A Study on Clinical Profile and Serodiagnosis of Scrub Typhus in Patients Attending a Tertiary Care Hospital, South India.

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ABSTRACT

Introduction: Scrub typhus is one of the differential diagnoses of acute febrile illnesses. It causes serious complications like renal failure, liver failure, acute respiratory distress syndrome (ARDS), Multiorgan dysfunction syndrome (MODS), meningitis and thrombocytopenia. High index of suspicion and early diagnosis of scrub typhus by serological test can greatly reduce the chances of life threatening complications.

Aim & Objectives: The aim of this study is to detect the IgM antibodies by Capture Enzyme linked immunosorbent assay (ELISA) and Weil-Felix agglutination test against Scrub typhus to compare the results of IgM ELISA with Weil-Felix test and to study the clinical complications associated with scrub typhus and its outcome.

Materials and Methods: Total of 150 blood samples were collected from clinically suspected Scrub typhus patients. IgM ELISA (commercial) and Weil-Felix agglutination test for Scrub typhus were performed on all serum samples.

Results: Among 150 patients, 34 (23 %) showed positivity by IgM ELISA, 32 (21%)showed positivity by both IgM ELISA and Weil-Felix test against OX K antigen and 84 (56%) samples were negative by both IgM ELISA and Weil-Felix test. The sensitivity of IgM ELISA and Weil-Felix agglutination test is 100 % and 48 % respectively.

Conclusion: Early diagnosis and treatment will prevent further complications and mortality due to Scrub typhus. This study suggests that IgM ELISA is highly sensitive for diagnosis of scrub typhus than Weil-Felix agglutination test.

Key Words: Scrub typhus, Weil-Felix agglutination Test, IgM Enzyme linked immunosorbent assay.

INTRODUCTION:

The Scrub typhus is an acute, febrile, infectious illness caused by Orientia (Rickettsia) tsutsugamushi. One million cases occur annually and mortality rates range from 0-30%, due to complications like MODS, pneumonitis, ARDS, Encephalitis, circulatory failure. Eschar is a pathognomonic sign of scrub typhus, can be found in a highly variable (7-97%) percentage of people usually found on the neck, axilla, chest, abdomen and groin.

The complications of scrub typhus develop after the first week of untreated illness. The complications of scrub typhus are renal failure, hepatic failure, acute respiratory distress
syndrome, septic shock, myocarditis, and meningo-encephalitis.\textsuperscript{5,6}

The immunofluorescence assay (IFA) is considered as 'gold standard' for diagnosing rickettsial infections. The most widely used serological test for rickettsial screening is the Weil-Felix test, although its reliability is suspected due to its poor sensitivity and specificity. Recent outbreaks are reported by detecting antigen-specific IgM or IgG antibodies by Enzyme linked immunosorbent assay (ELISA).\textsuperscript{14}

**AIM & OBJECTIVES:**

1. To detect the IgM antibodies against *Orientia tsutsugamushi* by IgM Capture Enzyme linked immunosorbent assay (ELISA).
2. To detect the IgM antibodies against *Orientia tsutsugamushi* by Weil-Felix agglutination test.
3. To compare the results of Weil-Felix agglutination test with IgM ELISA.
4. To study the clinical complications associated with scrub typhus and its outcome.

**MATERIALS AND METHODS:**

The study was a cross sectional study, conducted after getting Institutional ethics committee clearance. A total of 150 adult patients (age more than 18 years) with signs and symptoms of acute fever, eschar, breathlessness, cough, nausea, vomiting, myalgia, lymphadenopathy and headache suggestive of scrub typhus were included in this study.

The study was conducted for the period of one year at the Institute of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital (RGGGH), Chennai, a tertiary care hospital.

Under strict aseptic precautions, in a dry sterile test tube, 5ml of blood sample was collected from the patients with signs and symptoms suggestive of scrub typhus. After clot formation, samples were centrifuged at 3000x g for 10 minutes and serum was separated and preserved at -70°C and detection of IgM antibodies against *O.tsutsugamushi* was done by using commercial IgM Capture Enzyme linked immunosorbent assay kit (INBIOS, USA), quantitative detection of *O.tsutsugamushi* antibodies by using Proteus mirabilis OX K antigen by Weil-Felix agglutination test (Commercial OXK PROGEN ANTIGEN by Tulip Diagnostics). The results of Weil-Felix agglutination test were compared with IgM Capture ELISA.

**RESULTS:**

Totally 150 suspected cases of scrub typhus were included in this study. Of which 81 (54%) were male patients and 69 (46%) were female patients (Fig 1).

**Fig 1. Analysis of Sex Distribution**

IgM ELISA and Weil-Felix agglutination test were performed in all the one hundred fifty cases. Among 150 patients, 34 (23%) showed positivity by IgM ELISA, 32 (21%) showed positivity by both
IgM ELISA and Weil-Felix test against OX K antigen and 84 (56%) samples were negative by both IgM ELISA and Weil-Felix test (Table-1).

### Table 1. Positivity of Scrub typhus by IgM capture ELISA and Weil-Felix test (n=150)

<table>
<thead>
<tr>
<th>S.No</th>
<th>TEST</th>
<th>No. of positive patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Only IgM ELISA Positive</td>
<td>34 (23%)</td>
</tr>
<tr>
<td>2</td>
<td>Both IgM ELISA &amp; Weil-Felix test Positive</td>
<td>32 (21%)</td>
</tr>
<tr>
<td>3</td>
<td>Both IgM ELISA &amp; Weil-Felix test Negative</td>
<td>84 (56%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>150 (100%)</td>
</tr>
</tbody>
</table>

Table-2 shows that Four (12%) of these 32 patients were positive for Weil-Felix agglutination test with a titre of 1:1280, Eight patients (25%) with a titre of 1:640, Eight patients (25%) with a titre of 1:320, twelve patients (38%) with a titre of 1:160. If, the titre of $\geq 1:160$ was considered as significant.

