Dengue Haemorrhagic Fever in Thailand, 1998-2003: Primary or Secondary Infection

by

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Abstract
The pathogenesis of dengue haemorrhagic fever (DHF) is looked for in the serological records of DHF patients (mainly children between the ages of one month and 16 years) at Siriraj Hospital in Thailand over a six-year period beginning 1998 (covering two three-year cycles). Based on the primary and secondary infections by both the haemagglutination-inhibition assay (HI) test and the IgM capture enzyme-linked immunosorbent assay (ELISA) test, it was found that in 1998, 14 of the cases for which paired sera specimens were tested using both HI and ELISA (or 9.6% of 146 cases) had resulted from primary infections. In 1999, 2000, 2001, 2002 and the first half of 2003, three out of 57 cases (5.3%); six out of 48 cases (12.5%); 85 out of 293 cases (29%); 23 out of 90 cases (25.6%) and 16 out of 56 cases (28.6%), respectively, resulted from primary infections. The percentages of primary infections during the last three years are well above 14.0% reported for cases occurring in Bangkok between 1988 and 2003.

Keywords: Dengue haemorrhagic fever, serological tests, primary-secondary infection.

Introduction
Dengue fever (DF) is a rather benign febrile disease, afflicting mainly older children and adults[1] and often remaining unapparent in young children[2]. The sudden onset of fever and a variety of non-specific signs and symptoms characterize DF. The high fever lasts for two or three days, followed by additional symptoms. Its clinical presentations are similar to those of several other diseases, meaning thereby that many of the reported cases of DF could be due to other febrile illnesses and also that many

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Dengue infections are not recognized. During the 1977 epidemic in Santiago de Cuba\(^3\), only 3,012 out of 9,747 people who developed febrile illnesses and whose sera were tested, turned out to be infected with dengue fever. DF is caused by the dengue virus which belongs to the genus, Flavivirus, in the Flaviviridae family. There are four serotypes of this virus known as DEN-1, DEN-2, DEN-3 and DEN-4. Infection by any of the four serotypes causes similar clinical presentations, and confers permanent immunity to that particular serotype, but only a temporary one to the others.

The dengue virus is transmitted by the Aedes mosquitoes, i.e. Aedes aegypti and Aedes albopictus, in countries of South-East Asia. Introduction of the dengue virus by just one individual into a susceptible population residing in a locality where the above mosquitoes are prevalent can quickly lead to an epidemic. In many tropical countries, DF has now emerged as a major public health problem\(^4\).

The pathogenesis of dengue haemorrhagic fever (DHF) is still a matter of controversy. According to one school of thought\(^5\), pre-existing heterologous dengue antibodies recognize the infecting virus and form an antigen-antibody complex, which then binds the virus to the cell membrane of some leukocytes. Since the antibodies are only heterologous, the virus is not neutralized and is free to replicate once inside the cell. It is then thought that these cells secrete vasoactive mediators in response to dengue infection. These mediators cause an increased vascular permeability, which leads to hypovolemia and shock. Since the antibodies have to be pre-existing, this hypothesis terms it as the secondary infection or immune enhancement.

A cautionary note should be added here. It appears that the occurrence of DHF after a second infection depends on the strain of the serotype. During the 1996-1997 dengue epidemic in Belem Para, Brazil, none of the 24 patients who had been previously infected by the DEN-1 virus developed DHF after they had been re-infected by the DEN-2 virus\(^6\). Watts et al\(^7\) observed the same pattern during the 1995 epidemic in Iquitos, Peru. No cases of DHF/DSS were reported even though it was expected that between 887 to 10,247 cases would have occurred. The DEN-2 isolates were found to be of the American genotype (strain). Kochel et al\(^8\), attributed the non-occurrence of DHF/DSS to the presence of common envelope epitopes in both the American strain of the DEN-2 virus and the DEN-1 virus and the absence of these epitopes in the Asian strain of the DEN-2 virus. The common epitopes could have been acquired through the recombination between the American DEN-2 and the DEN-1 virus co-circulating in the Americas or through genetic drift (mutation).

