IRB Proposal/CRC Rotation
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5 May 2014

A. Study Purpose and Rationale

Dengue is the most prevalent arthropod-transmitted virus, with conservative estimates placing half of the world's population, including 128 countries on all continents, at risk of infection [1, 2]. While the clinical manifestation of dengue virus infection as dengue fever has been recognized for over 200 years [3-5], it was not until the 1950s that Dengue hemorrhagic fever, the potentially fatal form of dengue virus infection, became generally recognized following outbreaks in the Philippines and Thailand[6, 7]. Since the 1970s, endemic Dengue has spread from 9 nations to over 100, moved into urban areas, and explosive outbreaks of disease have become increasingly common [1]. Dengue fever and Dengue hemorrhagic fever are now considered major causes of morbidity and mortality in the subtropical and tropical regions of the world [8].

Dengue viruses, the causative agent of Dengue fever and Dengue hemorrhagic fever, are comprised of four distinct serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) and are members of the family Flaviviridae, genus *Flavivirus* [9-11]. Dengue virus infection of any of the four serotypes can manifest as a range of outcomes from clinically inapparent to death [11]. Individual outcomes are determined by multiple factors, including host susceptibility, virus genetics, cell-mediated immune response, and the presence or absence of cross-reactive antibodies [12, 13]. The majority of dengue infections is thought to be sub-clinical and can present atypically as an undifferentiated febrile illness [14, 15]. The remaining, more severe outcomes include classic dengue fever and dengue hemorrhagic fever. The pathogenesis of severe dengue disease is thought to be a consequence of a heightened immune response due to cross-reactive T-cell responses and/or enhancing dengue antibody during secondary dengue virus infection [16-18]. Cross reaction between pre-existing DENV antibodies and virus of a heterotypic serotype is due to the conservation of some viral envelope proteins across serotypes, which results in antibody binding to the virus particle without fully neutralizing it [19]. Epidemiologic and in vitro evidence suggests cross-reactive antibodies from other dengue serotypes can enhance disease severity during secondary infections [20-22]. This is referred to as antibody-dependent enhancement (ADE). It has also been inferred from hospital admissions data that severe dengue disease is rarer during third and fourth infections [23], suggesting that there is a protective effect conferred by cumulative cross-reactive antibodies.

Though more rare than in 2nd infections, there is little data available about severe dengue disease in 3rd and 4th infections. [23] I am proposing a retrospective cohort study that uses data from The Prospective Study of Dengue Virus Infection in Primary School Children in Kamphaeng Phet, Thailand. This was a two year cohort study initiated in January 1998 and published in 2002 [24], whose purpose was to study the epidemiology and immunology of inapparent to severe dengue disease and to identify risk factors for developing severe dengue disease after acquiring a secondary dengue infection. My study will focus on disease outcomes in relation to the presence of neutralizing antibodies to two or more Dengue serotypes. This study is designed to identify potential determinants of significant dengue infection in a cohort of patients exposed to two or more strains of Dengue virus (multi-typic serotype).

B. Study design and Statistical Analysis

This will be a retrospective cohort study. Baseline demographic information, height/weight, and blood sample were obtained every January. Evaluation of the entire study population (height/weight, blood sample for dengue serology) occurred three times per year. Active case surveillance occurred during the dengue season (June 1 – November 15). Evaluation including blood samples, oral temperature measurement, symptom

questionnaire were obtained from students who were absent from school or visited the school nurse with fever. A convalescent sample was taken 7 days later to diagnose dengue infection. For this study, all subjects with baseline (pre-season) serology positive for 2 or more strains of dengue virus will be identified as the primary study cohort.

Clinical definitions of serologically confirmed dengue virus infection

Inapparent dengue virus infection. Serologic indication of dengue infection during routine surveillance without febrile illness identified during active surveillance.

Acute dengue fever. Serologic indication of dengue infection with febrile illness without evidence of dengue hemorrhagic fever according to WHO criteria.

Acute dengue hemorrhagic fever. Serologic indication of dengue infection with febrile illness with evidence of dengue hemorrhagic fever according to World Health Organization criteria.

For the purpose of my study question, the cohort will be divided into significant infection, which will include acute dengue fever and acute dengue hemorrhagic fever, and non-significant infection which will include all inapparent dengue infections.

Statistical analysis

Statistical analyses will be performed by using STATA software for Windows (Version 11.2 StataCorp LP, College Station, TX, USA). Univariate analysis for each categorical variable will be performed with Chi-Square. Analysis of continuous variables (Age, BMI) will be performed with a T test. Odds ratios and confidence intervals will be calculated for all variables. Statistically significant variables will be used for the purpose of creating a multivariable logistic regression model.

Power Analysis

Sample sizes were pre-determined before the start of this retrospective secondary data analysis. Using the same principle as traditional power calculation for a 2 sided chi-square test we are able to determine effect size that the study is powered to detect at the 80% level with 0.05 significance. If we assume that a given variable is seen in 60% of significant infection, given the amount of people enrolled in each group, my study would be powered to detect an effect size of at least 14%. Alternatively, if we assume a variable occurred in 30% of significant infection, the study would be powered to detect an effect size of 14% above (or 12% below) this proportion.

C. Study Procedure

No procedures will be performed for the purpose of this study.

D. Study Drugs

N/A

E. Medical Device

N/A

F. Study Questionnaires

N/A

G. Study Subjects

Subjects in this study will be those that were participants in the original study "The Prospective Study of Dengue Virus Infection in Primary School Children in Kamphaeng Phet, Thailand".

- Inclusion
  Subjects that were included in this secondary data analysis were those that were exposed to at least 2 dengue serotypes prior to the beginning of the dengue season

H. Recruitment of Subjects

Subjects have already been recruited for the original 1998 study. There will be no additional recruitment of subjects for the purposes of this study.

I. Confidentiality of Study Data

Researchers for this study will be working with de-identified data, working only with study participant number. There will be no access given to key that would identify subjects.

J. Potential Conflict of Interest

There is no conflict of interest.

K. Location of Study

Twelve primary schools in Kamphaeng Phet, Thailand were selected to participate in this original prospective study on the basis of their reliable road access, desire to participate in the study, and location within a 3-hour driving radius from the field station laboratory at the Kamphaeng Phet Provincial Hospital. The secondary data analysis of data obtained from this original study will be performed at CUMC.

L. Potential Risks

No risks to study subjects in this secondary data analysis.

M. Potential Benefits

There is potential benefit to society from the completion of this study as it will add to the very scarce literature that exists on this topic. There are no potential benefits the subjects of this study.

N. Alternatives

N/A

O. Compensation to Subjects

No compensation will be provided to subjects

P. Costs to Subjects

No cost to subjects for this retrospective study

Q. Minors as Research Subjects

R. Radiation or Radioactive Substances

N/A

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