

Seroprevalence of Scrub typhus at a tertiary care hospital in Andhra Pradesh

A Ramyasree, *U Kalawat, ND Rani, A Chaudhury

Abstract

Introduction: Scrub typhus is a rickettsial infection which is caused by *Orientia tsutsugamushi* and transmitted by the bite of the chigger of a mite. Delay in diagnosis can be fatal otherwise the treatment is simple, doxycycline being the drug of choice. Indirect immunofluorescence is considered gold standard but it is not used in India as it is costly and also not available. There is need for rapid, economic and simple test for the diagnosis of scrub typhus. This study was taken up to study the seroprevalence of scrub typhus in Andhra Pradesh and to compare two commonly used serological methods; rapid test and IgM ELISA. **Materials and methods:** This is a prospective study in which 100 serum samples from clinically suspected cases collected over a period of 3 months were processed for the detection of IgM antibodies for scrub typhus by ELISA and Rapid test. Samples were also tested for leptospirosis and dengue fever which the other common causes of fever prevalent in this region. **Results:** Total number of samples processed was 100 of which 52 were males and 48 females. Among the hundred samples 39 were seropositive. Positivity was higher in the age group of patients between 16 and 30 yrs of age. There was 97% correlation between ELISA and rapid method. Of the 100 samples only three samples positive by ELISA were negative by rapid method. Fever was the most common manifestation and there was no eschar and no mortality reported. **Conclusion:** Scrub typhus should be included in the differential diagnosis of fever of unknown origin along with dengue, malaria and leptospirosis which are the other common endemic infections in this part of the country.

Key words: Elisa-enzyme linked immunosorbent assay, rapid test, scrub typhus

Introduction

Rickettsial infections are re-emerging with increased reports from different parts of the world. Scrub typhus is an acute febrile illness and delay in diagnosis is associated with considerable morbidity and mortality. Although it is a neglected disease in India, but in recent years there are reports from Maharashtra, Tamil Nadu, Karnataka, Kerala, Jammu and Kashmir, Uttaranchal, Himachal Pradesh, Rajasthan, Assam and West Bengal indicating the resurgence of the disease in this part of the world also.^[1-6]

Scrub typhus or Bush typhus is a rickettsial infection which is caused by *Orientia tsutsugamushi* and transmitted by some species of trombiculid mites ("chiggers", particularly a

Leptotrombidium deliense).^[3] The name is derived from the prevalence of the mites in areas of heavy scrub vegetation. The disease is endemic in the geographical region known as "tsutsugamushi triangle" which extends from northern Japan and far-eastern Russia in the north, to the territories around the Solomon Sea into northern Australia in the south, and to Pakistan and Afghanistan in the west. The bite of this mite leaves a characteristic black eschar that is useful to the doctor for making the diagnosis.^[2,7]

The observation of the eschar is often missed and other signs and symptoms of the disease are not characteristic thus posing the problem of delayed diagnosis by the clinician. In view of low index of suspicion, non-specific signs and symptoms, and absence of widely available sensitive and specific diagnostic tests, these infections are notoriously difficult to diagnose.^[8]

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.^[9] Among all the serological tests available Weil-Felix test is the cheapest and easily available, but this is notoriously unreliable. Indirect immunofluorescence test, the gold standard is beyond affordability specially in poor countries and needs expertise for interpretation as the choice of

*Corresponding author: (email: <ukalawat@yahoo.com>)

Department of Microbiology (AR, UK, NDR, AC), Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh, India.

Received: 28-09-2013

Accepted: 24-02-2014

Access this article online

Quick Response Code:



Website:

www.ijmm.org

DOI:

10.4103/0255-0857.148381

cut-off values for positive diagnosis is influenced by several factors such as antibody kinetics, geography, negative seroconversion and seasonality.^[10,11]

IgM ELISA has been evaluated and found to be quite satisfactory in comparison to the gold standard, but samples need to be pooled for ELISA which can lead to delayed diagnosis thus influencing the overall outcome. Rapid tests have come into vogue which are economic, rapid and single tests can be carried out.

This study was carried out to know the seroprevalence of scrub typhus in clinically suspected patients and to compare a rapid test which is simple, and economic test with IgM ELISA for the diagnosis of scrub typhus.

Material and Methods

This is a cross-sectional study carried out on serum samples from clinically suspected cases received over a period of 3 months extending from January to March 2013. The samples were processed for the detection of IgM antibodies for the diagnosis of scrub typhus by ELISA and Rapid test. Samples were also tested for dengue fever, typhoid fever and leptospirosis, tuberculosis and malaria.

