Japanese Encephalitis in Southeastern Nepal: Clinical Aspects in the 1986 Epidemic

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SUMMARY: A multi disciplinary team furthered the collaborative study of acute encephalitis in southeastern Nepal during a major epidemic which occurred in the monsoon period of 1986. Viral studies of serum and cerebro-spinal fluid (CSF) confirmed Japanese Encephalitis Virus (JEV) as the causative agent. Analysis of epidemiologic data suggests recent introduction of the virus to the regional population. Children accounted for the majority of all hospital admissions and had a markedly lower fatality rate from the infection than adults. Unfavourable prognostic indicators identified include a reduced conscious level on admission to hospital and a low serum or CSF IgM response to JEV.

Introduction

The mosquito-borne flavivirus Japanese encephalitis virus (JEV) is the causative agent of epidemic acute encephalitis which affects several Northern India States(1) bordering the fertile flatlands (Tarai) region of Southeastern Nepal (Fig 1). There is increasing epidemiological and serological evidence that this organism is also responsible for epidemics of acute encephalitis which occur during the latter period of the annual monsoon in Southeastern Nepal, August–October(2-5) and Leake C J, unpublished data. Sporadic cases of clinically presumed acute JE occur year round and this underlines the need for effective immunisation of the visitor to the region. A collaborative research project supported by the Royal Army Medical College, US Component, Armed Forces Research Institute Bangkok, and London School of Hygiene and Tropical Medicine was established in 1983 to study the disease in this region. A late and very wet monsoon occurred in Nepal during 1986 and this may have triggered the explosive onset of what proved to be an overwhelming epidemic of acute encephalitis.

Patients and Methods

Study site and case surveillance

Encephalitis case surveillance commenced on 21 August 1986 at the British Military Hospital (BMH), Dharan and at the civilian Koshi Zonal Hospital, Biratnagar, 25 miles distant to the south. Clinical case details of name, age, sex, address, admission date, date of death or discharge and condition at discharge were recorded in all patients with clinically suspected acute encephalitis admitted to the study hospitals. Similar information on 16 cases of acute encephalitis admitted in the immediate period predating the study was recovered from medical records. Most patients presented with headache and fever, and many displayed an alteration in conscious level.

Pre-admission symptom duration and clinical details including conscious level on admission and throughout the hospital stay were recorded and in a random group of 124 patients the clinical condition of survivors was assessed at time of hospital discharge. The assessment was based on a knowledge of the pre morbid health of the patient provided by attendants, usually relatives, and also a clinical evaluation of residual neurological dysfunction. For ease of recording patients were graded...
as follows; normal, improved, fair, marked deficit and fatal. The normal group had no higher functional or other neurological deficit, the improved group had only a mild residual disability, for example minor degrees of apparent intellectual loss, dysphasia or motor weakness of the limbs. The group listed as fair had a moderate neurologic loss but were continent and fully conscious, and the remaining group of survivors had severe impairment with features such as reduced consciousness, inability to communicate, incontinence of sphincters and marked paralysis of limbs. CSF and serum samples were collected in most cases during the first 48 hours of admission and again, whenever possible, during convalescence. Hospital surveillance was terminated on 8 October 1986.

JEV serology

Serum and CSF samples were held at -70°C in a dry ice cabinet before thawing for laboratory investigations. Immunoflogassay of serum and CSF IgM and IgG antibodies reactive with either JEV antigen or tetravalent dengue (DEN) antigen were measured by ELISA techniques(6-8). A standard weak positive control serum, specific for either JEV or DEN antigen, served as a reference of 100 units. The negative control was pooled flavivirus-naive serum from the United States of America. All sera were evaluated at a dilution of 1:100 and CSF at a dilution of 1:10. The JEV diagnostic criteria were presence of JEV IgM ≥ 50 units in CSF or ≥ 50 units in serum if the JEV IgM: DEN IgM ratio ≥ 1.2. The final reaction product was measured as the absorption at 492 nm and the cut off of 50 units represents a reading well outside the 99% confidence intervals of the negative control.

Virus isolation

Aliquots of previously unthawed CSF from all fatal cases were processed for virus isolation in AP-61 cells(9). Culture supernatants were blindly passaged twice and an indirect immunofluorescence assay (IFA) employing a JEV-specific mouse monoclonal antibody was performed at each passage to detect infection. The identity of virus isolates was confirmed using reference sera in a plaque reduction neutralization test.

Statistical treatment of human case data

The variables age, sex, CSF antibody levels, and serum antibody levels were studied by analysis of variance using all case records with complete data to determine if the mean admission mental status was the same for all factor quartiles. The same analysis of variance for the dependent variables of discharge status and case outcome was performed using the above independent variables plus admission mental status. F statistics were calculated using SPSS/PC+ software, SPSS Inc, Chicago, Illinois. When a significant F statistic was determined for an independent variable, the Scheffe multiple comparison test was used in this programme to determine pairwise comparisons of means that differed at the 0.05 level. Homogeneity of variance was tested for using the Cochrans C or Bartlett-Box F statistic.

Results

Encephalitis surveillance

An epidemic of encephalitis was witnessed during the period of the field study. A total of 266 patients with clinically suspected JE infection were admitted to a study hospital. The epidemic curve, based on hospital admission dates, for the period July-October is shown in Figure 2. In a six day period, 2–6 September, 37% (46/119) of all cases with serologically confirmed JE were admitted to hospital.

JEV serology

Cerebro-spinal fluid and/or serum from 124 patients was collected for analysis. In 96% (119/124) of these cases, levels of JEV-reactive IgM exceeding our diagnostic criteria for Japanese encephalitis were detected. Seventy nine percent (94/119) of JE cases were diagnosed by detection of JEV-reactive IgM in CSF. The remainder (25/119) were diagnosed by detection of serum flavivirus-reactive IgM that was at least 1.2 times

- 35 -
- 30 -
- 25 -
- 20 -
- 15 -
- 10 -
- 5 -

3 Day Periods 1986

*Two Cases presented during the first week of July

**In a random group of patients admitted 21 August–8 October 96% (119/124) had serologically confirmed acute JE.

Fig 2. Admissions to Koshi Zonal Hospital, Biratnagar & British Military Hospital, Dharan with clinically suspected Japanese Encephalitis, 10 July–8 October 1986.
more reactive with JEV antigen than with tetravalent DEN antigen (units of IgM anti-JEV/units of IgM anti-DEN ≥ 1.2.) The mean JEV to DEN IgM ratio for serum-diagnosed cases was 9.4 compared to 8.1 for CSF-diagnosed cases (90 cases were serum JEV-reactive IgM ≥ 50 units were analysed). The single instance where this IgM ratio was not observed was in a case confirmed as JE by virus isolation from CSF. Of the five remaining cases, two had no CSF collected and no convalescent serum was collected. The serologic data of the negative cases is shown in Table 1. One of the cases with no CSF available had a serologic response consistent with a dengue virus infection (case 117/86). The outcome was not known for one of the undiagnosed cases but there were no hospital fatalities nor were there serious neurologic deficits noted at discharge among the other four.

Virus isolation

Unthawed CSF from the 15 fatal cases was processed for virus isolation. After two passages in AP-61 cells, cultures of CSF from two patients reacted positively by IFA with a JEV-specific monoclonal antibody. The JEV IgM/IgG levels in these CSFs were 63/13 units and 23/9 units respectively.

Statistical analysis of case data

The age distribution of children and adults in the serologically confirmed JE cohort is shown (Fig 3). The modal age was 7 years. The mean age was 15.5 ± 14.0 years (± 1 S.D.) with a range of 86 years (3 months–86 years old).

In the larger group which also includes clinically diagnosed cases the overall male to female ratio was 1.7:1. (168/98). In cases of known outcome the overall admission mortality was 21%, (51/239), the mortality of cases aged over 13 years was 30%, (25/84) and in children aged 13 years and under the fatality figure was 17%, (26/155).

The hospital admission duration of all cases with a fatal outcome is shown in Figure 4. The modal day for death was the second and the mean survival duration of patients following admission was 3.6 days ± 2.8 days (± 1 S.D.) with a range of 12 days (0–12 days).

In 16.8 (20/119) of serologically confirmed cases, the precise discharge status is unknown either because they were not seen by the study physician at the time of discharge (11 cases), the field study ended before the patients were discharged (7 cases), or the patient’s family pressed for discharge before complete clinical stabilization, ie “left against medical advice” (2/119). The clinical condition in the remaining 99 cases is summarised in Table 2. Case data from the 15 fatal cases are shown in Table 3.

Prognostic indicators

To identify the clinical and laboratory prognostic indicators in serologically confirmed JE an analysis of variance was performed using the dependent variables of admission mental status, discharge status, and outcome, and the independent variables age, sex, quartiles of age, CSF JEV IgM, serum JEV IgM or IgG and also admission mental status (for the latter two analyses). Records of 90 cases were complete and included in the analyses. Twenty nine case records were excluded for the following reasons: outcome not known

Table 1

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age</th>
<th>Sex</th>
<th>CSF JEV IgM</th>
<th>Serum JEV IgM</th>
<th>Serum JEV IgG</th>
<th>CSF DEN IgM</th>
<th>Serum DEN IgM</th>
<th>Serum JEV-DEN IgM ratio</th>
<th>Admission Mental Status</th>
<th>Discharge Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>013/86</td>
<td>35</td>
<td>M</td>
<td>1</td>
<td>36</td>
<td>28</td>
<td>16</td>
<td>20</td>
<td>2.2</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>106/86</td>
<td>15</td>
<td>F</td>
<td>0</td>
<td>23</td>
<td>75</td>
<td>35</td>
<td>12</td>
<td>0.7</td>
<td>drowsy</td>
<td>improve</td>
</tr>
<tr>
<td>114/86(1)</td>
<td>18</td>
<td>M</td>
<td>2</td>
<td>46</td>
<td>73</td>
<td>23</td>
<td>4</td>
<td>2.0</td>
<td>drowsy</td>
<td>unknown</td>
</tr>
<tr>
<td>116/86</td>
<td>31</td>
<td>F</td>
<td>-</td>
<td>35</td>
<td>82</td>
<td>8</td>
<td>7</td>
<td>4.4</td>
<td>unknown</td>
<td>normal</td>
</tr>
<tr>
<td>117/86(2,3)</td>
<td>22</td>
<td>M</td>
<td>-</td>
<td>35</td>
<td>45</td>
<td>56</td>
<td>27</td>
<td>0.6</td>
<td>normal</td>
<td>normal</td>
</tr>
</tbody>
</table>

Note: 1. Serological response consistent with a dengue virus infection.
2. Lumbar puncture performed but CSF not submitted for analysis.
3. Left hospital against medical advice.
Fig 4. Duration from Hospital Admission to Death in Patients with Presumed Japanese Encephalitis*

* 1 case is excluded as date of death is unknown.

(20 cases), CSF not available for antibody measurements (6 cases), admission mental status not known (2 cases), and serum not available for antibody measurements (1 case). The admission mental status was independent of age, sex or any measured antibody level. The mean discharge status was worse for patients admitted with coma when compared with that of the group admitted with normal mental status. When outcome was graded as alive or dead, those patients with CSF IgM or serum IgM values in the lowest quartile were more likely to have a fatal outcome.

Discussion

For the third year in succession an epidemic of Japanese encephalitis occurred in 1986 in the Tarai region of Nepal. One hundred and twenty four human cases of encephalitis were studied serologically to define the aetiology and JEV -reactive IgM was detected in CSF and/or sera from 119 cases. This total of confirmed JE cases represents a much larger proportion of all 1986 encephalitis cases studied than that diagnosed as due to JEV infection in 1985(5). Much of the increase in diagnosis stems from more confident application of the JEV to DEN ratio to assess specificity of flavivirus – reactive IgM. The specificity of this measure is shown by the absence of a significant difference in the JEV to DEN IgM ratio of serum for CSF and serum diagnosed cases.

Table 2

Clinical outcome of serologically confirmed JE cases

<table>
<thead>
<tr>
<th>Discharge Status</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>24</td>
</tr>
<tr>
<td>Improved</td>
<td>54</td>
</tr>
<tr>
<td>Fair</td>
<td>3</td>
</tr>
<tr>
<td>Marked Deficit</td>
<td>3</td>
</tr>
<tr>
<td>Fatal</td>
<td>15</td>
</tr>
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</table>

n = 99

*In-patients at termination of study period (seven), patients not examined at time of discharge (eleven) and patients leaving against medical advice (two), are not included.

The timing of the epidemic in 1986 was similar to that witnessed in 1985, with the peak of hospital admissions occurring in late August and early September. Both years stand in contrast to the smaller epidemic of 1984 when human cases were not admitted to a study hospital until late October/early November(4).

The case fatality in this epidemic is in agreement with that seen in neighbouring regions(10) and elsewhere(11). The discrepancy in fatality rates recorded for serologically confirmed cases and all suspected cases may reflect a bias in sampling of serum and CSF, as many fatalities occurred within 24 hours of admission to a study hospital and before the daily round of serum and CSF collection. There is almost a two-fold difference in hospital fatality occurring in children, 17%, (26/155) and in cases aged 14 years and older, 30%, (25/84). Amongst adult cases the younger age groups had apparent higher morbidity and more fatalities were recorded. This supports the theory of recent introduction of the JE virus to the regional population. Fairly recent exposure to the virus is also suggested by the age distribution of cases which is strikingly different from that seen in Thailand(8). Thirty-six percent (97/266) of all cases recorded in this 1986 Nepal survey occurred in patients aged 14 years and over, whereas in regions of Thailand where JE is epidemic, few adult cases and an age 15 neutralizing antibody prevalence of over 90% is seen.

Mean admission mental status was the same for every subgroup analysed. The mean discharge status was better for those admitted with normal mental status and progressively worse for those admitted with a reduced conscious level. We did find, however, as Burke et al(8) suggested, that low serum or CSF anti-JEV IgM was associated with more frequent death. Thus while we were unable to find a relationship between the acute neurological deterioration as measured by admission mental status and JEV antibody levels, age or sex, a good discharge status was clearly associated with high serum IgM and a poor status was associated with a low serum or low CSF IgM response. Though analysis of cases of encephalitis in the 1983 JE epidemic in
Table 3

Data from Fatal Japanese Encephalitis Cases

<table>
<thead>
<tr>
<th>Age Years</th>
<th>Sex</th>
<th>Admission Mental Status*</th>
<th>CSF DENIgM</th>
<th>CSF DENIgG</th>
<th>Serum DENIgM</th>
<th>Serum DENIgG</th>
<th>Serum JEV IgM -DENIgM ratio</th>
<th>CSF Culture</th>
<th>Hospital Days</th>
<th>Pre-admission Symptoms (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>M</td>
<td>1</td>
<td>63</td>
<td>5</td>
<td>117</td>
<td>54</td>
<td>17</td>
<td>24</td>
<td>6.9</td>
<td>negative 10</td>
</tr>
<tr>
<td>35</td>
<td>F</td>
<td>3</td>
<td>149</td>
<td>16</td>
<td>118</td>
<td>24</td>
<td>23</td>
<td>25</td>
<td>5.1</td>
<td>negative 12</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>2</td>
<td>23</td>
<td>0</td>
<td>104</td>
<td>44</td>
<td>20</td>
<td>21</td>
<td>5.2</td>
<td>JEV 3</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>0</td>
<td>63</td>
<td>13</td>
<td>100</td>
<td>104</td>
<td>114</td>
<td>73</td>
<td>0.9</td>
<td>JEV 4</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>1</td>
<td>385</td>
<td>21</td>
<td>113</td>
<td>88</td>
<td>21</td>
<td>37</td>
<td>5.4</td>
<td>negative 1</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>1</td>
<td>20</td>
<td>13</td>
<td>106</td>
<td>138</td>
<td>33</td>
<td>29</td>
<td>3.2</td>
<td>negative 4</td>
</tr>
<tr>
<td>17</td>
<td>M</td>
<td>0</td>
<td>32</td>
<td>13</td>
<td>161</td>
<td>77</td>
<td>24</td>
<td>39</td>
<td>6.7</td>
<td>negative 7</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>1</td>
<td>17</td>
<td>3</td>
<td>93</td>
<td>130</td>
<td>23</td>
<td>23</td>
<td>4.0</td>
<td>negative 1</td>
</tr>
<tr>
<td>35</td>
<td>M</td>
<td>Unknown</td>
<td>604</td>
<td>121</td>
<td>156</td>
<td>41</td>
<td>77</td>
<td>91</td>
<td>2.0</td>
<td>negative 9</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>1</td>
<td>645</td>
<td>93</td>
<td>187</td>
<td>86</td>
<td>3</td>
<td>7</td>
<td>62.3</td>
<td>negative 4</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>2</td>
<td>29</td>
<td>5</td>
<td>167</td>
<td>137</td>
<td>19</td>
<td>30</td>
<td>8.8</td>
<td>negative 8</td>
</tr>
<tr>
<td>25</td>
<td>F</td>
<td>1</td>
<td>27</td>
<td>25</td>
<td>121</td>
<td>106</td>
<td>8</td>
<td>13</td>
<td>15.1</td>
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</tr>
<tr>
<td>3</td>
<td>M</td>
<td>3</td>
<td>137</td>
<td>1</td>
<td>167</td>
<td>69</td>
<td>25</td>
<td>43</td>
<td>6.7</td>
<td>negative 3</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>2</td>
<td>386</td>
<td>4</td>
<td>227</td>
<td>103</td>
<td>81</td>
<td>84</td>
<td>2.8</td>
<td>negative 4</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>64</td>
<td>43</td>
<td>46</td>
<td>52</td>
<td>1.4</td>
<td>negative 2</td>
</tr>
</tbody>
</table>

* Admission mental status

0 Normal
1 Drowsy, but responsive to voice commands
2 Light coma, unresponsive to voice but responsive to painful stimuli
3 Deep coma, unresponsive to voice or painful stimuli

Kampangphet Province, Thailand indicated that low serum JEV IgG was a predictor of a fatal outcome, we did not find this for groups of patients stratified by admission level of JEV IgG. Our population of patients may differ from those in Kampangphet in that the seroprevalence of cross reactive flavivirus antibody, due to previous dengue infection, is low. The actual impact of this difference on morbidity and mortality cannot be readily assessed without a denominator based study.

Treatment currently for confirmed cases of acute encephalitis is, when available, supportive only, and the burden this imposes on overstretched health care resources serves to state the pressing need for the design and application of an effective immunisation programme. The elucidation and where practicable, the rapid implementation of other preventive measures in the region is also important. In particular there is a need to identify the main primary virus reservoir and major mosquito vectors and to attempt to understand the dynamic factors necessary for significant transmission of JE virus to humans in the region.

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