulse</td>
<td>Thrombocytopenia &lt; 100,000, Hct rise ≥20%</td>
</tr>
</tbody>
</table>

**Figure 2** - WHO guidelines (2009) Dengue, guidelines for diagnosis, treatment, prevention and control.\(^2\)

**Criteria For Dengue ± Warning Signs**

- Probable Dengue
- Live in/travel to dengue endemic area
- Fever and 2 of the following criteria:
  - Nausea, vomiting
  - Rash
  - Aches and pain
  - Tourniquet test +
  - Leukopenia
  - Any warning sign

**Warning signs**

- Abdominal pain or tenderness
- Persistent vomiting
- Clinical fluid accumulation
- Mucosal bleed
- Lethargy, restlessness
- Liver enlargement > 2 cm
- Laboratory, increase in HCT concurrent with rapid decrease in platelet count

**Criteria For Severe Dengue**

- Severe plasma leakage leading to:
  - Shock (DSS)
  - Fluid accumulation with respiratory distress

- Severe bleeding as evaluated by clinician

- Severe organ involvement
  - Liver: AST or ALT > 1000
  - CNS: Impaired consciousness
  - Heart and other organs

Laboratory-confirmed dengue (Important when no sign of plasma leakage)

Requiring strict observation and medical intervention
FIGURE 3: Age- and gender-adjusted marginal mean cholesterol levels (mg/dl) by DENV infection status and day of illness; 3A. Trajectories of cholesterol levels by DENV infection status; 3B. Trajectories of LDL-C and HDL-C levels by DENV infection status. The day of fever onset was defined as day 1 of illness.

Abbreviations:
DENV: Dengue virus
LDL-C: Low-density lipoprotein cholesterol
HDL-C: High-density lipoprotein cholesterol

Biswas HH, Gordon A, Nuñez A, Perez MA, Balmaseda A, Harris E (2015) Lower Low-Density Lipoprotein Cholesterol Levels Are Associated with Severe Dengue Outcome. PLoS Negl Trop Dis 9(9): e0003904. doi:10.1371/journal.pntd.0003904.g002. Copyright: © 2015 Biswas et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
FIGURE 4: Age- and gender-adjusted marginal mean cholesterol levels (mg/dl) by dengue severity classification and day of illness; 4A, 4B, 4C. Trajectory of total serum cholesterol by dengue severity using the WHO 1997 classification, WHO 2009 classification, and IC, respectively. 4D, 4E, 4F. Trajectory of LDL-C and HDL-C by dengue severity using the WHO 1997 classification, WHO 2009 classification, and IC, respectively.
Abbreviations:
DF: Dengue fever
DHF: Dengue hemorrhagic fever
DSS: Dengue shock syndrome
DWS: Dengue with or without warning signs
SD: Severe dengue
IC: Standardized intervention categories
IC 1/IC 2: Mild dengue
IC 3: Severe dengue
LDL-C: Low-density lipoprotein cholesterol
HDL-C: High-density lipoprotein cholesterol

Biswa H, Gordon A, Nuñez A, Perez MA, Balmaseda A, Harris E (2015) Lower Low-Density Lipoprotein Cholesterol Levels Are Associated with Severe Dengue Outcome. PLoS Negl Trop Dis 9(9): e0003904. doi: 10.1371/journal.pntd.0003904.g003 Copyright: © 2015 Biswas et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Figure 5: Comparing lipid profile levels in control subjects and patients with DHF and DSS on day of admission to hospital.18

Abbreviations:
TC: Total cholesterol
HDL: High density lipoprotein
LDL: Low density lipoprotein
TG: Triglyceride
Mild DHF: Mild dengue hemorrhagic fever
DSS: Dengue shock syndrome