Original article:

Seroprevalence of Leptospirosis, Enteric fever and Dengue in patients with acute febrile illness in Tamil Nadu, India.

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ABSTRACT:

Introduction: In developing countries like India, malaria, Leptospirosis, Dengue and Typhoid presents as acute undifferentiated fever. Management of acute febrile illness differs and is mainly based on the causative infectious agents. In areas endemic for two or more infectious agents, co-infection is more common. The objective of the study was to estimate seroprevalence of Leptospirosis, Enteric Fever and Dengue in cases of acute febrile illness.

Methodology: Hundred patients with febrile illness who were referred for diagnosis of leptospirosis were included in the study. Their serum samples were tested for Leptospira using Microscopic Agglutination Test (MAT), Salmonella typhi using Widal rapid & tube agglutination test and Dengue virus by IgM ELISA. Demographic Characteristics were compared and analyzed.

Result: Out of 100 samples, 21 tested positive for Leptospirosis (21%), 17 for Typhoid (17%) and 8 for Dengue (8%). Four patients had Co-infections. Two of them tested positive for Leptospirosis & Typhoid, one for Leptospirosis & Dengue and 1 for Dengue & Typhoid. Forty eight percent (48 %) reported 1-5 days fever, 52% above 5 days, 86% intermittent fever. Out of 100 patients, 46 tested positive for one or more of the three tests. 54% were negative.

Conclusion: The laboratory diagnosis plays a very important role in determining the line of management in acute febrile illness. Possibility of co-infection with one or more causative organisms must also be borne in mind when treating patients with acute febrile illness.

Key words: Co-infection, Dengue, Leptospirosis

INTRODUCTION:
In developing countries, like India, Malaria, Leptospirosis, Dengue and Typhoid presents as acute undifferentiated fever [1]. Wide variety of causative agents and limited diagnostic tests to make prompt diagnosis pose major challenge in treating acute febrile illness in tropical countries [2]. The positivity rate of Leptospirosis among acute febrile illness is 25.6% in Southern India [3]. Leptospirosis is sometimes referred to as “The Great Mimicker” and it may be overlooked and under diagnosed due to its varied clinical presentations. In the early stages of the illness, leptospirosis is often indistinguishable from other common causes of acute febrile illnesses in the tropics such as dengue, malaria and typhoid (Enteric fever) [4]. Leptospirosis is a potentially serious but treatable disease [5]. They can cause considerable morbidity, mortality and economic burden to developing tropical nations [6]. Typhoid
and paratyphoid fever remain important public health problems globally and is a major cause of morbidity in the developing world. While both typhoid and paratyphoid fever share clinical features, paratyphoid fever tends to have a more benign course of illness. Without effective treatment, typhoid fever has a case-fatality rate of 10–30% [7]. Dengue has been rampant in parts of Tamil Nadu in the past two decades. The prevalence of dengue vector and silent circulation of dengue viruses have been detected in rural and urban Tamil Nadu, which is ever increasing [8]. Management of acute febrile illness differs and is mainly based on the causative infectious agents. In areas endemic for two or more infectious agents, co-infection is more common.

With this background, the study was designed to estimate the seroprevalence of Leptospirosis, Enteric Fever and Dengue infection in cases of acute febrile illness.

**MATERIALS & METHODS:**
This study was done in Department of Experimental Medicine, the Tamil Nadu Dr.M.G.R Medical University, Chennai which functions as a Leptospira Diagnostic Center. Approximately, the department receives 200 serum samples per month for diagnosis of Leptospirosis referred by the physicians from areas in and around Chennai city. This prospective study was conducted for a period of 6 months from March 2011 to August 2011. All patients with febrile illness who were referred for diagnosis of Leptospirosis were given a proforma to fill containing age, gender, occupation and locality. Their clinical signs and symptoms and past history was obtained. Five ml of blood was collected from patient and serum was separated. Every 12th sample was included in the study by the method of systematic random sampling. Informed written consent was obtained. A total of 100 serum samples were tested for Leptospirosis, Enteric fever and Dengue fever and proforma of the 100 patients were analyzed.

**Diagnosis of Leptospirosis:**
All the samples were tested for presence of antibody to Leptospira by Microscopic Agglutination Test (MAT) as per standard procedure[9]. Standard Leptospiral strain serovars (*icterohaemorrhagiae, australis, autumnalis, canicola*) which causes Leptospirosis in Chennai and saphrophytic serovar (*Patoc*) were included in MAT. A titer of 1:80 and above was considered positive.

**Diagnosis of Enteric Fever:**
All the samples were tested for Typhoid using Widal Rapid Slide Screening test. All the positive samples by Widal Rapid test were confirmed by Widal Tube Agglutination test. The test included the serovars *Salmonella typhi, Salmonella paratyphi A* and *Salmonella paratyphi B* causing enteric fever. A titer of 1:80 and above was considered positive.

**Diagnosis of Dengue:**
All the samples were tested for Dengue virus using IgM Microlisa. Tests were done as per the manufacturer’s instructions.

**RESULTS:**
Among the 100 patients, 56% were males and 44% were females. With regard to age distribution, 28 patients were < 20 years, 46 were between the ages of 20 to 45 years and 26 patients were > 45 years. Forty three percent (43%) of cases were from Chennai city and 57% from the surrounding areas. Twenty two percent (22%) were students, 18% were laborers, 18% were professionals and 4% were children and others (38%). Seventy four percent (74%) reported no history of fever in the family in recent times and 70% of patients reported the presence of mosquito in their locality.
contrast, 79% reported good drainage system. Among 100 patients with fever symptoms, 48% reported 1-5 days of fever and 52% above 5 days. Eighty six percent (86%) of the patients reported intermittent pattern of fever. Frequency of other symptoms is shown in Table: 1. Past history of febrile illnesses of the 100 patients revealed 11% had typhoid, 2% suffered from Dengue, 2% from Leptospirosis & 7% had jaundice. One patient reported past history of Typhoid, Leptospirosis, jaundice and 2 reported for Typhoid and Jaundice. Of the 100 serum samples tested, 21 were positive for Leptospirosis, 17 for Enteric fever and 8 for Dengue (Fig 1).

All the 21 positive patients for Leptospirosis reported high grade fever ranging from 3 days to 1 week and most of the patients had symptoms like headache, body pain, vomiting and abdominal pain. Out of 100 patients, 26 samples reported positive by Widal Rapid slide Agglutination test. On further confirmation, only 17 samples (36.9%) were positive by Widal Tube test. Thirteen patients (76.47%) had antibodies against Salmonella typhi followed by 3 patients for Salmonella paratyphi A (17.6%) and 1 patient for Salmonella paratyphi B (5.8%) (Fig 3). All the 17 positive patients reported high grade fever with headache, malaise, abdominal pain, joint pain and few patients had diarrhea. Eight patients (8%) were positive for dengue using the IgM ELISA. Dengue positive patients also reported high grade fever, head ache, vomiting, sore throat and some suffered from breathlessness. Four patients were diagnosed to have one or more infection. Two patients were positive for Leptospirosis and Typhoid, one patient for Leptospirosis and Dengue and one patient positive for Typhoid and Dengue (Table 4).

**DISCUSSION:** All the 100 patients enrolled in this study reported high grade fever for 3 days to a week. They were suspected to have Leptospirosis by general physicians and were referred for testing. Twenty one patients (21%) were seropositive for leptospirosis among the 100 samples tested. D. Deodharet al reported that 31% patients had serological evidence of leptospira among cases of acute febrile illness [3]. Smita B. Shekatkar al from Pondicherry had reported 60% of seropositivity for leptospirosis by MAT [10]. H Manochaet al from Uttar Pradesh had reported 7% of leptospirosis as a cause of acute febrile illness [11]. Clinically Leptospirosis presents as two forms: Anicteric leptospirosis is the most common form accounting for 85% and icteric leptospirosis or Weil’s syndrome occurring in 5 to 10% of cases. Anicteric leptospirosis has 2 phases, septicemic phase and immune phase. Septicemic phase lasts for 3-7 days. Fever is high and remitting. Headache is intense, unremitting and possibly throbbing. Anorexia, nausea, vomiting and abdominal pain occur in most patients [12]. In the present study, all 21 patients who had antibodies to Leptospira also had high grade fever ranging from 3 days to 1 week and most of the patients showed symptoms like headache, body pain, vomiting and abdominal pain.