### Table 2. Weil-Felix test positivity with titre (n=32)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Significant titre</th>
<th>No. of positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt; 1: 1280</td>
<td>4 (12%)</td>
</tr>
<tr>
<td>2</td>
<td>1:640</td>
<td>8 (25%)</td>
</tr>
<tr>
<td>3</td>
<td>1:320</td>
<td>8 (25%)</td>
</tr>
<tr>
<td>4</td>
<td>1:160</td>
<td>12 (38%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>32 (100%)</td>
</tr>
</tbody>
</table>

Male patients (62%) were most commonly affected than female. Positivity was higher in the age group of patients between 20 and 45 yrs of age.

Common symptoms were high grade fever of 7-14 days duration, nausea, vomiting, headache, myalgia, cough, breathlessness and reduced urine output.

Eschar was seen in 24 cases (16%) and the sites were shoulder, left mammary region, thigh, scrotum (figure-2) and groin.

### Table 3. Complications of Scrub typhus

<table>
<thead>
<tr>
<th>S.No</th>
<th>Complications</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Elevated liver enzyme</td>
<td>6 (16%)</td>
</tr>
<tr>
<td>2</td>
<td>ARDS</td>
<td>5 (14%)</td>
</tr>
<tr>
<td>3</td>
<td>MODS</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>4</td>
<td>Renal impairment</td>
<td>9 (25%)</td>
</tr>
<tr>
<td>5</td>
<td>Meningitis</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>6</td>
<td>Thrombocytopenia</td>
<td>13 (36%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>36 (100%)</td>
</tr>
</tbody>
</table>

Table-3 shows that the liver enzymes were elevated in 6 cases (16%), Multiple Organ Dysfunction Syndrome (MODS) was present in two patients (6%), renal impairment (9 patients, 25%), Acute respiratory distress syndrome (ARDS) (5 patients, 14%), meningitis (1 patients, 3%) and thrombocytopenia (13 patients, 36%) were some of the serious complications.
DISCUSSION:

Totally one hundred fifty cases were included in this study. Of which 81 (54%) were male patients and 69 (46%) were female patients. In our study most of the patients were presented with fever of more than 7 days duration, in addition to myalgia, headache, nausea, vomiting, cough, breathlessness, pedal oedema and reduced urine output.

Eschar is the most characteristic features of scrub typhus, but in our study, eschar was found in 24 out of the 66 positive cases. The common sites were shoulder, groin, thigh, scrotum and left mammary region.

More than 50% (36/66) of the patients had multi system involvement (Table-2). The liver enzymes were elevated in 6 cases (16%), Multiple Organ Dysfunction Syndrome (MODS) was present in two patients (6%), renal impairment (9 patients, 25%), Acute respiratory distress syndrome (ARDS) (5 patients, 14%), meningitis (1 patients, 3%) and thrombocytopenia (13 patients, 36%) were some of the important complications. Study conducted by Wang CC et al, Yen TH et al, and Thap. LC et al have reported mortality rate of 7-30%.7,8,9 Pandey et al from Himachal Pradesh reported 3 cases of ARDS due to scrub typhus.10 Tsay et al from Taiwan found 8 cases of ARDS, 3 cases of acute renal failure and one case each of myocarditis and septic shock.11

IgMcapture ELISA and Weil-Felix agglutination test were performed in all the one hundred fifty cases. Of which, 34 (23 %) samples showed positive by IgM capture ELISA,32 (21%) samples showed positive by both IgM ELISA and Weil-Felix test against OX K antigen and 84 (56%) samples showed negative by both IgM ELISA and Weil-Felix test. Four (12%) of these 32 patients positive for Weil-Felix agglutination test with a titre of 1:1280, eight patients (25%) with a titre of 1:640, eight patients (25%) with a titre of 1:320, and twelve patients (38%) with a titre of 1:160. Male patients (62%) were most commonly affected than females. Positivity was higher in the age group of patients between 20 and 45 yrs of age.

In comparison to IgM capture ELISA, Weil-Felix agglutination test results may be negative during the early stages of the disease because agglutinating antibodies are detectable only during the second week of illness.12 We found that the Weil-Felix agglutination test had 48% sensitivity and 100% specificity and positive predictive value at a titre of 1: 160. But ELISA test, provides positive results within 3-4 days after the onset of illness and it is more sensitive than Weil-Felix agglutination test. According to Issac et al, Weil Felix test has high specificity, even at a titer as low as 1:20.13 Hence patients with low titres need to be evaluated for scrub typhus by ELISA. Scrub typhus ELISA kits use Orientia tsutsugamushi recombinant p56kD type-specific antigen of Karp, Kato, Gilliam and TA716 strains and have more than 90% sensitivity and 90% specificity for detecting specific antibodies.14

CONCLUSION:

Scrub typhus is one of the differential diagnoses of the acute febrile illness. It causes important complications like renal failure, liver failure, ARDS, MODS, meningitis and thrombocytopenia. High index of suspicion and early diagnosis of scrub typhus by serological test can greatly reduce the chances of life threatening complications and can guide optimal therapy. Though eschar is pathognomonic of the disease, its absence does not
rule out the diagnosis of scrub typhus. The IgM capture ELISA is more sensitive test compared to Weil-Felix agglutination test. But the Weil-Felix agglutination test is more specific though it shows sensitivity of 56% only. However, Weil-Felix agglutination test is more economical when compared to ELISA and it can be used in resource poor settings, where facility for ELISA and other tests do not exist.

REFERENCES:


