Dengue in Jeddah, Saudi Arabia, 1994-2002

by

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Abstract

Dengue virus (DEN-2) was first isolated from a fatal case of dengue haemorrhagic fever (DHF) in an adult in Jeddah, Saudi Arabia, in 1994. A surveillance system for dengue was established in 1994 and since then clinical, virological, serological and RT-PCR techniques were used to confirm dengue cases and, as a guide, to implement vector control measures in risk areas.

During February 1994-December 2002, a total of 1,020 suspected clinical cases were examined by laboratory methods. Dengue virus infection was confirmed in 319 (31.3%) cases, 209 by virus isolation and the rest by serological techniques. DEN-2, DEN-1 and DEN-3 were detected between 1994 and 2002 in that order of frequency. Using IgG immunofluorescent assay or haemagglutination-inhibition (HI) test, the prevalence of dengue reactive antibodies in the suspected group was confirmed in 515 (50.5%) of the 1,020 samples tested.

The application of reverse transcriptase-polymerase chain reaction (RT-PCR) on culture-positive blood samples gave a specificity and sensitivity of 100% and allowed rapid diagnosis within one day.

All cases that were diagnosed by laboratory methods as dengue had leucopenia, thrombocytopenia and elevated liver enzymes, ALT-AST. There were only two fatal cases; one of dengue haemorrhagic fever and another of dengue shock syndrome.

It can be said that: (i) three dengue serotypes (DEN 1, 2 and 3) were detected in Jeddah; (ii) nearly all cases were non-complicated, 99.4% of them were of dengue fever; (iii) prevalence of dengue antibodies was in 50.5% of all suspected cases; and (iv) RT-PCR is a rapid, sensitive and effective method for diagnosis.

Limited rains, active case-finding and effective anti-mosquito measures helped to bring the disease under control and were probably responsible for the very small numbers of new indigenous dengue cases after the 1994 outbreak.

Keywords: Dengue, dengue fever, Jeddah, Saudi Arabia.
Introduction

Dengue fever (DF), and its more severe form known as dengue haemorrhagic fever (DHF), is the most important arthropod-transmitted viral disease of humans in the world today with one third of the world's population at risk. Epidemics of a dengue-like disease appeared in the Arabian Peninsula in the late 18th century. The disease was described in Zanzibar, Dar es Salam, the East African coast, Arabia (Aden, Mecca, Madera), and Jeddah, in Saudi Arabia (1). In 1994, the dengue virus was isolated in Jeddah, Saudi Arabia, from a fatal case of DHF at the Dr Fakeeh Hospital (2). Active surveillance was established in 1994, based on clinical, followed by laboratory, methods to evaluate the prevalence and incidence of dengue, the serotypes detected, and the efficacy of the laboratory methods used for its diagnosis. This paper (2) reviews the surveillance data from 1994-1999; and this report extends these results to 2002 and adds data obtained by reverse transcriptase-polymerase chain reaction (RT-PCR).

Materials and methods

Case selection

All cases suspected of having dengue, with non-specific fever, with no localizing signs or symptoms, were examined clinically and blood samples collected when suspected cases were first seen.

Virus isolation

Blood samples were inoculated on C6/36 and after the seventh day, cells were examined for cytopathogenic effects (CPE) and also examined by indirect immuno-

flourescent assay (IFA) for detection of dengue antigen (3,4).

Serological methods

Haemagglutination-inhibition (HI) antibody method of Clarke and Casals (5) was modified for use in microtiter format. IgM capture ELISA was applied according to the method described by Kuno et al. (6). Antibodies of the IgG type were detected using IFA (7).

Nucleic acid

RT-PCR was done using commercial kits for the extraction of viral RNA, amplification of cDNA and detection of amplified products. Viral RNA was extracted from tissue culture supernatant or serum using Qiaqen RNA kit (Qiaqen Germany, catalogue number 29504). Primers and probes were as described by Lanciotti (8). RT-PCR was done using Qiaqen one-step RT-PCR kit (Qiaqen, Germany, catalogue number 210212) in 50 ul volumes.

Amplified products were detected, using DNA enzyme immunoassay (DEIA) (Dia Sorin Biomedica Saluggia, Italy) according to the manufacturer's instructions.

Results

The number of clinically suspected cases was 1,020 (738 male and 282 female). Dengue virus infection was confirmed in 319 cases (31.3%). Virus isolation was obtained in 209 cases (65.5%) out of 319 cases and IgM capture ELISA detected 110 cases (34.5%). Three dengue serotypes, DEN-2, DEN-1 and DEN-3, were detected. Most of the cases (289, 90.6%) out of 319 were diagnosed in 1994 and the rest over the next eight years (Table 1).
Table 1. Distribution of suspected and confirmed dengue cases, 1994–2002

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of suspected cases</th>
<th>Number of confirmed cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994</td>
<td>673</td>
<td>289</td>
</tr>
<tr>
<td>1995</td>
<td>136</td>
<td>06</td>
</tr>
<tr>
<td>1996</td>
<td>57</td>
<td>02</td>
</tr>
<tr>
<td>1997</td>
<td>62</td>
<td>15</td>
</tr>
<tr>
<td>1998</td>
<td>31</td>
<td>00</td>
</tr>
<tr>
<td>1999</td>
<td>26</td>
<td>03</td>
</tr>
<tr>
<td>2000</td>
<td>17</td>
<td>00</td>
</tr>
<tr>
<td>2001</td>
<td>07</td>
<td>00</td>
</tr>
<tr>
<td>2002</td>
<td>11</td>
<td>04</td>
</tr>
<tr>
<td>Total</td>
<td>1020</td>
<td>319</td>
</tr>
</tbody>
</table>

The application of RT-PCR gave results comparable to virus isolation in all the samples that were tested. Typing of dengue isolates by RT-PCR, followed by hybridization using DEIA method gave the same results as culture followed by the use of type-specific monoclonal antibodies in an IFA (Table 2).

