July 2020

The Association between Dengue Virus Infection and Liver and Kidney Function among Cambodian Children

Ashley Moineau

THE ASSOCIATION BETWEEN DENGUE VIRUS INFECTION AND LIVER AND KIDNEY FUNCTION AMONG CAMBODIAN CHILDREN

A Thesis Presented

by

ASHLEY A. MOINEAU

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

May 2020

School of Public Health and Health Sciences

Department of Biostatistics and Epidemiology

THE ASSOCIATION BETWEEN DENGUE VIRUS INFECTION AND LIVER AND KIDNEY FUNCTION AMONG CAMBODIAN CHILDREN

A Thesis Presented

by

ASHLEY A. MOINEAU

Approved as to style and content by:

_____
Andrew A. Lover, Chair

_____
Cassandra N. Spracklen, Member

_____
Paula stamps, Graduate Program Director

Department of Biostatistics and Epidemiology

ABSTRACT

THE ASSOCIATION BETWEEN DENGUE VIRUS INFECTION AND LIVER AND KIDNEY FUNCTION AMONG CAMBODIAN CHILDREN

MAY 2020

ASHLEY A. MOINEAU, B.S., UNIVERSITY OF MAINE ORONO

M.S., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Dr. Andrew A. Lover

Severe liver and kidney dysfunction are prevalent in 10%-30% of the Southeast Asian population. Dengue virus infection has been reported as a modifiable risk factor for liver and kidney dysfunction, especially among children in Southeast Asia. Epidemiologic studies assessing this relationship are sparse, often failed to include children, and did not adjust for important covariates. Therefore, we evaluated the relationship between dengue virus infection and liver and kidney dysfunction among hospitalized children in Cambodia (n=551). Participants with a serologically confirmed dengue virus infection were categorized according to increasing severity of infection (i.e. dengue fever, dengue hemorrhagic fever, or dengue shock syndrome) using clinical assessment. Laboratory assays were used to assess liver (i.e. albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total protein) and kidney protein levels (i.e. creatinine and urea). Descriptive statistics were used to assess the impact of severity of dengue virus infection on kidney dysfunction. Additionally, descriptive statistics and linear mixed modeling were used to assess the impact of severity of dengue virus infection on liver dysfunction while adjusting for important risk factors. Approximately 75% of all participants had abnormal liver or kidney protein

level(s) over the first four days of follow-up. Overall, a negative association was observed between increasing dengue disease severity and albumin ($ß_{adj}$ = -0.08, 95% CI = -0.45 to 0.29), ALT ($ß_{adj}$ = -18.02, 95% CI = -51.59 to 15.55), and AST ($ß_{adj}$ = -7.18, 95% CI = -49.57 to 35.21) protein levels. A positive association was observed between increasing dengue disease severity and total protein levels ($ß_{adj}$ = 7.14, 95% CI = 1.15 to 13.13). While abnormal liver function (increases in ALT and/or AST levels and decreases in albumin and/or total protein levels) is a common clinical finding in dengue infections, we did not find evidence for a significant association with more severe forms of dengue virus infection (i.e. dengue hemorrhagic fever or dengue shock syndrome) and greater liver dysfunction as compared to patients with dengue fever in pediatric populations in Cambodia.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

CHAPTER I

BACKGROUND AND SIGNIFICANCE

A. Introduction

Liver and kidney dysfunction are general terms that refer to the improper functioning of the liver or kidneys. Liver (hepatic) and kidney (renal) dysfunction are characterized by certain traumatic damages to the filtering properties of these organs, often known as liver or kidney diseases and disorders. Common liver diseases and disorders include liver cirrhosis (scarring), liver inflammation, and non-alcoholic fatty liver disease (NAFLD), all of which can ultimately result in liver failure. Common kidney diseases and disorders include glomerulonephritis and interstitial nephritis, where the filtering units of the kidneys are dysfunctional. This often results in urine back-up in the kidneys, or in severe cases, kidney failure. Recent studies have estimated that liver and kidney disease impacts between 4.5% to 9.5%[1] and 8% to 10% of the population worldwide, respectively.[2] In Southeast Asia, these numbers vary markedly with 30% of the population estimated to have some form of fatty liver disease (a general term to describe the build-up of fat in the liver, liver inflammation, liver scarring, and/or liver failure).[3-4] There is an estimated incidence rate of 63 new fatty liver disease cases per one million people in Southeast Asia diagnosed per year.[5] Chronic kidney diseases are present in 10% to 20% of the population in Southeast Asia, with an estimated incidence rate of 200 to 300 new cases per one million people diagnosed per year.[6] Reporting for prevalence and incidence of liver and kidney disease are believed to be underestimates, as these syndromes are often clinically silent, and therefore, under-diagnosed.

Dysfunction of the liver or kidney can lead to more severe health issues such as liver failure, kidney failure, and death. Liver and kidney dysfunction can arise from various underlying health issues, especially in children, including genetic predisposition, autoimmune disorders, obesity, and persistent infection.[7,8]

Non-modifiable risk factors for liver dysfunction include inherited genetic conditions. One example is Alpha-1-antitrypsin deficiency (AATD), a disorder characterized by liver scarring, severe liver dysfunction, and often liver failure. AATD occurs in individuals who have two non-functional copies ("ZZ") within an exon of the *SERPINA1* gene that encodes the alpha-1 antitrypsin protein. Another example of an inherited genetic condition that can result in liver dysfunction is Wilson's disease.[9] Individuals diagnosed with Wilson's disease often experience liver scarring followed by gallstones, acute hepatitis, and liver failure as a result of mutations in the *ATP7B* gene.[9]

Common non-modifiable risk factors for serious kidney dysfunction among children include birth defects, such as renal dysplasia when the kidneys do not properly develop during fetal growth[10], and inherited genetic diseases, including Alport syndrome where mutations in the *COL4A3* and/or *COL4A4* genes often lead to end-stage renal disease.[10,11]

Among the most common modifiable risk factors for liver disease among children are obesity and viral hepatitis (HAV, HBV, or HCV).[9] Obesity has become a growing issue in children throughout the world, and is the leading cause of non-alcoholic fatty liver disease in children.[9] Chronic kidney disease in children is also often caused by modifiable factors such as diabetes and bacterial or viral infections.[10,11]

A potential risk factor for both liver and kidney dysfunction among children in Southeast Asia is dengue virus infection.[12,13] [14] Infections with this arbovirus vary greatly by severity and clinical course, and can be categorized into three disease categories listed in order of increasing severity: dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS).[15,16] As defined by WHO 1997 and 2009 dengue infection classification guidelines, the severity level of a dengue virus infection is characterized by various clinical factors,[32] as listed in Table 1. The 1997 WHO guidelines rely mainly on patient observational assessments, whereas the 2009 guidelines utilize both observational assessments as well as laboratory analyses (e.g. platelet counts, hematocrit measurements), and are most suitable for well-resourced health facilities.[32]

Four strains of dengue virus (DENV-1, DENV-2, DENV-3, and DENV-4) are known to cause infections in humans, but all produce similar symptomatology among infected patients.[16] An outbreak of dengue viral infections occurring in areas of Southeast Asia led to the diagnosis of over 100,000 cases of dengue virus infection in 2016 alone.[17] Although the proportion of children among cases in this outbreak cannot be ascertained from WHO reports, data suggest that dengue virus infection is most common among populations less than 15 years of age, especially in endemic areas such as Cambodia due to childrens' immature immune systems. While the direct physiological relationship between dengue virus infection and dysfunction of specific organs such as the liver and kidneys has not yet been fully characterized, it has been suggested that dengue virions may invade the liver and kidneys, potentially directly impairing liver and/or kidney function. This study therefore proposes to investigate the relationship between dengue disease severity as measured by disease category and risk of liver and kidney

dysfunction, as indicated by blood protein values, among a cohort of Cambodian children using clinical data collected between September 2011 and January 2013.

B. Physiology of the Relationship between Dengue Virus Infection and Abnormal Liver and Kidney Function

The direct physiological relationship between dengue virus infection and liver and kidney dysfunction has not yet been fully characterized, however one potential mechanism has been proposed: direct dengue virus invasion into liver and kidney tissue.

Dengue virus may directly invade and infect the liver and kidneys, leading to the pathogenesis of liver and kidney disorders.[12,13,16] Recent studies have proposed that, in response to dengue virus infection, the increased presence of Natural Killer (NK) cells in the liver and increases in interleukin-10, interleukin-17, and interleukin-22 are believed to contribute to hepatic cell apoptosis.[12,16] Viral invasion of the liver and kidneys can lead to hepatic sinusoidal obstruction, or the damage and death of endothelial cells in the liver,[18] as well as deposition of immune complexes within kidney cells.[13]

Recent or concurrent use of hepatotoxic drugs among dengue-infected individuals has also been shown to modify the effects of dengue virus infection on liver cells.[19] By definition, hepatotoxic drugs are known to negatively impact liver function,[20] and they can worsen the effects of dengue virus infection on liver function.[17,18] Hepatotoxic drugs include antibiotics and antiviral medications as well as commonly used anti-inflammatory medications (e.g. non-steroidal anti-inflammatory drugs) including pain/fever relievers (e.g. acetaminophen).[21]

C. Epidemiology of the Relationship between Dengue Virus Infection and Liver and Kidney Function

Five prior epidemiological studies have evaluated the relationship between dengue virus infection and liver and/or kidney function.[22-26] Four of these studies utilized a retrospective cohort design;[22,23,24,25] while the remaining study was a prospective cohort.[26] Two of the five studies included children in their study populations,[23,25] one of which included a population of children from Southeast Asia.[25] Epidemiologic studies assessing the relationship between severity of dengue virus infections and liver and kidney function are essential in order to properly monitor individuals with a dengue virus infection through the duration of their illness.

Only a single study analyzed blood creatinine and urea levels, which serve as indicators of kidney function;[22] while the other studies[23, 24, 25, 26] performed analyses of only blood liver protein levels (i.e. ALT, AST). In the study that assessed kidney protein levels by Wilder-Smith et al., adult patients diagnosed with dengue virus infection were compared to those diagnosed with severe acute respiratory syndrome (SARS) as the unexposed reference category.[22] Therefore, the authors did not use a three-level exposure of dengue disease severity in order to assess differences among dengue severity levels alone.[22] All five studies found that among patients with more severe dengue virus infections, an apparent increase in ALT and AST levels was found.[22-26]

In the only study to include children in their study population and also evaluate severity of dengue disease as the exposure of interest, Lee et al. utilized a retrospective cohort study design to analyze clinical records of 690 patients.[23] Adult and pediatric patients who presented to the Department of Infectious Diseases at Tan Tock Seng

Hospital in Singapore and tested positive for dengue virus infection between 2006 and 2008 were included in the analysis. Exposure was classified by dengue disease severity (DF, DHF, or DSS) based on the 1997 and 2009 WHO classification systems. Blood ALT and AST levels, which serve as indicators of liver function were extracted from patient records for outcome assessment. The median AST levels among DF, DHF, and DSS individuals were 93.0, 103.0, and 137.5 U/L, respectively. The median ALT levels among DF, DHF, and DSS individuals were 52.0, 60.0, and 74.0, respectively. Statistically significant increases in AST levels ($p= 0.01$) were observed, from Mann-Whitney U and Kruskal-Wallis tests, among patients categorized as having DSS, as compared to those with DF. This indicates that increasing severity of dengue disease was associated with an increased risk of liver dysfunction. This study by Lee et al. however did not stratify results by age to see if changes in ALT and AST levels differed between adults and children. These researchers also did not review indicators of kidney function (i.e. creatinine and urea).