Serological tests based on the detection of antibodies remain the most practical method of diagnosis of leptospirosis. Among the serological techniques, MAT remains the "gold standard" because of its unsurpassed diagnostic (serovar-/serogroup) specificity in comparison with other currently available tests [13]. In the present study, out of 21 leptospira positive patients, 10 had antibodies against L.australis and 6 had antibodies against L. canicola, 4 against Licterohemorrhagiae and one for L.autumnalis. S. Ratnamet al had reported that serogroup L. autumnalis was common in Chennai [14]. Ganesan A et al had reported L.australis was most common serovar followed by
**L. canicola** in a study conducted in the same department previously [15]. Smitha et al from Puducherry had reported that serogroup **L. icterohemorrhagiae** was found in 27% of their patients followed by **L. pomona** and **L. pyrogenes** [10]. Sharma, S et al from Andaman Islands had reported **L. grippotyphosa** as the commonest serotype followed by **L. australis** [16]. In the present study, patients were positive for leptospirosis during summer months. Sumathi G et al from Chennai had reported in the year 2008 that leptospirosis occurs throughout the year and number of cases increases during monsoon months [17].

Dengue is an acute viral infection with potential fatal complications. It is transmitted mainly by *Aedes aegypti* mosquito and also by *Ae. albopictus*. All four serotypes can cause the full spectrum of disease from a subclinical infection to a mild self limiting disease, the dengue fever (DF) and a severe disease that may be fatal, the dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS). The WHO 2009 classification divides dengue fever into two groups: uncomplicated and severe [18]. Manifestations of dengue infection range from asymptomatic or mild febrile illness to circulatory failure and death from dengue hemorrhagic fever (DHF) [19]. In the present study, 8 out of 100 patients (8%) were positive for Dengue by IgM ELISA. Megan E. Reller et al reported that Dengue accounted for 6.3% (54/859) of acute febrile illnesses in Srilanka [20]. A study in Vietnam by H L Phuong reported that dengue is responsible for one third of fever cases presented to the public primary health services [21]. Piyush Tripathi et al in Uttar Pradesh reported 21% of their study patients had antibodies to Dengue [22]. Gunasekaran P et al from Chennai reported 15.5% of adult patients had antibodies to dengue for a period of three years from 2006 to 2008 [23]. In the present study, Dengue positive patients also reported high grade fever, head ache, vomiting, sore throat and some suffered from breathlessness. Dengue usually presents with symptoms of flu-like illness such as high-grade fever, generalized body ache, arthralgia, myalgia, nausea, and vomiting as well as maculopapular rashes. The symptoms of dengue may mimic other diseases such as leptospirosis and malaria which are also prevalent in areas where dengue is endemic [24].

In this study initially 26 samples reported positive by Widal Rapid slide Agglutination test. On further confirming, only 17 samples (36.9%) were positive by Widal Tube Agglutination test. Typhoid fever continues to be a global health problem with an estimated 16 million cases worldwide and 600,000 deaths each year. The disease is endemic in many developing countries [25]. The signs and symptoms of uncomplicated typhoid fever are nonspecific and a definitive diagnosis can be made by isolation of *Salmonella typhi* from blood or bone marrow. In areas where bacterial culture facilities are not available, Widal test remains the specific diagnostic test. The Widal test has been in use for more than a century as an aid in the diagnosis of typhoid fever. Serological diagnosis relies classically on the demonstration of a rising titer of antibodies in paired samples 10 to 14 days apart [26]. R. Shyamala et al in their study had reported 9% of seropositivity of Widal test among clinically suspected typhoid patients [27]. In this study, 17 cases of enteric fever was diagnosed from March to August. Of the 17 cases, 13 were due to *Salmonella typhi*. This could be due to onset of monsoon season in the month of July and August and lack of proper drainage leading to water contamination with fecal material. A study from Kolkata by Sur D...
et al also showed a peak of the disease from July to September [28].

In the present study, 8 patients were positive by widal tube test in their first week of illness. Christopher M et al also reported that widal was positive in 90% of patients with blood culture-positive typhoid fever and admitted during the first week of their illness. This could be due to regular subclinical sensitization to Salmonella in endemic areas [26]. In this study, 4 co-infections were noticed, 2 for Leptospirosis and typhoid, 1 for Leptospirosis and Dengue, 1 for dengue and typhoid. This could be explained by 2 ways. One by the fact that all the three infections are endemic in Chennai and the patients would have had infections with one or two causative agents.

On the other hand results of serology may be a false positive reaction caused by cross-reacting antibodies or non-specific polyclonal immunoreactivity [28]. Moreover, IgM antibody against dengue infection stays in circulation for a period of two to three months. Levett P N et al from Barbados had also reported that 2 out of 25 leptospirosis patients had serological evidence of acute dengue infection [29].

L. S. Yong et al from Malaysia had also reported a case of co-infection with Dengue, Leptospirosis and malaria in their patient [4]. A study in Kerala by Anil Kumar et al reported 17 cases (1.3%) of co-infections due to both leptospirosis and dengue [30]. Farid Ahmed et al from Bangladesh had reported a case co-infections of typhoid fever with Hepatitis A, Hepatitis E and dengue [31].

There are a few limitations to this study. The study was undertaken in a Diagnostic Testing Laboratory in the University rather than a hospital. In a hospital setup, the case history could have provided more information. The follow up of patients were not possible in case of extending the study in depth. Nearly half of the patient had fever for less than 5 days, absence of IgM antibody may not represent true Negatives. This could have been overcome by doing the serological test in paired serum samples. If paired serum samples were tested, which it would have demonstrated the increasing titers of antibodies. Financial constraints did not permit us to perform NS1 antigen in diagnosing Dengue fever. Blood culture was not done in the present study to confirm the diagnosis of typhoid and paratyphoid fevers. The present study was carried out in 100 samples. Entire workup for diagnosing the etiology of acute febrile illness could have been performed. In spite of malaria being the most frequent cause of acute febrile illness in Chennai, tests for malaria was not done.

CONCLUSION:

The laboratory diagnosis plays a very important role in determining the line of management in acute febrile illness. In resource limited setting, meticulous history taking, careful clinical examination and right choice of laboratory tests to detect the infections endemic in an area is crucial in diagnosing the cause of Acute Febrile Illness. Possibility of co-infections with one or more causative organisms must also be borne in mind when treating patients with acute febrile illness.
Table: 1 Presenting symptoms

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>55</td>
</tr>
<tr>
<td>Rigor and chills</td>
<td>50</td>
</tr>
<tr>
<td>Cough</td>
<td>48</td>
</tr>
<tr>
<td>Joint pain</td>
<td>39</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>28</td>
</tr>
<tr>
<td>Muscle ache</td>
<td>29</td>
</tr>
</tbody>
</table>

Table: 2 Distribution of gender in Leptospirosis, Typhoid and Dengue Positive patients.

<table>
<thead>
<tr>
<th></th>
<th>MALE</th>
<th>FEMALE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptospirosis</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Enteric Fever</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Dengue</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>24(52.15%)</td>
<td>22(47.8%)</td>
</tr>
</tbody>
</table>

Table: 3 Distribution of Age in Leptospirosis, Typhoid and Dengue Positive patients.

<table>
<thead>
<tr>
<th>AGE</th>
<th>LEPTOSPIROSIS</th>
<th>Enteric Fever</th>
<th>DENGUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20 years</td>
<td>5</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>20-45 years</td>
<td>9</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>&gt;45 years</td>
<td>7</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>17</td>
<td>8</td>
</tr>
</tbody>
</table>

Table: 4 Co-infections in Seropositive cases (n=4)

<table>
<thead>
<tr>
<th>Co-infections</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptospirosis and Typhoid</td>
<td>2</td>
</tr>
<tr>
<td>Leptospirosis and Dengue</td>
<td>1</td>
</tr>
<tr>
<td>Typhoid and Dengue</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
</tr>
</tbody>
</table>
Fig. 1. DISTRIBUTION OF LEPTOSPIROSI S, TYPHOID AND DENGUE AMONG PATIENTS (n=100)
Fig. 2. DISTRIBUTION OF SEROVARS IN LEPTOSPIROSIS POSITIVE PATIENTS (n=21)
Fig. 3. DISTRIBUTION OF SEROTYPES IN ENTERIC FEVER PATIENTS (n=17)

REFERENCES:
5. HUMAN LEPTOSPIROSIS: GUIDANCE FOR DIAGNOSIS, SURVEILLANCE AND CONTROL. WHO. International Leptospirosis Society.