The other school of thought\(^5\) maintains that the mutation of the viruses could have produced viruses with greater virulence and therefore greater epidemic potential. DHF would then be due to the appearance of these mutant strains among the circulating virus. This second hypothesis does not presuppose the presence of pre-existing antibodies and so the DHF/DSS infection would be the result of a primary infection. In an attempt to contribute to this debate, we
reviewed the serological status of children suffering from DHF who were admitted to the Paediatrics ward of Siriraj Hospital (a tertiary-care medical centre with a 300-bed facility in Bangkok, Thailand) between 1998 and mid-2003. A similar review of children admitted to the Department of Paediatrics, Chulalongkorn Hospital, Bangkok, between 1985 and 1995 was made recently.

Materials and methods

Criteria for primary and secondary infections

The World Health Organization (WHO)\(^4\) has established a set of criteria to determine whether a case of dengue fever is due to primary or a secondary infection. The determination is based on the results of either HI tests or ELISA tests or both, done on a paired set of sera taken at least seven days apart, one in the acute phase and the other in the convalescence phase. The criteria for primary infection are that, for a paired set of sera specimens there should be a fourfold increase in the IgM antibody response and HI titers of any of the DEN serotypes and the IgM/IgG ratio should be ≥1.8 and/or the HI titers in the convalescence phase should be <1,280. The criteria for determining secondary infection are that the IgM/IgG ratio should be <1.8 and/or the HI titers in the convalescence phase should be ≥2,560.

Patients

Admission to the ward was based on the clinical presentation of DHF as per the case definition of WHO\(^4\). Serological tests, i.e. haemagglutination inhibition (HI) assay\(^10\) and IgM/IgG enzyme-linked immunosorbent assay (ELISA)\(^11\), were used to determine whether the patients had dengue virus infection. Attempts were made to isolate the virus on Toxorhynchites mosquito to identify the serotype of the virus responsible for the illness.

Results

The results of the laboratory survey are given in the Table. Of the 1,183 patients admitted, the serological tests established that 1,082 of them were confirmed as of DHF. A total of 214 patients were determined to be due to primary infections, 291 due to secondary infection and 577 remained undetermined. One hundred and one patients turned out not to be sick with DHF. The virus responsible for the infection was isolated in 373 cases. The predominant virus was DEN-1 (162), followed by DEN-2 (121), DEN-3 (70) and DEN-4 (17). Multiple viruses were found in three patients (not included in the table). On the basis of the serological tests and using the WHO criteria for primary and secondary infections, our study found that in 1998, 14 of the cases for which paired sera specimens were tested by both tests (or 9.6% of 146 cases) had resulted from primary infection. In 1999, 2000, 2001, 2002 and the first half of 2003, three out of 57 cases (5.3%), six out of 48 cases (12.5%), 85 out of 293 cases (29%), 23 out of 90 cases (25.6%) and 16 out of 56 cases (28.6%), respectively, had resulted from primary infection.
Table. Summary of the serological records of DHF patients admitted to Siriraj Hospital, 1998-2003

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of patients admitted</th>
<th>Number sick with DHF</th>
<th>Number of cases where virus were isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>DEN-1</td>
</tr>
<tr>
<td>1998</td>
<td>318</td>
<td>281</td>
<td>51</td>
</tr>
<tr>
<td>1999</td>
<td>137</td>
<td>112</td>
<td>9</td>
</tr>
<tr>
<td>2000</td>
<td>84</td>
<td>71</td>
<td>13</td>
</tr>
<tr>
<td>2001</td>
<td>334</td>
<td>334</td>
<td>49</td>
</tr>
<tr>
<td>2002</td>
<td>186</td>
<td>186</td>
<td>23</td>
</tr>
<tr>
<td>2003*</td>
<td>121</td>
<td>121</td>
<td>17</td>
</tr>
</tbody>
</table>