In summary, all five epidemiological studies were limited in regard to study population, as only two[23,25] of the five[22-26] studies included children. Four studies examined the association between stages of dengue disease severity and liver function,[23-26] but only one study[22] assessed the impact of dengue disease severity on kidney protein levels. No covariates were assessed or included in the analyses of any of the five studies,[22-26] likely resulting in biased association measures due to uncontrolled confounding, particularly by age and recent non-steroidal anti-inflammatory drugs (NSAID) use.

D. Study Objectives, Significance and Innovation

This study proposes to assess the relationship between dengue disease severity as measured by disease category and risk for liver and kidney dysfunction, as measured by changes in blood protein levels, adjusting for multiple clinical factors. Specifically, the aim of the study is:

> Specific aim: Use data collected from pediatric patients in Phnom Penh, Cambodia to evaluate the relationship between dengue disease severity classification and liver and kidney protein laboratory levels.
>
> Hypothesis 1: Children diagnosed with more severe disease (dengue hemorrhagic fever and dengue shock syndrome) will have statistically significant differences in liver function, defined by higher mean blood ALT and AST levels and lower mean blood albumin and total protein levels, as compared to children who have dengue fever.
>
> Hypothesis 2: Children diagnosed with more severe disease (dengue hemorrhagic fever and dengue shock syndrome) will have statistically significant differences in liver function, defined by higher mean blood creatinine and urea protein levels, as compared to children who have dengue fever.

These analyses will contribute to the sparse epidemiological findings on the association between dengue virus infection among children in Southeast Asia and liver/kidney function.

This study is **innovative** because it is the first, to our knowledge, to analyze longitudinally the association between dengue disease severity and liver and kidney dysfunction among children in Cambodia. It is also **innovative** in its ability to adjust for multiple covariates, due largely to the prospective study design.

This study is **significant** because it utilized an under-studied population at increased risk for both the exposure and outcome. Few studies have looked at the association between dengue disease severity and risk of liver and kidney dysfunction among children, and this study can contribute to a more comprehensive understanding of the relationship between dengue virus infection and kidney and liver function in pediatric populations.

CHAPTER II

STUDY DESIGN AND METHODS

A. Study Design and Population

We evaluated the relationship between dengue disease severity and liver and kidney function among children from the Cambodian Dengue study: a prospective cohort study conducted between September 20, 2011 and January 15, 2013.[27] Participants were enrolled at the National Paediatric Hospital (NPH), Phnom Penh, Cambodia. All children aged 1-15 years presenting to the internal medicine or emergency wards of the NPH with a current fever or history of fever in the last seven days were initially considered as potential participants for this study upon their admission to NPH. Children were eligible to participate if they met either the 1997 or 2009 WHO criteria for dengue viral infection.[15,16] The exclusion criteria for the Cambodian Dengue study has been described previously,[27] and included no indication of fever or history of fever in the last week upon admission, and eligibility forms assessing clinical symptoms not being completed. Of the 1,228 children who were initially screened for inclusion, 704 participants were considered eligible due to information obtained from eligibility forms that clinically assessed patient symptoms using WHO 1997 and 2009 dengue case definitions. Ultimately, 704 children met eligibility requirements, consented to participation in the study, and were included in the final dataset.

Of these 704 participants included in the dataset, this analysis excluded 146 who were serologically negative for a dengue virus infection. Additionally, 12 participants were excluded due to missing WHO 1997 dengue infection classifications. This resulted in a final analytic population of 551 participants (Table 3).

Clinical and demographic data were collected from all enrolled participants at time of admission by NPH staff. Blood samples for liver and kidney protein analyses were collected from children upon admission and for each subsequent day of hospital stay, for up to seven consecutive days. All virological and serological blood assays were performed at the virology department of Institut Pasteur in Phnom Penh, Cambodia; all routine clinical laboratory analyses were performed at NPH.

Ethics: The study was conducted according to the ethical principles of the Declaration of Helsinki of October 2002. The Cambodian National Ethics Committee for Health Research approved the overall study protocol after institutional review (approval number 123-NECHR, 22 August 2011), with fully informed consent in Khmer for all participants and/or guardians.

B. Exposure Assessment

Blood samples were collected from all eligible participants upon admission and were immediately centrifuged. Serum samples were shipped the same day for serologic and virologic testing for dengue virus infection and other potential arboviral infections. Serologic testing for infection included IgG / IgM Capture ELISA and hemagglutination inhibition assays. Virologic testing was performed through direct virus isolation, RT-PCR, and NS1 antigen detection by ELISA. A combination diagnostic kit that detects NS1 antigen and IgM and IgG antibodies was also utilized. Participants who tested negative for dengue virus infection by virological and serological testing were excluded from the analyses.

NPH staff utilized clinical assessment to classify patients by severity of dengue virus infection using both the 1997 and 2009 WHO classification systems, upon patient

admission to NPH.[15,16] Patients presenting with symptoms of a dengue virus infection were classified into one of three disease categories based on clinical judgement: DF, DHF, or DSS.

Subsequently, the results of serologic and virologic blood assays, as well as clinical assessment criteria using the 1997 WHO dengue classification system were applied to categorize participants by exposure status into three severity categories (DF, DHF, or DSS) for our categorical severity variable. Due to small cell frequencies, a second categorical variable was created for multivariable analyses that categorized participants by dichotomous disease severity categories. In other words, participants were categorized as having severe dengue if they were clinically diagnosed with DHF or DSS.

The combination diagnostic test kit used in this study has been shown to be valid for detecting dengue virus infection by NS1 antigen and IgM and IgG antibodies. In a field validity study, Andries et al. found a sensitivity of 85.7% (95% CI: 78.4 to 91.3), specificity of 83.9% (95% CI: 66.3 to 94.5), positive predictive value (PPV) of 95.6% (95% CI: 90.0 to 98.5), and a negative predictive value (NPV) of 59.1% (95% CI: 43.2 to 73.7) for dengue virus infection among hospital laboratories in Cambodia. Additionally, the national reference laboratory at Institut Pasteur (Cambodia) found the sensitivity, specificity, PPV, and NPV of the combination diagnostic kit for dengue virus infection to be 94.4% (95% CI: 88.9, 97.7), 90.0% (95% CI: 73.5, 97.9), 97.5% (95% CI: 93.0, 99.5), and 77.1% (95% CI: 59.9, 89.6), respectively.[28] When used in conjunction with clinical assessment of symptoms, false negative results were not found to impact patient management.

The RT-PCR method used to detect the dengue virus has been well-validated in prior studies. In a validity study by Hue et al., the limit of detection (LOD) and specificity of RT-PCR for dengue RNA detection and quantification in human blood samples was assessed.[29] The specificity for both viral particle and plasmid DNA amplification was 100%. The authors concluded the RT-PCR method had good sensitivity for all four strains of dengue virus.

Mosquito C6/36 and Vero E6 cell lines were used for direct viral culture in this study; the validity of this procedure has been described elsewhere and is a widely used technique for directly identifying the presence of virus in culture.[30]

C. Outcome Assessment

Blood was collected from all participants upon admission and for up to seven consecutive days, or until discharge. Liver protein levels assessed included serum ALT, AST, albumin, and total protein. Kidney protein levels measured included serum creatinine and urea levels.

Additionally, liver and kidney protein levels were categorized into normal and abnormal values, by sex, using the cut-off points for abnormal laboratory values shown in Table 2. These cut-off points for high versus low values have been adapted from *The Harriet Lane Handbook*, *21st Edition*.[31]

D. Covariate Assessment

Risk factors associated with both dengue virus infection and with abnormal liver and kidney function were assessed as possible confounders. These covariates included: age, sex, body mass index (BMI), recent NSAID use, clinical diagnosis of influenza-like illness (ILI) or acute respiratory illness (ARI), confirmed diagnosis of malaria or

chikungunya, prior dengue virus infection, time of fever onset prior to admission, infusion prior to admission, and all blood parameters collected (i.e. complete blood count, glucose level, sodium level).[7-11, 31] Data for age, height and weight (used to calculate BMI), and sex were collected upon patient admission to NPH. Clinical diagnosis of ILI/ARI, malaria, or chikungunya were determined from lab data collected at patient discharge. Prior use of NSAIDs was self-reported upon admission, and NSAID use on each day of hospitalization was also documented daily by NPH staff for 12 consecutive days or until discharge. Prior dengue virus infection, time of fever onset prior to admission (days), and infusion prior to admission were self-reported by participants, or guardians, at time of admission. Blood count parameters such as leukocyte and platelet counts were analyzed upon admission, and on each day of hospitalization for 12 consecutive days or until discharge. Blood glucose, sodium, potassium, and calcium levels were analyzed only upon admission.

CHAPTER III

DATA ANALYSIS

Specific aim: Use data collected from pediatric patients in Phnom Penh, Cambodia to evaluate the relationship between dengue disease severity and liver and kidney protein levels.

All statistical analyses were conducted using SAS version 9.4. Multiple imputation was conducted to account for missing data among all covariates as well as among the liver and protein outcome measures. This multiply imputed dataset was used for multivariable model building only. The significance of each of the four multivariable models was assessed using Wald tests for model fit.

A. Descriptive Analysis

The total number and percent of study participants who tested negative for dengue virus infection and the number and percent of those missing dengue disease severity classification using 1997 WHO guidelines was assessed. The number and proportion of those diagnosed with DF, DHF, and DSS by clinical assessment was also calculated. The mean and SD of liver protein levels (i.e. albumin, ALT, AST, and total protein) and kidney protein levels (i.e. creatinine and urea) were calculated for up to seven days of follow up. The distribution of all continuous laboratory analyses (e.g. liver and kidney protein levels, complete blood counts, etc.) followed normal distributions, and therefore did not require transformation of values.

B. Bivariate Analysis

Cross-tabulations of all covariates by both our exposure (dengue disease severity assessed by clinical assessment) and our outcome (liver and kidney protein levels) were performed. Chi-square (X^2) tests were used to calculate p-values for categorical variables. Where small cell frequencies (n<5) were encountered, the Fisher's Exact Test was utilized. To calculate p-values for our continuous variables, ANOVA was used.