Detection IgM antibodies by ELISA- was done using In Bios International TM IgM ELISA.

Detection of IgM antibodies by Rapid method was done using SD Bioline Tsutsugamushi, one-step scrub typhus antibody test. Clinical features of the patients were retrieved from hospital medical records. Statistical analysis was done using SPSS 11.5 version. *P*-value was calculated using Chi-square test.

Results

Among the 100 samples tested, 39 were positive either by ELISA or rapid method. Out of the 100 samples, 52 were males and 48 from females. Age and sex distribution of patients and their seropositivity is shown in Figure 1. There was 97% correlation between ELISA and rapid method. Of the 100 samples, only 3 samples positive by ELISA were negative by rapid method [Figure 2]. *P*-value was less than 0.01 which is significant.

Fever was the most common manifestation (100%). Other presentations included cough, vomiting. There was no eschar but rash was present in three patients [Table 1]. Seizures were present in two patients, hepatic dysfunction and oliguria in seven patients. No mortality was reported.

Discussion

Rickettsial infections are re-emerging with increasing reports from India and other countries with considerable mortality and morbidity. Scrub typhus is a rickettsial infection caused by *Orientia tsutsugamushi*.

Table 1: Clinical presentations of patients (or) Common symptoms and their percentages

Clinical features	No. of patients
Fever	100
Rigor	100
Vomiting	50
Cough	16
Chills	58
Headache	30
Eschar	-
Skin rash	3
Pain abdomen	10
Seizures	2
Body pain	14
Oliguria	7
Mortality	-

The prevalence of scrub typhus varies from 0-8% to 60% in different countries.^[11,12] In a study from Thailand, the positivity for scrub typhus was 59.5% with highest prevalence in 40-49 (77.7%) year age group with no difference between the two sexes.^[13] Sarma *et al.*, tested 150 samples from patients with fever of unknown origin of which 52 were found to be positive for OX K antibodies by Weil-Felix test.^[14] Gurung *et al.*, tested 204 patients with fever of unknown origin of which 63 were confirmed positive of which 42 were males and 21 were females.^[15] In our study, among the 100 patients tested 39 were positive. Of these 39 positive patients, 23 were males and 16 were females. Highest seropositivity was observed in 46-60-years age group.

Fever with chills and rigour was the most common presentation in our study which is similar to a hospital-based study in Taiwan and a study by Dass *et al.*, from the state of Meghalaya, India.^[16]

Other presentations included vomiting (50%), headache (30%) and cough (16.7%). One patient had hepatic dysfunction, rash was present in three, seizures in two and oliguria in seven patients. No mortality was reported.

Fever, cough and vomitings have been reported as chief complaints in other studies also.^[16-19] Kammili *et al.*, from Secunderbad, India tested for the prevalence of scrub typhus among patients who were provisionally diagnosed as dengue fever. Among the 100 patients studied, 19 were found to be positive for antibodies for scrub typhus by rapid immune-chromatography and Weil-Felix test. Seropositivity was equal among males and females with preponderance in old age group. Chief complaints included arthralgia (2 patients), haemorrhagic manifestations (3 patients) and rash (3 patients).^[20]

Boorugu *et al.*, reported a case from Andhra Pradesh. The patient presented with fever which was associated with myalgia,

loose stools and dry cough. Patient was finally diagnosed with scrub typhus associated with thrombocytopenia, hepatitis, hypoalbuminaemia and bilateral pleural effusion.^[21]

A study from this area reported fever and headache as the most common manifestation (more than 50%), followed by cold and cough (40%) with no rash. Among 200 subjects tested 39 were reactive for rickettsial infections by Weil-Felix test. Among these 36 were reactive for OXK antigens.^[22]

A study from Chennai reported seizures, signs of consolidation, thrombocytopenia, elevated serum alkaline phosphates and renal failure in 25%, 40%, 37.5%, 52% and 33% patients, respectively. 6.25% of patients died of multiorgan failure.^[23]

Although there was no eschar among patients in our study but rash was observed in three cases. Several studies have reported eschar and rash in different populations from different parts of the world. The prevalence of the rash has been reported varying from 8.9% to 90% by different authors.^[19,24]