*From January - June 2003

Regarding the results of virus isolation, of the 121 cases where virus was isolated in 1998, 40.0% were of DEN-1, 24.2% were of DEN-2, 34.2% were of DEN-3 and 1.7% were of DEN-4. In 1999, the respective percentages were 31.0, 44.8, 10.3 and 13.8. In 2000, the percentages changed to 42, 42, 16.1 and 0, respectively. In 2001, they were 44.5, 32.7, 15.5 and 14.1. In 2002, they were 46.0, 44.0, 8.0 and 2.0 respectively. For the first half of 2003, the percentages were 58.6, 31.0, 0 and 10.3 respectively. Comparing the percentages year by year, we can quantify the relative amount of the virus in circulation during that year. The relative abundance of DEN-1 virus appears to be increasing year after year, while that of DEN-3 appears to be decreasing. Overall, DEN-2 appeared to be the second-most abundant serotype in circulation throughout the study period.

The age distribution of the patients suffering from DHF is given in the Figure. This looks similar to the one of the DHF/DSS patients admitted to Yangon Children’s Hospital, Myanmar, between 1995 and 1996, but is different from that of the children admitted to the Children’s Hospital in Bangkok between 1995 and 1998. Halstead et al proposed that this group should be the one to study for understanding primary infections. They found that infants with DHF/DSS constituted 4.9% of the patients in their study group. Only eight infants were recorded in our study group. A similarly small number of infants was seen in the study group of Pancharoen et al. Also shown in the Figure is the age distribution of the DHF cases resulting from primary infection. In all the three studies, DHF infections in infants were primary infections.
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Discussion

The period covered in the present study spans two complete three-year cycles in the month of incidence of DHF in Bangkok, Thailand, between January 1998 and June 2003. Hays et al.\(^{(13)}\) carried out a spectral density analysis of the data and found an annual variation and a super-annual variation (of three years). The 1998 epidemic was one of the peak years in the annual occurrence of DHF in Thailand. Based on this, Hays predicted during a dengue fever conference held in December 2000 in Chiang Mai that 2001 would be a peak year for DHF. This was borne out by the increase observed in the incidence of DHF in Bangkok in that year. Many of us also made similar predictions\(^{(14)}\). As observed in this study, the incidence of DHF peaked in 1998 and then decreased in 1999 and again went down in 2000. It rose sharply in 2001 (a peak year in the three-year cycle) and then dropped in 2002. The decrease appeared to be continuing in 2003. Based on the previous trends, it is expected that there will be a rise in the incidence of DHF in 2004.
In the present study, dengue virus was isolated only in 34.5% of the 1,082 DHF patients confirmed by serological examinations. This is far below the percentage isolated by Vaughn et al\(^\text{[15]}\), who were able to isolate the virus in 98% of their patients. Their study was done in 1994. The difference in the percentages is due to the fact that Vaughn et al carried out their isolation within three days of the beginning of the high fever. Using similar criteria for differentiating between primary and secondary infections as the cause of the DHF illness, Vaughn et al established that only 8% of the acute dengue illnesses were due to primary infections (92% were due to secondary infections).

The relative abundance of the four serotypes observed by Vaughn et al, in 1994 was DEN-1 (20.3%), DEN-2 (28.8%), DEN-3 (16.9%) and DEN-4 (33.9%). Combining these numbers with the relative abundance observed in our study and in 1960\(^\text{[16]}\), we observed that there was permanent circulation of the four serotypes in Thailand. This is likely to be the cause of the short intervals between the high epidemic peaks, compared to what is observed, for example, in Polynesia where mono-serotype epidemics occur at an interval of at least six-to-seven years\(^\text{[17]}\). Looking at the relative abundance of all the four serotypes on a year-to-year basis, we found that the relative abundance of DEN-4 was fluctuating the most, followed by DEN-3. No significant differences (p<0.05) were observed between confirmed primary and secondary infections for any serotype during any year.

The percentages of primary infections during the last three years are well above 14.0 reported for cases occurring in Bangkok between 1988 and 1995\(^\text{[9]}\). This raises the question: Does the fact that the percentages of DHF/DSS arising from primary infections have almost doubled during the last three years signify that the dengue viruses have become more virulent or are there other factors at play?

References