C. Multivariable Analysis

Linear mixed modeling was used to model the relationship between dengue disease severity and abnormal liver protein levels for a total of seven days of patient hospital admission. Due to the small number of participants with values for kidney protein outcomes (creatinine and urea levels) analyzed in this cohort, multivariable regression analyses were performed only for liver protein outcomes (i.e. albumin, ALT, AST, and total protein). Separate models were created for each of the four continuous liver protein metrics. Covariates included in the model that caused a 20% change in the coefficient for dengue disease severity were considered as confounding variables and were included in all final models. Unadjusted and adjusted coefficient estimates are reported with corresponding 95% confidence intervals.

CHAPTER IV

RESULTS

Characteristics of the study participants are described in Table 6. Of the 551 participants included in this study, 456 (82.8%) were clinically diagnosed with DF, 91 (16.5%) were diagnosed with DHF, and 4 (0.7%) were diagnosed with DSS. The mean age (SD) of children in this cohort for those diagnosed with DF, DHF, and DSS was 7.9 (3.38), 8.43 (3.82), and 5.5 (1.29), respectively (ANOVA: $p= 0.15$). Those clinically diagnosed with more severe forms of dengue (i.e. dengue hemorrhagic fever or dengue shock syndrome) had significantly lower platelet counts upon admission to NPH, as compared to those diagnosed with dengue fever ($p<0.001$). Although only one participant diagnosed with DSS had their sodium level analyzed upon admission, this value was significantly lower than the mean sodium levels among those diagnosed with DF or DHF ($p=0.04$). There was no evidence for differences in any of the other examined covariates compared across the three levels of dengue disease severity (Table 6).

Results from the liver protein laboratory analyses are presented in Tables 4, 7 and 8. Overall, upon admission a significantly greater proportion of those diagnosed with DF had abnormal ALT and AST protein levels (both with $p <0.0001$; Table 4). Similarly, a significantly higher percentage of those diagnosed with DHF had abnormal ALT and AST levels upon admission (both with $p<0.0001$; Table 4). Participant liver protein levels at admission were more likely to be abnormal (81.0 to 100% of all participants) with the exception of total protein, which was more likely to be within the normal range (Table 7). The mean age (SD) of those with abnormal albumin, ALT, AST, and total protein levels was 9.33 (3.06), 7.99 (3.42), 8.02 (3.40), and 7.28 (3.03), respectively (Table 8). A higher

percentage of those with abnormal ALT levels upon admission were female (52.7%; p=0.0005), however no statistically significant differences between participant sex were observed for any other liver or kidney protein outcome (Table 8). Additionally, participants with abnormal total protein levels at admission were significantly more likely to have had an infusion prior to admission (67.8%; p=0.0007); have a significantly lower platelet count (p<0.0001) and mean corpuscular volume (p=0.012); and have a significantly higher hematocrit percent (p=0.045) and hemoglobin value (p=0.025; Table 8). No other statistically significant differences in participant characteristics were observed across the four liver protein outcomes (Table 8).

Results from the kidney protein laboratory analyses are presented in Tables 5, 7, and 9. Few participants had their creatinine (n=6) or urea (n=3) levels measured upon admission (Table 5). However, among those with measurement values, creatinine levels were more likely to be abnormal (100%) and urea levels were more likely to be normal (100%; Table 7). Because of the small number of participants with kidney protein level measurements, statistical differences in creatinine and/or urea levels by participant characteristics could not be assessed (Table 9).

In general, over the seven days of follow-up, the absolute number (Fig. 1) and percent (Fig. 2) of those with any abnormal liver protein level who were diagnosed with DF was greater than that of those diagnosed with severe dengue (dengue hemorrhagic fever or dengue shock syndrome). However, a larger proportion of participants with severe dengue had at least one abnormal liver protein measure on days 2 (DF: 12.72%, severe dengue: 31.58%), 4 (DF: 2.19%, severe dengue: 5.26%), and 5 (DF: 0%, severe

dengue: 2.11%). Few participants had protein measurements taken on days 4-7; thus, these results should be interpreted with caution.

Day1 (day of admission) through day 3 of hospital stay contained the largest number of participants with analyzed ALT and AST levels. Upon admission and on day 2 of hospital stay, the average ALT (Figure 5) and AST (Figure 6) levels remained consistent, around 85-90 IU/L. On day 3 of hospital stay, the average protein levels were seen to decrease significantly to around 50 IU/L for both ALT (Figure 5) and AST (Figure 6) levels. High variation was observed for ALT and AST protein levels analyzed on all days of hospital stay among those clinically diagnosed with DF compared to those clinically diagnosed with severe dengue (Figure 5 and Figure 6), likely due to the significantly larger number of participants in the DF exposure category.

None of the participants with dengue fever had measurement values for creatinine or urea. However, a distribution of the absolute number (Figure 3) and proportion (Figure 4) of participants with severe dengue who had at least one abnormal kidney protein value on each of the seven days of follow up are presented. A clear increase in the number of individuals (11) with severe dengue who had abnormal kidney protein level(s) is observed on day two of hospital stay.

Table 10 shows the associations between dichotomized dengue disease severity and liver protein outcomes. We observed a significant increase in mean total protein level among those diagnosed with severe dengue as compared to those diagnosed with dengue fever in both unadjusted and adjusted models (B_{adj} = 7.14, 95% CI = 1.15 to 13.13). We also observed a significant decrease in mean albumin levels among participants with severe dengue disease compared to those with dengue fever; however, this association

became insignificant following adjustment for covariates (B_{adj} = -0.08, 95% CI = -0.45 to 0.29). Finally, we also observed a nominally significant decrease in the mean ALT and AST protein levels among those diagnosed with severe dengue as compared to those diagnosed with dengue fever (ALT: B_{adj} = -18.02, 95% CI = -51.59 to 15.55; AST: B_{adj} = -7.18, 95% CI = -49.57 to 35.21). Because of the small sample size, we were not able to evaluate the association between dengue severity and creatinine or urea protein levels in a fully-adjusted analysis.

CHAPTER V

DISCUSSION

In this prospective cohort study of pediatric dengue virus infections in Cambodia, descriptive statistics were used to assess the impact of severity of dengue virus infection on kidney dysfunction. Additionally, descriptive statistics and linear mixed modeling were used to assess the impact of severity of dengue virus infection on liver dysfunction while adjusting for important risk factors. We observed one statistically significant positive association between dengue disease severity and total protein levels. Three non-significant inverse associations were observed between dengue disease severity and albumin, ALT, and AST protein levels. Although it was not possible to assess an association between dengue disease severity and kidney protein levels using multivariable models, a large proportion of those diagnosed with severe forms of dengue virus infections had abnormal creatinine and urea levels on each day of hospital stay, with an apparent increase in the number and percent of participants with abnormal levels on day 2 of hospital stay.

There are few epidemiological studies that have assessed the quantitative association between severity of dengue virus infection and liver protein outcomes[23, 24, 26] and even fewer that have assessed an association with kidney protein outcomes.[22] All three studies that specifically examined severity of dengue virus infections and the association with liver protein outcomes found statistically significant increases in either the median or the mean ALT and AST levels among those diagnosed with more severe forms of dengue virus infection, compared to those diagnosed with DF throughout the duration of hospital stay. However, in general, the previous studies had significantly

larger populations and comprehensive follow-up data available for a minimum of 10 days of hospital stay, whereas the present study analyzed lab values collected for up to seven days of follow-up. Two studies[24, 26] excluded participants less than 14 years of age, limiting their analyses to a population that is comparatively older (mean age: 21 and 26.4) than our study population. The third study[23] included a population of adults and children, however the mean age of the population was 35 years. The mean age of participants in our study was 8 years for those diagnosed with DF or DHF and 6 years for those diagnosed with DSS. Because our study is the first to look at an exclusively pediatric population, our results may provide age-specific association measures between dengue disease severity and liver and kidney dysfunction.

We observed an overall decrease in average ALT and AST levels and an increase in average total protein levels among those diagnosed with more severe forms of dengue virus infections as compared to those diagnosed with DF. Because liver dysfunction is characterized in part by increases in ALT and AST levels and decreases in total protein levels, our results do not suggest an overall increase in liver dysfunction among those clinically diagnosed with severe forms of dengue virus infections (i.e. DHF or DSS) compared to those clinically diagnosed with DF. Despite the small number of participants with measured albumin levels, we did observe a decrease in the mean albumin level over the 7 days of follow up among those clinically diagnosed with severe dengue compared to those clinically diagnosed with DF. Although statistical significant effects were not found in this study, these results are consistent with previous literature[23,24,26] and suggest potential for decreased liver function among those with more

severe forms of dengue virus infections, as blood albumin levels are known to decrease in response to liver dysfunction.

This study is not without limitations. One important limitation is loss to follow up. Studies involving the prospective collection of hospital data are prone to differential loss of participants based on patient diagnosis, symptomatology, and overall patient health. The large proportion of missing data in the present study suggest that participants were either discharged from NPH at different time points or passed away. Those with less severe dengue virus infections (i.e. DF) who had adequate liver and kidney function were likely discharged from the hospital sooner than those who had more severe forms of dengue virus infections and/or impaired liver and kidney function. This form of differential loss to follow up would result in an attenuation of any measured association.

Non-differential misclassification of participant outcome could have also occurred in our study, leading to an underestimation of the association between dengue disease severity and liver and kidney dysfunction. Participants were diagnosed with DF, DHF, or DSS using clinical judgement alone, therefore children in this study could have been incorrectly classified if their symptomatology was difficult to assess.

The time of patient admission to NPH does not necessarily correlate with time of infection with dengue virus, or time of disease onset (i.e. DF, DHF, DSS) in our study. Children admitted to NPH may have had, in general, more severe cases of dengue virus infection compared to the general pediatric population. It is also possible that children initially diagnosed with DF upon admission developed more severe symptoms later in their hospital stay. This would have incorrectly classified patients as having DF, whose data actually represent someone with DHF or DSS. It is also possible that severely ill

patients admitted to NPH who were initially classified as having DHF or DSS improved during their hospital stay, and had characteristics that were more closely related to a diagnosis with DF. This could have also misclassified participants in our study later in their hospital stay.

Despite the potential limitations, our study has several strengths. The prospective study design and collection of follow-up data limit the potential for biases such as recall bias or temporal bias that are common in other study designs. Our ability to adjust for multiple covariates makes our study unique from other studies that also looked at severity of dengue virus infections and liver and kidney dysfunction, and allows us to report a multivariable-adjusted effect estimate comparing those diagnosed with DF with those diagnosed with more severe forms of dengue virus infection. Our study population was also an under-studied, but high-risk, cohort of children in Southeast Asia.