The prevalence of IgG antibodies by IFA assay showed 515 (50.5%) of the total tested cases (1,020) had IgG antibodies in the first sample submitted for laboratory diagnosis. IgM detected 110 cases, mostly by the end of the first week of infection.

Table 2. Results of RT-PCR followed by DEIA compared to gel electrophoresis after nested PCR and IFA following virus culture

<table>
<thead>
<tr>
<th>Samples</th>
<th>RT-PCR followed by DEIA</th>
<th>Nested PCR followed by gel electrophoresis</th>
<th>Virus culture followed by IFA detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEN-1 positive serum samples</td>
<td>28/28</td>
<td>28/28</td>
<td>28/28</td>
</tr>
<tr>
<td>DEN-2 positive serum samples</td>
<td>40/40</td>
<td>40/40</td>
<td>40/40</td>
</tr>
<tr>
<td>DEN-3 positive serum samples</td>
<td>12/12</td>
<td>12/12</td>
<td>12/12</td>
</tr>
<tr>
<td>Control negative serum samples</td>
<td>0/30</td>
<td>0/30</td>
<td>00/30</td>
</tr>
<tr>
<td>Dengue infected C6/36 cells</td>
<td>20/20</td>
<td>20/20</td>
<td>20/20</td>
</tr>
<tr>
<td>Dengue non-infected C6/36 cells</td>
<td>00/20</td>
<td>00/20</td>
<td>00/20</td>
</tr>
</tbody>
</table>

DEIA = DNA enzyme immunoassay, IFA = Indirect immunofluorescent assay
Discussion and conclusions

Dengue fever made its first appearance in Jeddah in 1994; by the end of the year, 289 cases had been diagnosed. Since that time, continuous surveillance showed that dengue was no longer endemic in Jeddah. Jeddah is a central area where a large number of pilgrims visiting Mecca transit every year for Haj (visiting Islamic holy places). The Haj provides a fertile opportunity for the introduction and exchange of infectious agents among pilgrims, including dengue viruses. Pilgrims come from many countries in South-East Asia, India, Indonesia, Malaysia, and Pakistan. Some of these persons may be in the incubation period of dengue infection and may actually be viremic during the time of Haj, infecting mosquitoes and subsequently infecting other pilgrims and residents. Also, pilgrims come from Africa, so in Jeddah and Mecca, there may be an exchange of dengue viruses among pilgrims. However, the little (less than 60 mm/year) rain and dry weather most of the year does not favour efficient spread of dengue. A case-control study indicated that water storage container facilities in or near construction sites were the focal points of dengue virus transmission in Jeddah as, in spite of the low rainfall, water storage containers served as the breeding sites for Aedes aegypti, the vector species.[12]

It was during 1994 that two DEN-2 isolates were obtained and one of these was from a fatal haemorrhage case. Immediately after the isolation of DEN-2 virus surveillance was established by the Ministry of Health and information and guidelines were passed on to clinicians in the region. Clinically-suspected cases based on WHO criteria were sampled and blood was tested at the Dr Solimon Fakeeh Hospital by virus isolation, IgM capture ELISA, and finally by an IFA (later RT-PCR was applied). Clinically, all cases were found to be of non-complicated dengue fever, except for two cases that were fatal. Despite the high prevalence of dengue antibody in the examined suspected cases, among the confirmed cases only these two showed DHF or DSS. Inoculation of acute phase sera on C6/36 cells detected 209 cases, mostly in the first days of the disease, a common finding elsewhere[4,9,14]. Most of the confirmed cases were adults between 15-40 years of age and there were more male than female, a pattern consistent with the exposure at construction sites in Jeddah. The male/female ratio was similar to observations made in other dengue endemic areas in Brazil, Puerto Rico and, more recently, in the Philippines.

Three dengue serotypes were detected over the nine-year period from 1994 to 2002; DEN-2 (138 cases), DEN-1 (58 cases), and DEN-3 (13 cases). There were no DEN-4 isolates. The paucity of cases of DHF in the population in Jeddah could be attributed to factors such as viral, racial or nutritional.

Typing of the disease in this study was done using specific monoclonal antibodies[3,4]. Most isolates were in 1994 (186) and the rest of the isolates were in the following eight years. IgM capture ELISA detected 110 cases that were culture negative, presumably because of the delay in collecting the blood samples. Samples were often taken by the end of the first week of the presentation of symptoms and signs. The IgM ELISA has a high sensitivity and specificity for dengue infection[9,10,11]. Application of RT-PCR, followed by DNA enzyme immunoassay for the detection of amplified products, gave comparable results.
to virus isolation on tissue culture followed by typing with monoclonal antibodies. The main advantages of the RT-PCR method is its rapidity, done in one day, sensitivity and specificity of diagnosis.

The city of holy Mecca is 75 km from Jeddah. During Haj, large numbers of pilgrims come from disease-endemic areas all over the world, with the possibility of introduction of dengue viruses. Rapid and frequent travel between Jeddah and other endemic areas of the world favours the continued introduction of other strains and serotypes.

It is important to maintain dengue surveillance at high levels in order to detect early dengue activity and to take effective steps for the control of the vector.

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References