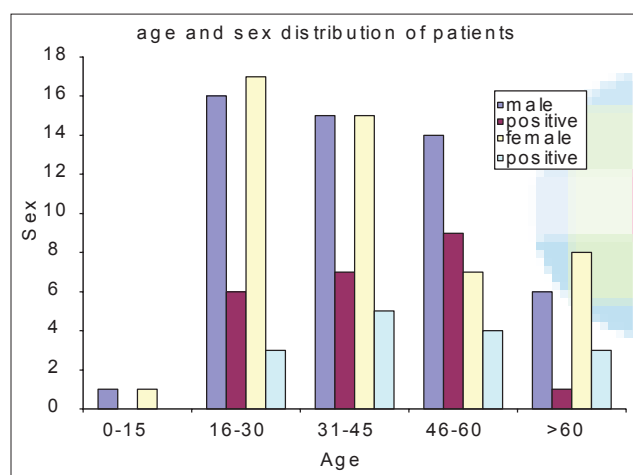


Figure 1: Age and sex distribution of patients

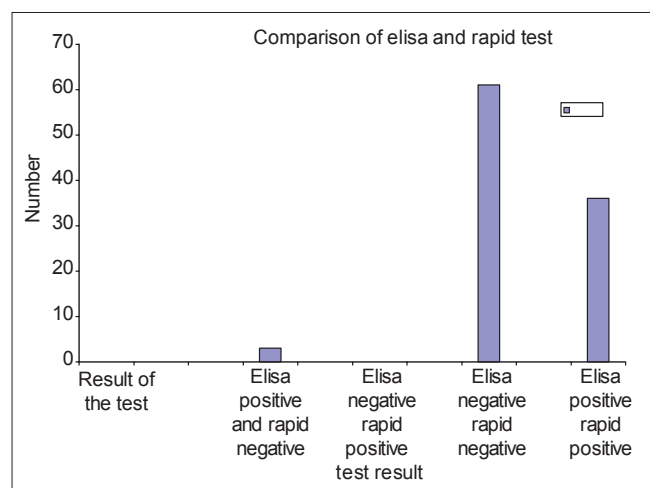


Figure 2: Results of ELISA and rapid test

The diagnosis of scrub typhus poses a problem due to low index of suspicion, non-specific signs and symptoms, absence of the specific presentation of an eschar and the lack of diagnostic facilities in India.

A battery of tests is available like Weil-Felix, indirect immunofluoresce, PCR, culture and ELISA all of which have their own limitations. In poor countries some of these tests like PCR, indirect immunofluorescence which provide accurate and specific diagnosis are either not available or are too expensive. Weil-Felix is a commonly used inexpensive serological test which lacks both sensitivity and specificity.^[25] There is need for a rapid, technically simple and economic test. ELISA is an easy and comparatively economic test but the results of this test may not be available on the same day as samples need to be pooled for testing thus causing delayed diagnosis and treatment, which at times may be fatal. A rapid method which can provide the diagnosis on the same day can really make a difference in the overall outcome.

Therefore, we compared the rapid method with IgM ELISA for the diagnosis of scrub typhus. Prakash *et al.*, evaluated Dot enzyme immunoassay (EIA), an immunoglobulin (Ig) M enzyme-linked immunosorbent assay (ELISA) and the Weil-Felix test for diagnosis of scrub typhus. They reported sensitivity of 100, 86.5 and 43.5% with Dot EIA, IgM ELISA and Weil-Felix test, respectively. False-positive reactions were observed in patients with falciparum malaria, pulmonary tuberculosis, *S. viridans* septicaemia and typhoid fever using Dot EIA and IgM ELISA.^[26] In our study two patients were also positive for malaria, two for dengue fever and four for leptospirosis which are other febrile illnesses endemic in this area. Whether the scrub typhus positivity was due to cross-reactions or there were coinfections, could not be ascertained as further confirmatory tests were not carried out. No patient tested positive for typhoid or tuberculosis.

Chinprasatsak *et al.*, evaluated a rapid dot blot assay for rapid diagnosis and reported the sensitivity and specificity of 87% and 94%, respectively. They concluded that the dot blot immunoassay dipstick was accurate, rapid, easy to use and relatively inexpensive. No false positives were observed.^[27]

Jang *et al.*, evaluated IgM ELISA for the diagnosis of scrub typhus and reported sensitivity of 96.3% for IgG IFA-positive samples and of 100% for IgM IFA-positive samples. The specificity of the IgM capture ELISA was 99%, for IgM-positive samples.^[28]

Gurung *et al.*, used ELISA and rapid method for the diagnosis of scrub typhus. In their study one sample positive by rapid method was negative by ELISA.^[15] In our study using IgM ELISA and rapid test, there was 97% correlation between the two tests. The difference was statistically significant indicating the superiority of ELISA as compared

to rapid test. Therefore, it can be concluded that in centres with high workload, ELISA should be used but with less number of samples rapid method may be used for early diagnosis of scrub typhus.