Overall, the present study found that those diagnosed with more severe forms of dengue virus infections have lower average albumin, ALT, and AST protein levels and higher average total protein levels when compared to those diagnosed with a less severe dengue virus infection. Overall, this is not suggestive of large increases in liver dysfunction among those clinically diagnosed with severe dengue virus infections, compared to those diagnosed with DF. A large percentage of those with severe dengue virus infections were also observed to have abnormal kidney protein levels (creatinine or urea). Although only one of our effect estimates reached statistical significance, it is important to note the significant loss to follow up for liver and kidney protein levels that reduced the analytic population size for each subsequent day of hospital stay. This study provides preliminary results that lay the groundwork for future studies assessing the

association between dengue disease severity and liver and kidney function among children. Further studies utilizing larger populations of children and comprehensive follow up for liver and kidney protein levels are warranted and will help explain the extent to which severity of dengue virus infection influences liver and kidney function among children with a dengue virus infection.

Table 1. WHO 1997 and 2009 Dengue Infection Classification Guidelines

WHO Guideline Year:	1997	2009
	DF: Headache, myalgia, leukopenia, rash, hemorrhagic manifestations	Dengue w/out Warning Signs: nausea, rash, aches, leukopenia, positive tourniquet test
	DHF: Fever or history of fever (2-7 days), positive tourniquet test, bleeding from various sites, thrombocytopenia, plasma leakage	Dengue w/ Warning Signs: abdominal tenderness/pain, persistent vomiting, fluid accumulation, bleeding, lethargy, liver enlargement, increase in hematocrit, decreased platelet count
	DSS: All signs of DHF plus hypotension for age, rapid/weak pulse, cold and clammy skin	Severe Dengue: severe plasma leakage, shock, fluid accumulation, respiratory distress, severe bleeding, severe organ involvement and failure

Clinical criteria used to classify patients by dengue disease severity, according to WHO 1997 and 2009 guidelines for dengue diagnosis and treatment. Information in this table was adapted from Narvaez et al.

Table 2. Cut-off Points for Defining Abnormal Liver and Kidney Protein Levels; CDS 2011-2013, n=551

Protein	Abnormal values-Male	Abnormal values-Female
ALT	>45 IU/L	>30 IU/L
AST	>40 IU/L	>35 IU/L
Albumin	<36 g/L	<36 g/L
Total protein	<56 g/L	<56 g/L
Urea	>6.4 mmol/L	>6.4 mmol/L
Creatinine	>62 µmol/L	>62 µmol/L

Cut-off points for pediatric patients, by sex, adapted from the *Harriet Lane Handbook, 21st edition*
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; IU, international units.

Table 3. Number and Percent in Final Sample; CDS 2011-2013, n=551

	Total	Percent[a]
Original Study Sample	704	100%
Excluded		
Negative testing for dengue infection[b]	146	20.70%
Missing WHO 1997 dengue infection classification	12	1.70%
Final Sample Size	551	77.60%

[a]Percent of original population
[b]Negative testing for Dengue infection determined by serological and virological laboratory assays

Table 4. Distribution of Dengue Disease Severity by Liver Protein Levels; CDS 2011-2013, n=551

	Liver Protein Levels											
	Albumin			ALT			AST			Total Protein		
Dengue Disease Severity	Normal	Abnormal	p-value	Normal	Abnormal	p-value	Normal	Abnormal	p-value	Normal	Abnormal	p-value
Dengue Fever	0 (0%)	0 (0%)	n/a	72 (18.56%)	316 (81.44%)	<0.0001***	56 (14.43%)	332 (85.57%)	<0.0001***	285 (74.8%)	96 (25.2%)	<0.0001***
Dengue Hemorrhagic Fever	0 (0%)	3 (100%)	n/a	5 (8.93%)	51 (91.07%)	<0.0001***	8 (14.29%)	48 (85.71%)	<0.0001***	7 (100%)	0 (0%)	n/a
Dengue Shock Syndrome	0 (0%)	0 (0%)	n/a	1 (50%)	1 (50%)	n/a	0 (0%)	2 (100%)	n/a	0 (0%)	1 (100%)	n/a

Normal and abonrmal liver protein levels are from admission blood analyses only. Numbers presented are total number of participants with correpsonding percent in each dengue disease severity category. Fisher's exact tests were used to calculate p-values, however p-values could not be calculated for comparison groups with <1 data point (indicated by value of "n/a").

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

***p<0.0001

Table 5. Distribution of Dengue Disease Severity by Kidney Protein Levels; CDS 2011-2013, n=551

	Kidney Protein Levels			
	Creatinine		Urea	
Dengue Disease Severity	Normal	Abnormal	Normal	Abnormal
Dengue Fever	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Dengue Hemorrhagic Fever	0 (0%)	6 (100%)	3 (100%)	0 (0%)
Dengue Shock Syndrome	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Normal and abnormal liver protein levels are from admission blood analyses only. Numbers presented are total number of participants with corresponding percent in each dengue disease severity category. P-values could not be calculated as all comparison groups contained <1 data point.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table 6. Distribution of Covariates According to Dengue Disease Severity; CDS 2011-2013, n=551

Characteristics	Dengue Disease Severity			p-value
	Dengue Fever (n=456)	Dengue Hemorrhagic Fever (n=91)	Dengue Shock Syndrome (n=4)	
Age	7.90 (3.38)	8.43 (3.82)	5.50 (1.29)	0.15
Sex				0.16
Male	236 (51.75%)	37 (40.66%)	2 (50.00%)	
Female	220 (48.25%)	54 (59.34%)	2 (50.00%)	
BMI[a]				0.27
Underweight	195 (42.76%)	27 (29.67%)	2 (50.00%)	
Normal weight	211 (46.27%)	51 (56.04%)	2 (50.00%)	
Overweight	21 (4.61%)	7 (7.69%)	0 (0%)	
Obese	28 (6.36%)	6 (6.59%)	0 (0%)	
Prior NSAID Use				1.00
Yes	1 (0.22%)	0 (0%)	0 (0%)	
No	454 (99.56%)	91 (100%)	4 (100.00%)	
Clinical Diagnosis ARI/ILI				0.53
Yes	3 (0.66%)	1 (1.10%)	0 (0%)	
No	453 (99.34%)	90 (98.90%)	4 (100.00%)	
Prior Dengue Virus Infection				0.56
Yes	3 (0.66%)	1 (1.10%)	0 (0%)	
No	237 (51.97%)	51 (56.04%)	3 (100.00%)	
Infusion Prior to Admission				0.43
Yes	221 (48.46%)	35 (38.46%)	2 (50.00%)	
No	218 (47.81%)	53 (58.24%)	2 (50.00%)	
Leukocyte Count	5.26 (3.00)	5.89 (3.99)	6.88 (1.76)	0.15
Lymphocyte Count	1.91 (1.22)	2.26 (1.83)	1.80 (1.38)	0.07

Table 6. Distribution of Covariates According to Dengue Disease Severity; CDS 2011-2013, n=551 (cont.)

Characteristics	Dengue Disease Severity			p-value
	Dengue Fever (n=456)	Dengue Hemorrhagic Fever (n=91)	Dengue Shock Syndrome (n=4)	
Platelet Count	95.62 (50.68)	74.88 (38.00)	73.50 (89.47)	0.001**
Neutrophil Count	2.63 (1.71)	3.08 (1.62)	3.80 (n/a)	0.69
Hemoglobin Value	13.03 (1.50)	14.33 (0.95)	13.50 (n/a)	0.22
Mean Corpuscular Volume	77.95 (5.91)	72.50 (6.61)	77.00 (n/a)	0.18
Mean Corpuscular Hemoglobin	25.16 (2.32)	24.50 (1.91)	25.00 (n/a)	0.85
Glucose Level	3.90 (n/a)	4.86 (1.48)	3.90 (n/a)	0.69
Sodium Level	140.00 (n/a)	140.33 (4.51)	127.00 (n/a)	0.04*
Potassium Level	4.20 (n/a)	4.57 (0.84)	4.30 (n/a)	0.88
Calcium Level	2.00 (n/a)	2.17 (0.26)	2.00 (n/a)	0.69

Totals/means are from admission laboratory data only. Numbers presented are total number of participants with corresponding percent in each dengue disease severity category for categorical characteristics or mean with correpsonding standard deviation for continuous characteristics. Characteristics with only one data point have standard deviation values of "n/a". χ-square tests were used to calcualte p-values for categorical characteristic variables. Fisher's exact tests were used in place of χ-sqaure tests when small cell frequencies were encountered. ANOVA tests were used to calculate p-values for continuous characteristic variables.

[a]BMI classification based on WHO and CDC criteria where underweight = <5th percentile, normal weight = 5th to <85th percentile, overweight = 85th to <95th percentile, obese = ≥ 95th percentile.

Abbreviations: ARI: acute respiratory illness; ILI: influenza-like illness

*p<0.05; **p<0.01

Table 7. Distribution of Conitnuous and Categorical Liver and Kidney Protein Levels; CDS 2011-2013, n=551

		Mean	SD	Total (%) Normal Levels	Total (%) Abnormal Levels
Liver:					
Albumin:					
	Admission Level	3.66	1.15	0 (0%)	3 (100.00%)
	Day 2 Level	5.4	1.52	0 (0%)	5 (100.00%)
ALT:					
	Admission Level	82.28	49.88	78 (17.49%)	368 (82.51%)
	Day 2 Level	78.02	62.93	17 (18.48%)	75 (81.52%)
	Day 3 Level	73.29	51.93	8 (19.05%)	34 (80.95%)
	Day 4 Level	101.13	79.49	3 (20.00%)	12 (80.00%)
	Day 5 Level	4.86	2.41	7 (100.00%)	0 (0%)
	Day 6 Level	3.5	2.12	2 (100.00%)	0 (0%)
	Day 7 Level	2.67	2.89	3 (100.00%)	0 (0%)
AST:					
	Admission Level	89.75	55.5	64 (14.35%)	382 (85.65%)
	Day 2 Level	89.88	71.22	17 (18.48%)	75 (81.52%)
	Day 3 Level	72.67	48.69	7 (16.67%)	35 (83.33%)
	Day 4 Level	111.27	66.4	3 (20.00%)	12 (80.00%)
	Day 5 Level	5.14	2.61	7 (100.00%)	0 (0%)
	Day 6 Level	4.5	0.71	2 (100.00%)	0 (0%)
	Day 7 Level	2.33	2.31	3 (100.00%)	0 (0%)
Total Protein:					
	Admission Level	62.34	10.29	292 (75.06%)	97 (24.94%)
	Day 2 Level	5.29	2.05	0 (0%)	17 (100.00%)
	Day 3 Level	5.78	0.83	0 (0%)	9 (100.00%)
	Day 5 Level	6.00	0	0 (0%)	2 (100.00%)
Kidney:					
Creatinine:					
	Admission Level	81.00	9.40	0 (0%)	6 (100.00%)
	Day 2 Level	93.58	33.67	2 (16.67%)	10 (83.33%)
	Day 3 Level	65.25	17.23	2 (50.00%)	2 (50.00%)
	Day 4 Level	72.67	20.43	1 (33.33%)	2 (66.67%)
	Day 5 Level	5.00	0.00	2 (100.00%)	0 (0%)
Urea:					
	Admission Level	4.10	1.18	3 (100.00%)	0 (0%)
	Day 2 Level	14.08	23.57	6 (75.00%)	2 (25.00%)
	Day 3 Level	2.50	0.71	2 (100.00%)	0 (0%)
	Day 4 Level	5.50	4.95	1 (50.00%)	1 (50.00%)
	Day 5 Level	6.00	4.24	1 (50.00%)	1 (50.00%)

Percentages represent number of participants with analyzed protein level on each day of hospital stay divided by the total number of individuals with the analyzed protein level on the same day of hospital stay. Data presented only for days of hospital stay where ≥ 1 participant had analyzed protein levels.

