

Original Research Article

Rickettsial neglected zoonoses: prevalence of scrub typhus at central Karnataka

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Received: 12 June 2017

Accepted: 08 July 2017

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ABSTRACT

Background: Fever of unknown Origin (FUO) has many multiple causes such as enteric fever, malaria, dengue, tuberculosis, brucellosis. But scrub typhus is less known cause in Indian scenario. The present study reports the prevalence of scrub typhus at central Karnataka and compares the sensitivity and specificity of Weil-Felix test and the IgM ELISA in the detection of infection.

Methods: 368 serum samples of FUO cases were collected. Weil-Felix test was performed and also analyzed for IgM antibodies to *Orienta tsutsugamushi* by IgM ELISA test along with haematological and biochemical investigations.

Results: Out of 368 patients of fever of unknown origin, 94 cases were positive by OXK antigens by Weil Felix test and 61 were positive by ELISA test for ST IgM antibodies. Fever was the most common clinical presentation occurring in ST IgM ELISA positive cases, followed by myalgia in 90.1% cases, headache in 77%, hepatomegaly in 65.5%, splenomegaly in 62.2% and rashes were seen in 29.5% patients. Eschar was seen in 13.1% patients, pneumonia in 3.2% and meningo-encephalitis in 1.6%. Sensitivity and specificity of WFT in relation to IgM ELISA at a titre of 160 was 81.97% and 85.67% respectively.

Conclusions: With the growing number of cases detected in India, scrub typhus is fast emerging as a public health threat and also due to limited diagnostics leading to underreporting, Weil Felix test could be used in adjunct with Enzyme-linked immunosorbent assay and blood parameters in the diagnosis of rickettsial diseases.

Keywords: Rickettsial diseases, Scrub typhus, ST IgM ELISA, Thrombocytopenia, Weil felix test

INTRODUCTION

Rickettsial diseases are the most-sneaky emerging and re-emerging diseases and notoriously difficult to diagnose.

In India, rickettsial diseases have been documented since 1930s in Assam.¹ Rickettsial diseases are a group of infections caused by the obligate intracellular bacteria.² Rickettsia is the Gram-negative coccobacilli, commonly

transmitted to humans by arthropod vectors.^{2,3} Factors contributing for under diagnosis of rickettsial diseases are due to its non-specific clinical presentation which could be confused with other common tropical infections like dengue, enteric fever, leptospirosis, malaria etc. and also due to limited diagnostic facilities.⁴ Therefore, clinical suspicion is of paramount importance in the diagnosis of typhus fever. Confirmation of the diagnosis is important as the treatment is different but may not always be possible.⁵ In most cases of typhus, treatment is given based on clinical suspicion or a positive Weil-Felix test.⁶ In clinically-suspected cases of typhus fever, a rising titre of OXK, OX2, and OX19 antigens supports the diagnosis but confirmation of the diagnosis may be difficult.⁷⁻⁹ Enzyme-linked immunosorbent assay along with Weil-Felix test and blood parameters could be adjunct in the diagnosis of rickettsial diseases. Prevalence of scrub typhus is a cause of concern in central Karnataka. Understanding local patterns of scrub typhus and factors that place individuals at risk is pivotal to future preventive measures against scrub typhus. Here, we present results of 368 cases of fever of unknown origin at Central Karnataka

METHODS

Ethical clearance: Protocol was approved by Institutional Ethical Review Board of S. S. Institute of Medical Sciences and Research Centre.

368 Serum samples of Fever of Unknown Origin (FUO) cases presented to S.S Institute of Medical Sciences and Research Centre were collected from the month of October 2014 to March 2015. IgM ELISA Scrub Typhus and Weil-Felix test were carried out on sera of suspected cases.

Clinical and demographic data were collected through interviewing the patients or their attendants and meticulous physical examination of the patients conducted by their treating physician. Reports of haematological and biochemical investigations were obtained.

IgM ELISA scrub typhus

The kit for detection of scrub typhus was procured from InBios International Inc. Seattle, WA.

USA. Scrub Typhus IgM ELISA Kit is an immunosorbent assay to test for exposure to *Orientia tsutsugamushi*. The Scrub Typhus IgM ELISA Kit is a qualitative, membrane-based immunoassay for the detection of IgM antibodies in human plasma/serum to *Orientia tsutsugamushi* derived recombinant antigen.

Wells of each plate on the Scrub Typhus IgM ELISA test have been coated with unique recombinant antigen mix. The serum sample is diluted and applied to each well. After incubation and washing, the wells of this Scrub

Typhus IgM ELISA Kit are treated with IgM enzyme HRP-conjugate. After a second incubation and washing step, TMB substrate is added.

An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by absorbance measurement at 450nm. The absorbance measured is directly proportional to the concentration of IgM antibodies to *Orientia tsutsugamushi* present in the patient's serum.

Weil-Felix test

Weil-Felix is a nonspecific agglutination test which detects anti-rickettsial antibodies in patient's serum. Weil-Felix test is based on cross-reactions which occur between antibodies produced in acute rickettsial infections with antigens of OX (OX 19, OX 2, and OXK) strains of *Proteus* species.⁵⁻⁷ Dilution of patient's serum is tested against suspensions of the different *Proteus* strains.

Weil-Felix test (PROGEN, Tulip Diagnostics (P) Ltd., Verna, Goa, India) was performed according to the manufacturer's instructions. Titers of more than 1:160 for OXK were considered diagnostically significant.^{8,9}

The data was statistically analyzed on SPSS 16 software by using Chi-square (X^2) test and the probability value (p value) of less than 0.05 were considered significant.

RESULTS

Out of 368 patients of FUO, ST IgM antibodies were detected in 61 patients by ELISA. Among these 61 diagnosed cases of ST, 27 were males and 34 were females. Positivity of ST was significantly higher among females who were suffering from fever of unknown origin in comparison to males ($P < 0.05$). The age of the patients ranged between 2 months to 76 years. Positivity of ST IgM antibody was highest among the age group 31-40 years followed by 41-50 years and 11-20 years (Table 1).

Table 1: Age wise distribution of patient positive for scrub typhus by IgM ELISA.

Age	Number
1-10	6
11-20	10
21-30	5
31-40	20
41-50	16
>51	4
Total	61

Various clinical features are summarized in Table 2. Fever was the most common clinical presentation occurring in all patients. There was no specific pattern of fever and height of fever ranged from 38°C to 40°C. Other common clinical features were myalgia (90.1%),

headache in 77%, hepatomegaly in 65.5%, splenomegaly in 62.2% and rashes were seen in 29.5% patients. Eschar was seen in 13.1% patients, pneumonia in 3.2% and meningo-encephalitis in 1.6%.

The laboratory parameters are depicted in Table 3. Increased leucocyte count was seen in 43 patients. A platelet counts less than 40,000/mm³ was observed in 8 patients and platelet between 40,000-80,000/mm³ was observed in 33 patients and platelet count greater than 1,00,000/mm³ was seen in 20 patients. AST was raised in 74% patients, ALT in 60.6%, Creatine kinase raised in 34.4% of the patients.

Table 2: Clinical features of patient’s positive for scrub typhus by IgM ELISA.

Clinical Feature	Number (%)
Fever	61 (100)
Myalgia	55 (90.1)
Headache	47 (77.0)
Hepatomegaly	40 (65.5)
Splenomegaly	38 (62.2)
Vomiting	29 (48.0)
Seizures	21 (34.0)
Abdominal pain	19 (31.0)
Rash	18 (29.5)
Eschar	8 (13.1)
Diarrhoea	7 (11.4)
Pneumonia	02 (3.2)
Meningo-encephalitis	01 (1.6)

Table 3: Laboratory investigations in patient positive for scrub typhus by IgM ELISA.

Investigations	Numbers (%)
Leucocytosis	27 (44.2)
Platelet Count ≤40,000	8 (13.1)
Platelet Count 40,000 - 80,000	33 (54.0)
Platelet Count > 1,00,000	20 (32.7)
Aspartate Aminotransferase >40 U/L	46 (75.4)
Alanine Aminotransferase >40 U/L	37(60.6)
Creatinine Kinase >175 U/L	21(34.4)
Partial thromboplastin time >2 fold	12(19.6)

Table 4: Comparison of weil felix test with Scrub typhus by IgM ELISA.

