Leptospirosis is a zoonosis with multisystem involvement caused by the pathogenic strains of *Leptospira* spp. that affects humans and animals. It is considered to be the most widespread zoonotic disease in the world. Leptospirosis occurs as epidemic as well as sporadic outbreaks. Leptospirosis is a disease of the environment; transmission depends on interactions between humans and mammalian reservoir hosts. Animals may serve as carriers thus transmitting the disease while in humans the disease is usually acute that can result in severe or fatal disease.

Taylor and Goyal reported the first case of leptospirosis from India in 1929 from Andaman and Nicobar Islands. There has been a significant increase in the reported cases of leptospirosis from India since 1980s. Epidemics have been increasingly reported from Orissa, Maharashtra, Karnataka, Tamil Nadu and Kerala. In Kerala, it is colloquially called Elippani (rat fever). The high prevalence of leptospiral infection of cattle represents potential threats to human health and agricultural economics.

Calicut Medical College caters to the need of Kozhikode district and adjacent districts and covers a population of more than eight million. People living in the rural area have contact with livestock and cattle.

Epidemics of leptospirosis occur in monsoon seasons. The present study was done on early diagnosis of leptospirosis during the period July 2003 to December 2003, a period when there is the usual pattern of heavy rainfall.

Materials and Methods

Human patients

265 patients, visiting the out-patient department of Calicut Medical College from July 2003 to December 2003, inclusive of all age-groups and occupation-groups, consisting of

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agricultural workers, manual laborers, housewives, students and office workers were included in the study. Patients had symptoms of fever and myalgia with headache, vomiting, jaundice, conjunctival suffusion and abdominal symptoms.

**Blood Culture**

Fresh blood samples were collected from all antigen positive and antibody positive patients and EMJH medium was inoculated and incubated at room temperature for four to six weeks.

**Leptospiral antigen and antibody detection tests**

All the serum samples were tested for IgM antibody by PanBio IgM ELISA kit.

Diagnostic kits, based on the principle of sandwich enzyme-linked immunosorbent assay (ELISA) were provided by Microbiology division, Defence Research and Development Establishment, (DRDE) Gwalior. This consisted of a microtitre plate coated with polyclonal leptospiral antibody, upon which the antigen capture was bound by the second antibody that consisted of a group of monoclonal antibodies specific for pathogenic strains of *Leptospira* spp. This consisted of monoclonal antibodies against the following serovars: Australis, Autumnalis, Bataviae, Canicola, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Pomona, Tarassovi and Grippotyphosa + Hardjo. Detection was done by rabbit anti-mouse peroxidase conjugate, using ortho-phenylenediamine hydrochloride and hydrogen peroxide as substrate.

The antigen detection was also devised as Dot ELISA strips. Nitrocellulose strips, coated with both control and polyclonal leptospiral antibody were used for detection.

**Polymerase chain reaction (PCR) analysis**

Serum was centrifuged and DNA was extracted from the pellet, using QIA DNA extraction kit. Polymerase chain reaction (PCR) was done using Hookey’s primers

\[
P1 – 5’ CGC TGG CGG CGC GTG TTA AA 3’ (20 mer)
\]

\[
P2 – 5’ TTC ACC GCT ACA CCT GGA A 3’ (19mer)
\]

The PCR products were analysed on a 0.8% agarose gel

Serovar identification of clinical isolates.

The clinical isolates obtained from blood cultures were killed by keeping in a boiling water bath for three minutes and tested with a panel of serovar- specific monoclonal antibodies, using protocol as for antigen detection.

**Results**

The (Fig. 1) summarizes the results of the tests performed with the blood samples from 265 patients. Leptospiral antigen was detected in 51 blood samples (Fig. 2), all of which were also positive by PCR (Fig. 3), while 6 samples were positive for IgM antibody. Out of the 95 samples subjected to blood culture, leptospires were isolated in 27 samples. One of the isolates was a saprophyte and was obtained from a sample that was antigen negative. Table 1 summarizes the detection of leptospiral antigen (and culture, if any) / antibody with reference to time of collection of the blood sample after the onset of fever. It can be observed that high culture positivity was evident in samples collected in the early stages after infection. Among the 26 clinical isolates, the serovars included Australis (1), Hardjo (3), Autumnalis (1), Hebdomadis (3), Bataviae (1), Icterohaemorrhagiae (2), Canicola (1), Pomona (7),
Grippotyphosa (1), Tarassovi (3), unidentified (3), thus making Pomona as the most common serovar. Table 2 shows the occupation and the different symptoms in these seven patients with Pomona as the causative serovar.

Discussion

In leptospirosis, there is poor correlation between the infecting leptospiral serovar and clinical presentations. In human leptospirosis, though microscopic agglutination test (MAT) provides information about the infecting serovar, isolation and typing of the organism is mandatory for definite confirmation.

In this study on 265 patients, 51 patients who were antigen and PCR positive were taken as confirmed cases of leptospirosis. MAT was not done as all the samples were collected in the acute phase of the disease. 49 of the 51 antigen positive patients responded to treatment with penicillin, amoxycillin or doxycycline while two patients died due to severe multi-organ dysfunction syndrome at the time of admission.

Acknowledgements

Dr. H V Batra (DRDE, Gwalior India) is acknowledged for providing the antigen detection kits and for the help provided through out the study.