Abbreviations: SD, standard deviation; ALT: alanaine aminotransferase; AST: aspartate aminotransferase

Table 8. Distribution of Covariates by Liver Protein Levels; CDS 2011-2013, n=551

	Liver Protein Levels											
	Albumin			ALT			AST			Total Protein		
Characteristics	Normal	Abnormal	p-value	Normal	Abnormal	p-value	Normal	Abnormal	p-value	Normal	Abnormal	p-value
Age	n/a	9.33 (3.06)	n/a	7.83 (3.57)	7.99 (3.42)	0.71	7.66 (3.67)	8.02 (3.40)	0.44	8.03 (3.41)	7.28 (3.03)	0.05
Sex						0.0005**			0.79			1.00
Male	0 (0%)	2 (66.67%)	n/a	54 (69.23%)	174 (47.28%)		34 (53.13%)	194 (50.79%)		149 (51.03%)	50 (51.55%)	
Female	0 (0%)	1 (33.33%)	n/a	24 (30.77%)	194 (52.72%)		30 (46.87%)	188 (49.21%)		143 (48.97%)	47 (48.45%)	
BMI[a]						0.77			0.15			0.17
Underweight	0 (0%)	0 (0%)	n/a	31 (39.74%)	162 (44.02%)		23 (35.94%)	170 (44.5%)		138 (47.26%)	39 (40.21%)	
Normal weight	0 (0%)	2 (66.67%)	n/a	38 (48.72%)	161 (43.75%)		33 (51.56%)	166 (43.46%)		119 (40.75%)	50 (51.55%)	
Overweight	0 (0%)	0 (0%)	n/a	5 (6.41%)	19 (5.16%)		6 (9.38%)	18 (4.71%)		13 (4.45%)	5 (5.15%)	
Obese	0 (0%)	1 (33.33%)	n/a	4 (5.13%)	26 (7.07%)		2 (3.13%)	28 (7.33%)		22 (7.53%)	3 (3.09%)	
Prior NSAID Use						1.00			1.00			0.25
Yes	0 (0%)	0 (0%)	n/a	0 (0%)	1 (0.27%)		0 (0%)	1 (0.26%)		0 (0%)	1 (1.03%)	
No	0 (0%)	3 (100.00%)	n/a	78 (100.00%)	366 (99.73%)		64 (100.00%)	380 (99.74%)		291 (100.00%)	96 (98.97%)	
Clinical diagnosis ARI/ILI						0.08			0.06			0.58
Yes	0 (0%)	0 (0%)	n/a	2 (2.56%)	1 (0.27%)		2 (3.13%)	1 (0.26%)		3 (1.03%)	0 (0%)	
No	0 (0%)	3 (0%)	n/a	76 (97.44%)	367 (99.73%)		62 (96.88%)	381 (99.74%)		289 (98.97%)	97 (100.00%)	
Prior dengue virus infection						0.91			0.27			0.40
Yes	0 (0%)	0 (0%)	n/a	0 (0%)	4 (1.90%)		0 (0%)	4 (1.86%)		3 (1.83%)	0 (0%)	
No	0 (0%)	2 (100.00%)	n/a	46 (100.00%)	207 (98.10%)		42 (100.00%)	211 (98.14%)		161 (98.17%)	48 (100.00%)	
Infusion prior to admission						0.30			0.48			0.0007**
Yes	0 (0%)	2 (66.67%)	n/a	31 (41.33%)	180 (50.85%)		26 (41.94%)	185 (50.41%)		134 (47.52%)	61 (67.78%)	
No	0 (0%)	1 (33.33%)	n/a	44 (58.67%)	174 (49.15%)		36 (58.06%)	182 (49.59%)		148 (52.48%)	29 (32.22%)	
Leukocyte count	n/a	5.07 (1.83)	n/a	5.21 (2.94)	5.43 (3.36)	0.59	5.30 (2.91)	5.41 (3.35)	0.80	5.27 (3.22)	5.81 (3.23)	0.59
Lymphocyte count	n/a	4.70 (4.75)	n/a	2.01 (1.56)	1.98 (1.32)	0.84	2.09 (1.57)	1.97 (1.33)	0.50	1.92 (1.36)	2.20 (1.35)	0.09
Hematocrit percent	n/a	41.33 (4.93)	n/a	40.21 (3.90)	40.32 (4.88)	0.85	39.83 (4.10)	40.38 (4.81)	0.39	40.13 (4.58)	41.22 (4.71)	0.0454*
Platelet count	n/a	69.67 (31.26)	n/a	102.49 (53.26)	90.93 (47.75)	0.06	96.27 (59.09)	92.40 (47.03)	0.56	99.53 (49.36)	73.18 (43.46)	<0.0001***

Table 8. Distribution of Covariates by Liver Protein Levels; CDS 2011-2013, n=551 (cont.)

	Liver Protein Levels											
	Albumin			ALT			AST			Total Protein		
Characteristics	Normal	Abnormal	p-value	Normal	Abnormal	p-value	Normal	Abnormal	p-value	Normal	Abnormal	p-value
Neutrophil count	n/a	n/a	n/a	2.70 (2.09)	2.59 (1.57)	0.60	2.75 (2.28)	2.58 (1.56)	0.51	2.59 (1.56)	2.87 (2.18)	0.18
Hemoglobin value	n/a	n/a	n/a	12.94 (1.41)	13.04 (1.54)	0.64	12.78 (1.46)	13.06 (1.52)	0.20	12.91 (1.47)	13.30 (1.56)	0.0254*
Mean Corpuscular Volume	n/a	n/a	n/a	76.70 (6.15)	77.90 (5.93)	0.13	76.61 (6.55)	77.86 (5.87)	0.16	78.29 (5.65)	76.56 (6.38)	0.0122*
Mean Corpuscular Hemoglobin	n/a	n/a	n/a	24.67 (2.70)	25.18 (2.26)	0.11	24.52 (2.63)	25.18 (2.29)	0.06	25.20 (2.28)	24.77 (2.33)	0.11
Glucose level	n/a	4.10 (n/a)	n/a	3.90 (0.10)	4.44 (0.73)	0.24	3.90 (0.14)	4.39 (0.72)	0.37	3.95 (0.21)	3.90 (n/a)	0.88
Sodium level	n/a	143.50 (0.71)	n/a	136.67 (8.74)	140.00 (4.99)	0.41	141.50 (3.54)	138.82 (6.15)	0.57	n/a	127.00 (n/a)	n/a
Potassium level	n/a	3.90 (0.28)	n/a	3.93 (0.47)	4.56 (0.72)	0.19	3.75 (0.49)	4.54 (0.68)	0.15	n/a	4.30 (n/a)	n/a
Calcium level	n/a	2.05 (0.21)	n/a	2.05 (0.13)	2.16 (0.25)	0.49	2.08 (0.19)	2.15 (0.25)	0.71	2.20 (n/a)	2.00 (n/a)	n/a

Totals/means are from admission laboratory data only. Numbers presented are total number of participants with corresponding percent in each liver protein category for categorical characteristics or mean with corresponding standard deviation for continuous characteristics. Characteristics with only one data point have standard deviation values of "n/a". Normal and abnormal protein levels with characteristic values of "n/a" indicate missing data. χ-square tests were used to calcualte p-values for categorical characteristic variables. Fisher's exact tests were used in place of χ-sqaure tests when small cell frequencies were encountered. ANOVA tests were used to calculate p-values for continuous characteristic variables. P-values could not be calculated for comparison groups with <1 data point (indicated by value of "n/a" in p-value columns).

Abbreviations: ARI: acute respiratory illness; ILI: influenza-like illness

[a]BMI classification based on WHO and CDC criteria where underweight = <5th percentile, normal weight = 5th to <85th percentile, overweight = 85th to <95th percentile, obese = ≥ 95th percentile.

*p<0.05, **p<0.01, ***p<0.0001

Table 9. Distribution of Covariates by Liver Protein Levels; CDS 2011-2013, n=551

	Kidney Protein Levels[b]	
	Creatinine	**Urea**
Characteristics	Abnormal	Normal
Age	9.83 (3.13)	8.67 (4.16)
Sex		
Male	1 (16.67%)	1 (33.33%)
Female	5 (83.33%)	2 (66.67%)
BMI[a]		
Underweight	2 (33.33%)	0 (0%)
Normal weight	4 (66.67%)	3 (100%)
Overweight	0 (0%)	0 (0%)
Obese	0 (0%)	0 (0%)
Prior NSAID Use		
Yes	0 (0%)	0 (0%)
No	6 (100%)	3 (100%)
Clinical diagnosis ARI/ILI		
Yes	0 (0%)	0 (0%)
No	6 (100%)	3 (100%)
Prior dengue virus infection		
Yes	0 (0%)	0 (0%)
No	3 (100%)	2 (100%)
Infusion prior to admission		
Yes	4 (66.67%)	2 (66.67%)
No	2 (33.33%)	1 (33.33%)
Leukocyte count	8.07 (4.24)	4.90 (1.77)

Table 9. Distribution of Covariates by Liver Protein Levels; CDS 2011-2013, n=551 (cont.)

	Kidney Protein Levels[b]	
	Creatinine	**Urea**
Characteristics	Abnormal	Normal
Lymphocyte count	2.83 (1.30)	2.20 (1.51)
Hematocrit percent	45.17 (5.64)	41.00 (4.36)
Platelet count	52.50 (29.53)	63.67 (40.02)
Neutrophil count	n/a	n/a
Hemoglobin value	n/a	n/a
Mean Corpuscular Volume	n/a	n/a
Mean Corpuscular Hemoglobin	n/a	n/a
Glucose level	5.08 (0.84)	4.35 (0.35)
Sodium level	141.50 (4.23)	144.00 (1.00)
Potassium level	4.42 (0.74)	3.83 (0.23)
Calcium level	2.15 (0.17)	2.03 (0.16)

Totals/means are from admission laboratory data only. Numbers presented are total number of participants with corresponding percent in each liver protein category for categorical characteristics or mean with corresponding standard deviation for continuous characteristics. Normal or abnormal protein levels with characteristic values of "n/a" indicate missing data. P-values could not be calculated as all comparison groups contained <1 data point.

Abbreviations: ARI: acute respiratory illness; ILI: influenza-like illness

[a]BMI classification based on WHO and CDC criteria where underweight = <5th percentile, normal weight = 5th to <85th percentile, overweight = 85th to <95th percentile, obese = ≥ 95th percentile.

Table 10. Unadjusted and Multivariable Beta Estimates and 95% Confidence Interval (CI) of Dengue Disease Severity by Abnormal Liver Protein Levels; CDS 2011-2013, n=551

	Unadjusted		Adjusted[a]		Unadjusted		Adjusted[b]		Unadjusted		Adjusted[c]		Unadjusted		Adjusted[d]	
	Albumin		Albumin		ALT		ALT		AST		AST		Total Protein		Total Protein	
Dengue Disease Severity	Beta	95% CI	Beta	95% CI	Beta	95% CI	Beta	95% CI	Beta	95% CI	Beta	95% CI	Beta	95% CI	Beta	95% CI
Dengue Fever	0	Referent	0	Referent	0	Referent	0	Referent	0	Referent	0	Referent	0	Referent	0	Referent
Severe Dengue	-0.07	-0.12, -0.02	-0.08	-0.45, 0.29	-0.38	-2.26, 1.51	-18.02	-51.59, 15.55	1.83	-0.24, 3.90	-7.18	-49.57, 35.21	-7.09	-7.91, -6.28	7.14	1.15, 13.13

Severe Dengue refers to clinical diagnosis with either dengue hemorrhagic fever or dengue shock syndrome. Linear mixed modeling was used to obtain beta estimates with corresponding 95% confidence intervals. Results expressed as the change in mean protein level (Beta) for those clinically diagnosed with severe dengue comapred to those clinically diagnosed with dengue fever. Covariates that produced a 20% or greater change in the beta coefficient for each liver protein level were retained in each adjusted model. Statistical significance is represented

[a]Adjusted for age, BMI, for prior infusion, leukocyte count, lymphocyte count, hematocrit percent, platelet count, neutrophil count, hemoglobin, mean corpuscular hemoglobin, and glucose, sodium, and calcium levels.

[b]Adjusted for age, BMI sex, prior infusion, leukocyte count, lymphocyte count, hematocrit percent, platelet count, hemoglobin, mean corpuscular hemoglobin, and glucose, sodium, and calcium levels.

[c]Adjusted for age, BMI, sex, prior infusion, leukocyte count, lymphocyte count, hematocrit percent, platelet count, neutrophil count, hemoglobin, mean corpuscular hemoglobin, and glucose, sodium, potassium and calcium levels.

[d]Adjusted for age, BMI, sex, platelet count, hematocrit percent, mean corpuscular volume, mean corpuscular hemoglobin, and glucose and potassium levels.

Abbreviations: ALT, alanine aminotransferase; AST: aspartate aminotransferase.

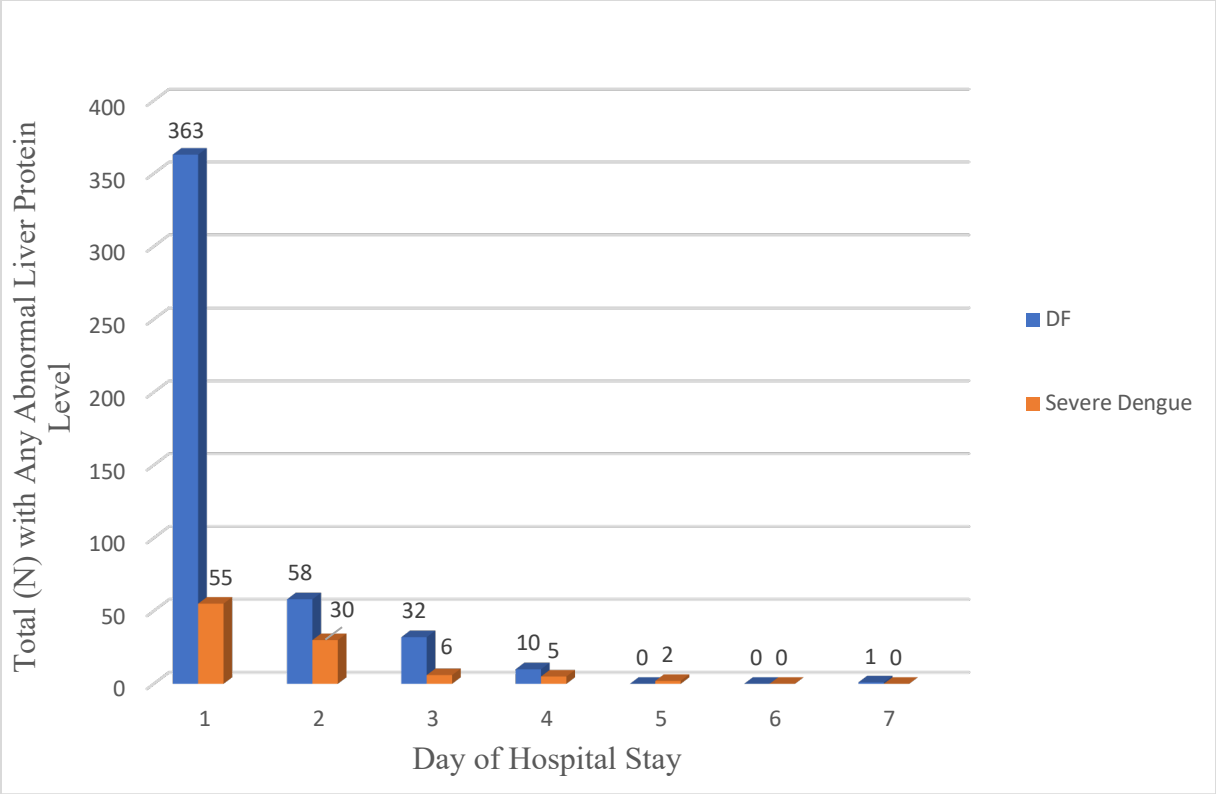

Figure 1. Total Number of Participants with (Any) Abnormal Liver Protein Level by Day of Hospital Stay; CDS 2011-2013, n=551

Data presented are total number of participants on each day of hospital stay who have one or more abnormal liver protein levels (i.e. albumin, ALT, AST, and/or total protein). Results are stratified by dengue disease severity. Abnormal liver protein level cut-points were adapted from the *Harriet Lane Handbook, 21st Edition.* Day 1 of hospital stay refers to day of admission.

Abbreviations: DF, dengue fever.

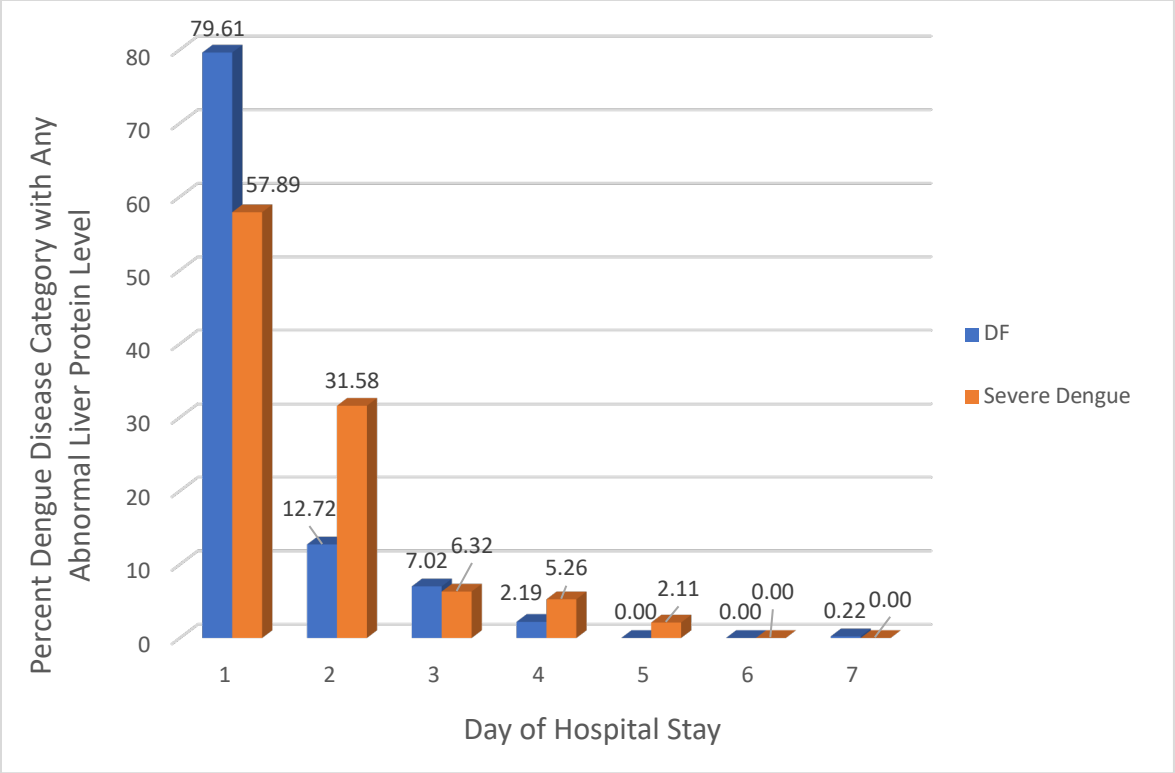

Figure 2. Percent of Participants with (Any) Abnormal Liver Protein Level by Day of Hospital Stay; CDS 2011-2013, n=551

Data presented are total number of participants with one or more abnormal liver protein levels (i.e. albumin, ALT, AST, and/or total protein) divided by the total number of participants in each dengue disease severity category to yield percent values. Results are stratified by dengue disease severity. Abnormal liver protein level cut-points were adapted from the *Harriet Lane Handbook, 21st Edition.* Day 1 of hospital stay refers to day of admission.

Abbreviations: DF, dengue fever.

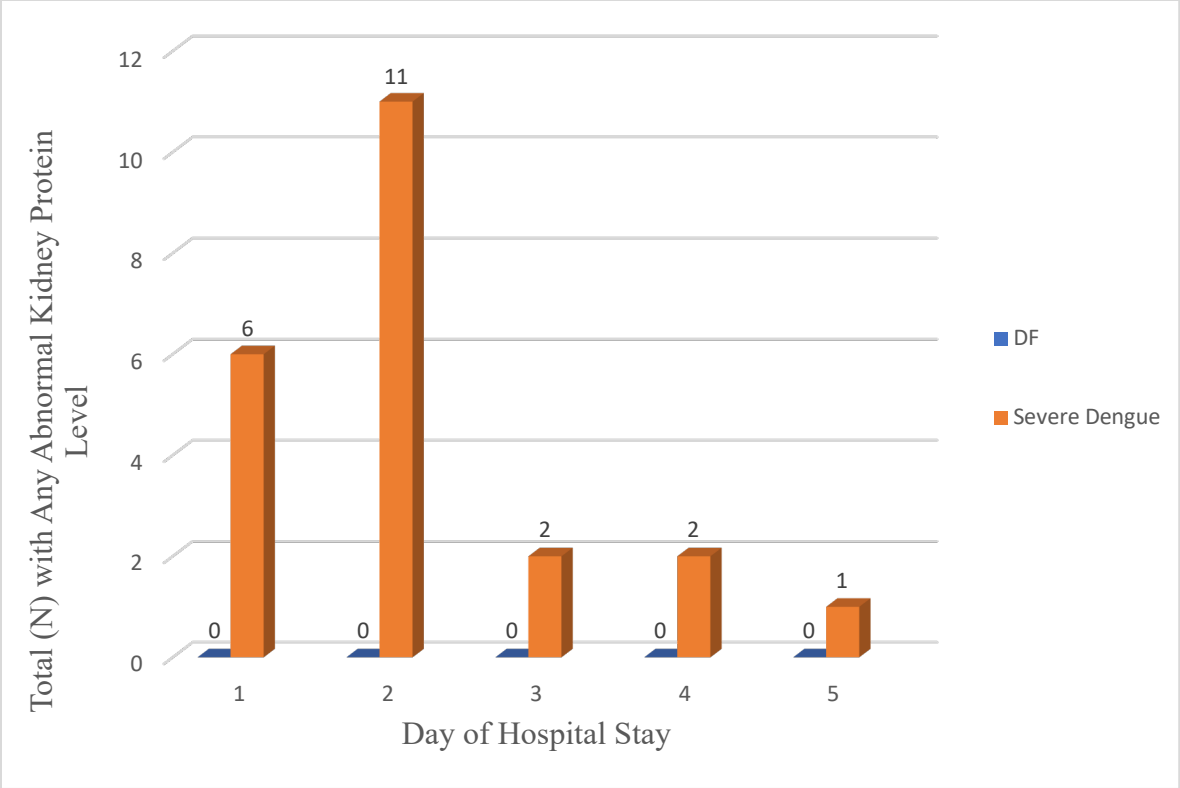

Figure 3. Total Number of Participants with (Any) Abnormal Kidney Protein Level by Day of Hospital Stay; CDS 2011-2013, n=551

Data presented are total number of participants on each day of hospital stay who have one or more abnormal kidney protein levels (i.e. creatinine and/or urea). No participants clinically diagnosed with DF had creatinine or urea levels in the abnormal range. Abnormal kidney protein level cut-points were adapted from the *Harriet Lane Handbook, 21st Edition.* Day 1 of hospital stay refers to day of admission.
Abbreviations: DF, dengue fever.

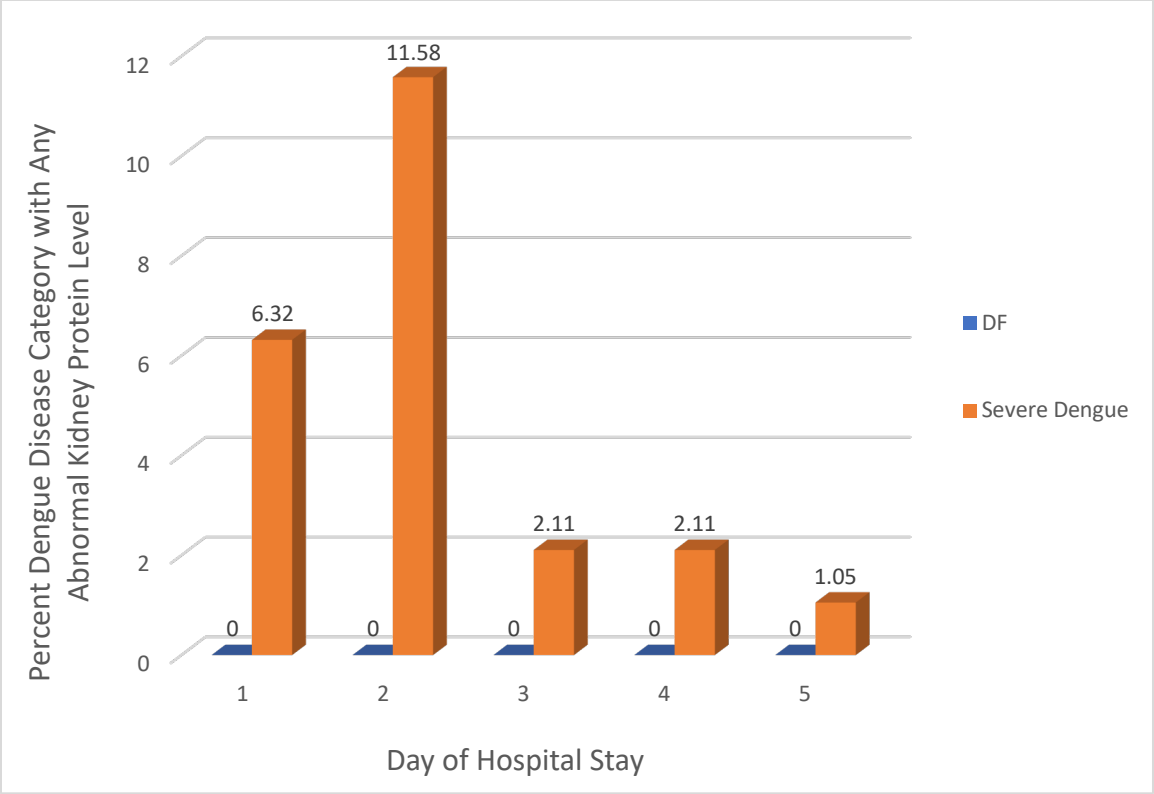

Figure 4. Percent of Participants with (Any) Abnormal Kidney Protein Level by Day of Hospital Stay; CDS 2011-2013, n=551

Data presented are total number of participants with one or more abnormal kidney protein levels (i.e. creatinine or urea) divided by the total number of participants in each dengue disease severity category to yield percent values. Results are stratified by dengue disease severity. Abnormal kidney protein level cut-points were adapted from the *Harriet Lane Handbook, 21st Edition.* Day 1 of hospital stay refers to day of admission.
Abbreviations: DF, dengue fever.

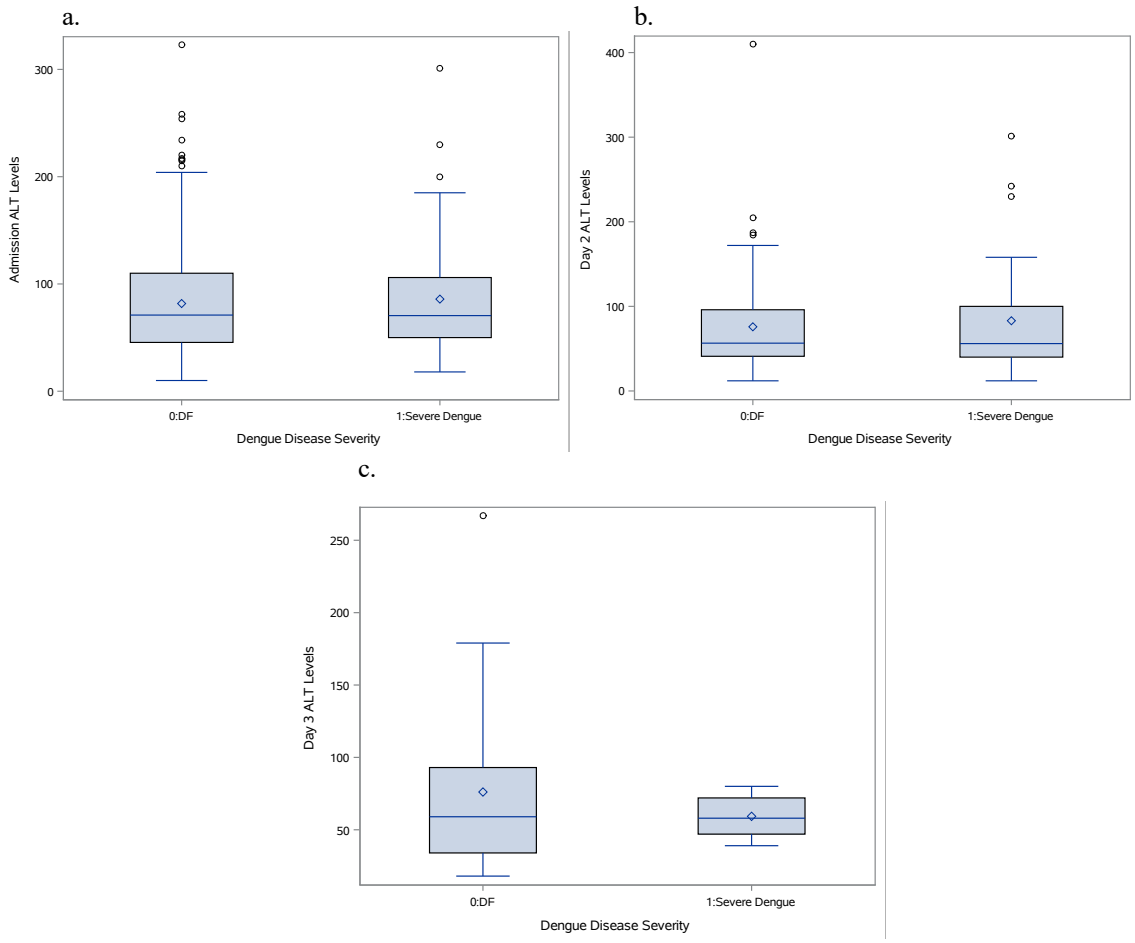

Figure 5. Distribution of ALT Levels by Dengue Disease Severity; CDS 2011-2013, n=551

Boxplots are presented to represent the median and deviation from the median for ALT levels on day of hospital admission (a), day 2 (b), and day 3 (c) of hospital stay, stratified by two-level dengue disease severity. ALT levels are expressed as IU/L.
Abbreviations: DF, dengue fever; IU, International Units.

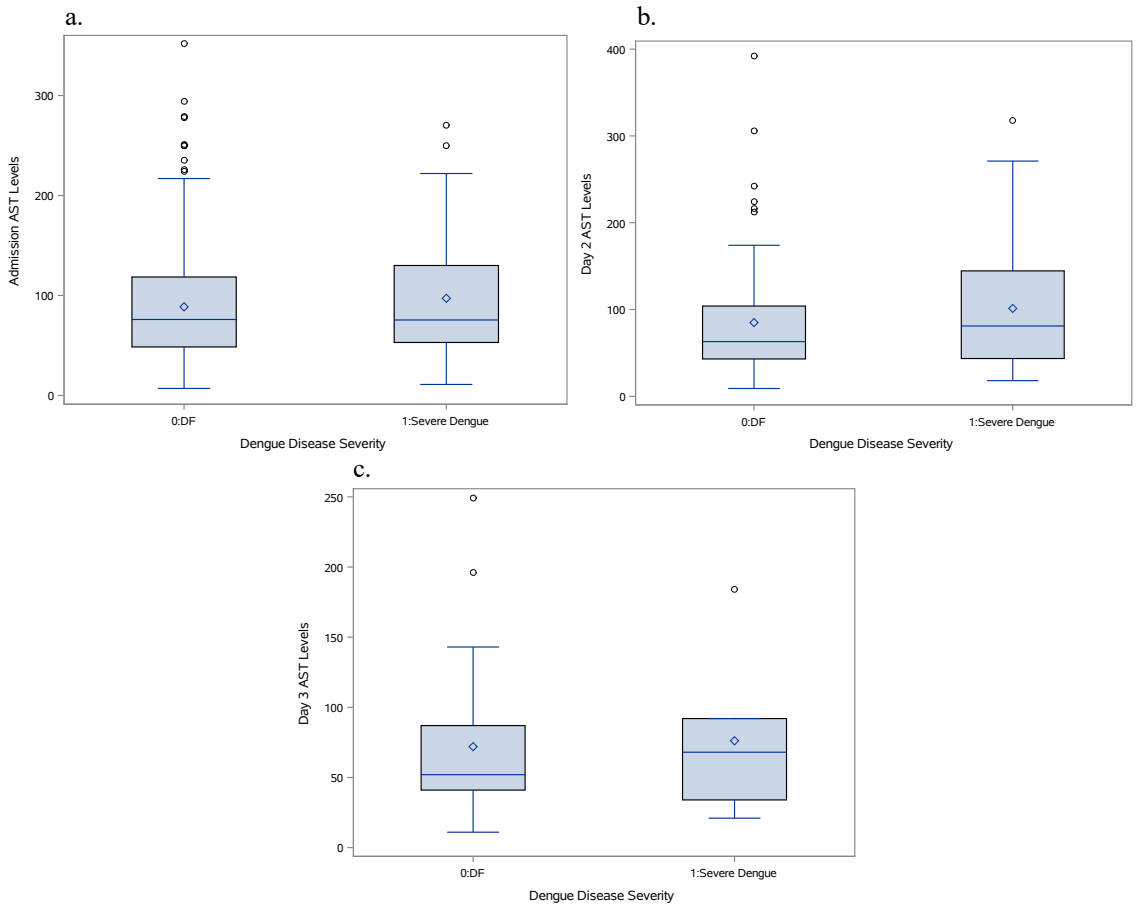

Figure 6. Distribution of AST Levels by Dengue Disease Severity; CDS 2011-2013, n=551

Boxplots are presented to represent the median and deviation from the median for AST levels on day of hospital admission (a), day 2 (b), and day 3 (c) of hospital stay, stratified by two-level dengue disease severity. AST levels are expressed as IU/L.
Abbreviations: DF, dengue fever; IU, International Units.

BIBLIOGRAPHY

1. Lim YS, Kim WR. The Global Impact of Hepatic Fibrosis and End-Stage Liver Disease. *Clin Liver Dis*. 2008;12(4):733-746. doi:10.1016/j.cld.2008.07.007

2. World Kidney Day Staff. Chronic Kidney Disease. World Kidney Day. https://www.worldkidneyday.org/facts/chronic-kidney-disease/. Published 2019. Accessed March 10, 2019.

3. Gross A. Liver Disease: A Growing Problem in Asia. medtechintelligence. https://www.medtechintelligence.com/feature_article/liver-disease-a-growing-problem-in-asia/. Published 2018. Accessed May 10, 2019.

4. Mayo Clinic Staff. Nonalcoholic fatty liver disease. Mayo Clinic. https://www.mayoclinic.org/diseases-conditions/nonalcoholic-fatty-liver-disease/symptoms-causes/syc-20354567. Published 2019. Accessed May 11, 2019.

5. Thanapirom K, Treeprasertsuk S, Soonthornworasiri N, et al. The incidence, etiologies, outcomes, and predictors of mortality of acute liver failure in Thailand: A population-base study. *BMC Gastroenterol*. 2019;19(1):1-7. doi:10.1186/s12876-019-0935-y

6. Jha V, Garcia-Garcia G, Iseki K, et al. Chronic kidney disease: Global dimension and perspectives. *Lancet*. 2013;382(9888):260-272. doi:10.1016/S0140-6736(13)60687-X

7. Mayo Clinic Staff. Liver Disease. Mayo Clinic. https://www.mayoclinic.org/diseases-conditions/liver-problems/symptoms-causes/syc-20374502?p=1. Published 2019. Accessed March 10, 2019.

8. Mayo Clinic Staff. Chronic Kidney Disease. Mayo Clinic. https://www.mayoclinic.org/diseases-conditions/chronic-kidney-disease/symptoms-causes/syc-20354521. Published 2019. Accessed March 10, 2019.

9. Pinto RB, Schneider ACR, da Silveira TR. Cirrhosis in children and adolescents: An overview. *World J Hepatol*. 2015;7(3):392-405. doi:10.4254/wjh.v7.i3.392

10. American Kidney Fund Staff. Cause of kidney disease in children. American Kidney Fund. https://www.kidneyfund.org/kidney-disease/kidney-disease-in-children/causes-of-kidney-disease-in-children/. Published 2019. Accessed May 10, 2019.

11. Becherucci F, Roperto RM, Materassi M, Romagnani P. Chronic kidney disease in children. *Clin Kidney J*. 2016;9(4):583-591. doi:10.1093/ckj/sfw047

12. Fernando S, Wijewickrama A, Gomes L, et al. Patterns and causes of liver involvement in acute dengue infection. *BMC Infect Dis*. 2016;16(1):1-9. doi:10.1186/s12879-016-1656-2

13. Lizarraga KJ, Nayer A. Dengue-associated kidney disease. *J Nephropathol*. 2014;3(2):57-62. doi:10.12860/jnp.2014.13

14. Verhagen LM, de Groot R. Dengue in children. *J Infect*. 2014;69(S1):S77-S86. doi:10.1016/j.jinf.2014.07.020

15. World Health Organization. *Guidelines for Treatment of Dengue Fever/Dengue Haemorrhagic Fever in Small Hospitals*. Vol 1.; 1999. doi:10.1002/jbio.201200501

16. World Helath Organization. *Dengue Guidelines for Diagnosis, Treatment, Prevention, and Control*. Vol 1.; 2010. doi:10.1176/pn.41.1.0029b

17. World Health Organization Staff. Dengue and severe dengue. WHO. https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue. Published 2019. Accessed May 10, 2019.

18. Khongphatthanayothin A, Mahayosnond A, Poovorawan Y. Possible cause of liver failure in patient with dengue shock syndrome. *Emerg Infect Dis*. 2013;19(7):1161-1163. doi:10.3201/eid1907.121820

19. Souza LJ de, Alves JG, Nogueira RMR, et al. Aminotransferase changes and acute hepatitis in patients with dengue fever: analysis of 1,585 cases. *Braz J Infect Dis*. 2004;8(2):156-163. doi:10.1590/S1413-86702004000200006

20. Stefan David M, James P Hamilton M. Drug-induced Liver Injury. *US Gastroenterol Hepatol Rev*. 2010;6(1):73-80.

21. Björnsson ES. Hepatotoxicity by drugs: The most common implicated agents. *Int J Mol Sci*. 2016;17(2). doi:10.3390/ijms17020224

22. Wilder-Smith A, Earnest A, Paton NI. Use of Simple Laboratory Features to Distinguish the Early Stage of Severe Acute Respiratory Syndrome from Dengue Fever. *Clin Infect Dis*. 2004;39(12):1818-1823. doi:10.1086/426029

23. Lee LK, Gan VC, Lee VJ, Tan AS, Leo YS, Lye DC. Clinical relevance and discriminatory value of elevated liver aminotransferase levels for dengue severity. *PLoS Negl Trop Dis*. 2012;6(6):1-8. doi:10.1371/journal.pntd.0001676

24. Kittitrakul C, Silachamroon U, Phumratanaprapin W, Krudsood S, Wilairatana P, Treeprasertsuk S. Liver function tests abnormality and clinical severity of dengue

infection in adult patients. *J Med Assoc Thail*. 2015;98:S1-S8.

25. Laoprasopwattana K, Jundee P, Pruekprasert P, Geater A. Outcome of severe dengue viral infection-caused acute liver failure in Thai children. *J Trop Pediatr*. 2016;62(3):200-205. doi:10.1093/tropej/fmv099

26. Trung DT, Thao LTT, Hien TT, et al. Liver involvement associated with dengue infection in adults in Vietnam. *Am J Trop Med Hyg*. 2010;83(4):774-780. doi:10.4269/ajtmh.2010.10-0090

27. Lover AA, Buchy P, Rachline A, et al. Spatial epidemiology and climatic predictors of paediatric dengue infections captured via sentinel site surveillance, Phnom Penh Cambodia 2011-2012. *BMC Public Health*. 2014;14(1):1-8. doi:10.1186/1471-2458-14-658

28. Andries AC, Duong V, Ngan C, et al. Field Evaluation and Impact on Clinical Management of a Rapid Diagnostic Kit That Detects Dengue NS1, IgM and IgG. *PLoS Negl Trop Dis*. 2012;6(12). doi:10.1371/journal.pntd.0001993

29. Kien Duong Thi Huea, Trung Vu Tuana, Hanh Tien Nguyen Thia, Chau Tran Nguyen Bicha, Huy Huynh Le Anha, Bridget A. Willsa C. Validation of an internally controlled one-step real-time multiplex RT-PCR assay for the detection and quantitation of dengue virus RNA in plasma. *J Virol Methods*. 2011;177(2):168-173. doi:10.1016/j.jviromet.2011.08.002.Hue

30. Vong S, Khieu V, Glass O, et al. Dengue incidence in urban and rural cambodia: Results from population-based active fever surveillance, 2006-2008. *PLoS Negl Trop Dis*. 2010;4(11):2006-2008. doi:10.1371/journal.pntd.0000903

31. Kahl L, Hughes H. TABLE 27.1: REFERENCE VALUES. Harriet Lane Handbook. Elsevier. https://www.unboundmedicine.com/harrietlane/view/Harriet_Lane_Handbook/309269/all/TABLE_27_1:_Reference_Values. Published 2018. Accessed April 4, 2020.

32. Narvaez F, Gutierrez G, Pérez MA, et al. Evaluation of the traditional and revised WHO classifications of dengue disease severity. *PLoS Negl Trop Dis*. 2011;5(11):1-8. doi:10.1371/journal.pntd.0001397