Scrub typhus by IgM ELISA	Weil Felix test		Total
	Positive	Negative	
Positive	50	44	94
Negative	11	263	274
	61	307	368

Out of these 61 patients’ positive by ST IgM ELISA, 50 were also positive by Weil Felix OXK agglutination test. The diagnostic titre of Weil Felix considered positive was titre 160. Sensitivity and Specificity of WFT in relation to

IgM ELISA at a titre of 160 was 81.97% and 85.67% respectively. The positive predictive accuracy was 53.19% and negative predictive accuracy was 95.99% with respect to IgM ELISA (Table 4).

DISCUSSION

Among the patients who were positive for IgM ST ELISA, 55.7% were female and 44.3% were male and majority of patients were from rural area (72.1%). Commonest age group involved was of the age 31 to 40 years. Scrub typhus often appears as a nonspecific febrile illness. Diagnosis and surveillance can be challenging, particularly in the absence of advanced laboratory diagnostic techniques.^{10,11} The common clinical features include fever, headache, rash, lymphadenopathy and in some presence of an eschar could be pathognomonic, but it is typically unnoticed or misdiagnosed.¹²⁻¹⁴ In very few cases pulmonary involvement could occur and it is the principal cause of death in complicated disease.^{10,11} In the present study fever was the commonest symptom followed by myalgia, headache, hepatomegaly and splenomegaly. Rashes were seen in 29.5% of IgM positive cases, eschar was seen in 13.1%, pneumonia in 3.2% and meningo-encephalitis in 1.6%. Our finding is in correlation with other studies.^{7,8,12,15}

The sharing of antigens between rickettsia and Proteus is the basis of Weil Felix test. It demonstrates antibodies to *Proteus vulgaris* strain OX 19, OX2 and OX K. Most of the Western research has advised against performing this test for diagnosis of rickettsial infections.³

The poor sensitivity of the Weil Felix test is well established but a good correlation between the results of the Weil Felix test and detection of IgM antibodies by an indirect immunofluorescence assay is often observed.⁵ In our study we compared the Weil Felix test with the Enzyme linked immuno sorbent assay for detection of IgM for scrub typhus. Among 368 sera tested for rickettsia by Weil Felix, 94 sera were positive for OXK. Out of 94 Weil Felix OXK positive cases, 50 sera were positive for IgM ST ELISA and 11 were positive in Weil Felix negative cases. Totally 61 cases were positive among 368 cases of fever of unknown origin indicating 16% of incidence of scrub typhus at central Karnataka. All 61 IgM positive cases revealed the clinical features suggestive of scrub typhus and complete hemogram and liver parameters were deranged.

Failure of timely diagnosis leads to significant morbidity and mortality. With timely diagnosis treatment is easy, affordable and often successful with dramatic response to antimicrobials.

As antimicrobials effective for rickettsial diseases are usually not included in empirical therapy of nonspecific febrile illnesses, treatment of rickettsial diseases is not provided unless they are suspected. Several tests are available with their own advantages and limitations.

Among all the serological tests available Weil-Felix test is the cheapest and easily available, but this is notoriously unreliable. In our study Weil Felix test had sensitivity and specificity of 81.7% and 85.7% in diagnosis of scrub typhus when compared to ST IgM ELISA at a titre of 160. Our finding corroborates with other studies.^{8,16,17}

Hence, Weil Felix test could be used in adjunct with Enzyme-linked immunosorbent assay and blood parameters in the diagnosis of suspected rickettsial diseases.

ACKNOWLEDGEMENTS

Authors would like to thank Vision Group of Science and Technology, Government of Karnataka for their support.

Funding: Project funded by Vision Group of Science and Technology, Government of Karnataka

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: VinodKumar CS, Patil S, Prasad BS, Kalappanavar NK, Jayaraj SG, Niranjan K, et al. Rickettsial neglected zoonoses: prevalence of scrub typhus at central Karnataka. Int J Res Med Sci 2017;5:3672-5.