References


Table 1: Culture and positivity of detection of antigen and antibody determined by sample collection after onset of fever

<table>
<thead>
<tr>
<th>Days*</th>
<th>Ag (+), IgM (-)</th>
<th>Ag+, IgM (-)</th>
<th>Ag (+), IgM (+)</th>
<th>Ag (-), IgM (-)</th>
<th>Ag (-), IgM (+)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-4</td>
<td>14 (37)</td>
<td>12 (31.5)</td>
<td>0</td>
<td>0</td>
<td>12 (31.5)</td>
<td>38</td>
</tr>
<tr>
<td>5-10</td>
<td>9 (16.7)</td>
<td>9 (16.7)</td>
<td>2 (3.7)</td>
<td>4 (7.4)</td>
<td>29 (53.7)</td>
<td>54</td>
</tr>
<tr>
<td>&gt;10</td>
<td>1 (3.3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (66.7)</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>24 (25.3)</td>
<td>21 (26.3)</td>
<td>2 (2.1)</td>
<td>4 (4.2)</td>
<td>43 (45.3)</td>
<td>95</td>
</tr>
</tbody>
</table>

*After onset of fever, Figures in parentheses are in percentage

Table 2: Clinical presentation of all the patients with Pomona as the causative serovar

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Age</th>
<th>Sex</th>
<th>Presenting features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labourer</td>
<td>54</td>
<td>M</td>
<td>Fever, myalgia, jaundice, hepatomegaly, MODS (death)</td>
</tr>
<tr>
<td>House wife</td>
<td>50</td>
<td>F</td>
<td>Fever, myalgia, conjunctival congestion, headache, vomiting</td>
</tr>
<tr>
<td>Student</td>
<td>18</td>
<td>M</td>
<td>Fever, myalgia, conjunctival congestion, vomiting</td>
</tr>
<tr>
<td>Sedentary worker</td>
<td>70</td>
<td>F</td>
<td>Fever, myalgia, vomiting</td>
</tr>
<tr>
<td>Labourer</td>
<td>30</td>
<td>M</td>
<td>Fever, myalgia, conjunctival congestion, headache, vomiting, arthralgia</td>
</tr>
<tr>
<td>Labourer</td>
<td>32</td>
<td>M</td>
<td>Fever, jaundice, vomiting, bleeding, renal failure</td>
</tr>
<tr>
<td>Sedentary worker</td>
<td>74</td>
<td>F</td>
<td>Fever, diarrhoea</td>
</tr>
</tbody>
</table>

MODS - Microscopic observation broth drug susceptibility
A two-year study of the efficacy of azithromycin in the treatment of leptospirosis in humans

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Azithromycin, a macrolide antibiotic is highly effective against both gram-positive and gram-negative organisms. In-vitro studies have shown activity of azithromycin against leptospires. Though penicillin administered intravenously is a drug of choice in the treatment of leptospirosis, some patients are apprehensive to accept hospitalization when they are ambulatory and also the risk of penicillin sensitivity exists in some patients.

Hence this study was conducted to study the efficacy of azithromycin in human leptospirosis cases.

Materials and Methods

The study was conducted from March 2004 to February 2006 at two private clinics and two hospitals in Chennai. Patients suspected of leptospirosis were diagnosed by dark field microscopy (DFM) and MAAT at Weil and Pasteur Lepto Lab Pvt Ltd.

A total of 682 leptospirosis positive patients, tested negative for typhoid and malaria were included in the study. These patients, who were ambulatory and had no involvement of vital organs like lungs, kidney and heart were treated with azithromycin.

Azithromycin was administered orally at a dose of 15 mg/kg body weight in divided doses, twice a day for one week. Patients were reviewed after one week to evaluate the remission of symptoms. Based on the feedback of the patients, the response to azithromycin was classified as good, mild and no response. If there was a good resolution of clinical signs after one week of treatment, the patients were advised to continue the same medication for one more week. In those cases in which there was a mild improvement in condition or if the symptoms did not subside after one week of azithromycin administration, the patients were advised to go for administration of parenteral antibiotics.

The symptoms in patients were studied as follows: fever, abdominal pain and vomiting; fever and myalgia (calf, thigh and lower back muscles); fever, headache, neck pain and conjunctival suffusion; fever and skin rashes; Jaundice.

Results and Discussion

72% of the patients (491/ 682) responded to azithromycin, with complete subsiding of the clinical symptoms. In 14.95% of the patients (102 patients), there was only a mild degree of alleviation of clinical symptoms, while 89 patients (13.05%) showed no response to treatment with azithromycin (Table 1).

Table 1: Azithromycin treatment for leptospirosis: response of patients with specific symptoms of the disease

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Number of patients</th>
<th>Good response</th>
<th>Mild response</th>
<th>No response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever, abdominal pain and vomiting</td>
<td>18</td>
<td>2</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>Fever and myalgia</td>
<td>17</td>
<td>13</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Fever, headache, neck pain and conjunctival suffusion</td>
<td>19</td>
<td>12</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Fever and skin rashes</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Jaundice</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>682</td>
<td>491</td>
<td>102</td>
<td>89</td>
</tr>
</tbody>
</table>

Source of Support: Nil. Conflict of Interest: None declared.