Scrub typhus infections have been reported from neighbouring states like Tamil Nadu, Kerala, Karnataka and Orissa. The climatic conditions in Andhra Pradesh are more or less similar to these states therefore, it is highly likely that the various infections prevalent may be similar. Our study provides an evidence for the seropositivity of scrub typhus in this state.

To the best of our knowledge, this the first study on the seroprevalence of scrub typhus by Rapid and the ELISA method from Andhra Pradesh. It is recommended that larger sample based studies may be taken up which may throw better light on the seroprevalence and clinical presentations of scrub typhus in this region. It is also recommended that scrub typhus should be included in the differential diagnosis of fever of unknown origin along with leptospirosis and dengue fever which are other endemic diseases in this region, which will help in proper diagnosis, timely and adequate treatment and avoidance of the complications which are associated with high mortality.

Acknowledgement

We are thankful to all the clinicians who have sent samples during the study period.

References

- Rathi N, Rathi A. Rickettsial infections: Indian perspective. *Indian Paediatr* 2010;47:157-64.
- Mahajan SK, Rolain JM, Kashyap R, Bakshi D, Sharma V, Prasher BS, *et al.* Scrub typhus in Himalayas. *Emerg Infect Dis* 2006;12:1590-2.
- Sundhinda BK, Vijaykumar S, Kutti AK, Tholpadi SR, Rajan AS, Mathai E, *et al.* Rickettsial spotted fever in Kerala. *Natl Med J India* 2004;17:51-2.
- Mathai E, Lloyd G, Cherian T, Abraham OC, Cherian AM. Serological evidence of the continued presence of human rickettsioses in southern India. *Ann Trop Med Parasitol* 2001;95:395-8.
- Kamarasu K, Malathi M, Rajagopal V, Subramani K, Jagadeeshramasamy D, Mathai E. Serological evidence for wide distribution of spotted fevers and typhus fever in Tamil Nadu. *Indian J Med Res* 2007;126:128-30.
- Mittal V, Gupta N, Bhattacharya D, Kumar K, Ichhpujani RL, Singh S, *et al.* Serological evidence of rickettsial infections in Delhi. *Indian J Med Res* 2012;135:538-41.
- Chang WH. Current status of tsutsugamushi disease in Korea. *J Korean Med Sci* 1995;10:227-38.
- Vivekanandan M, Mani A, Priya YS, Singh AP, Jayakumar S, Purty S. Outbreak of scrub typhus in Pondicherry. *J Assoc Physicians India* 2010;58:24-8.
- Kaore NM. Laboratory diagnosis of scrub typhus. *J K Science* 2010;12:72-5.
- Blacksell SD, Bryant NJ, Paris DH, Doust JA, Sakoda Y, Day NP. Scrub typhus serologic testing with the indirect immunofluorescence method as a diagnostic gold standard: A lack of consensus leads to a lot of confusion. *Clin Infect Dis* 2007;44:391-401.
- Taylor AC, Hill J, Kelly DJ, Davis DR, Lewis GE Jr. A serological survey of scrub, tick, and endemic typhus in Sabah, East Malaysia. *Southeast Asian J Trop Med Public Health* 1986;17:613-9.
- Chanyasanha C, Kittigul L, Puenchitton S, Sangasuwan P. Antibodies titers to rickettsial diseases in blood donor in Bangkok by Indirect immunoperoxidase technique. *Proc Natl Epidemiol Semin Bangkok* 1992;167-9.
- Chanyasanha C, Kaeburong K, Chenchittikul M, Sujirarat D. Seroprevalence of scrub typhus infection in patients with pyrexia at some malaria clinics in three western provinces of Thailand. *Asian Pac J Allergy Immunol* 1998;16:119-25.
- Sharma A, Mahajan S, Gupta ML, Kanga A, Sharma V. Investigation of an outbreak of scrub typhus in the himalayan region of India. *Jpn J Infect Dis* 2005;58:208-10.
- Gurung S, Pradhan J, Bhutia PY. Outbreak of scrub typhus in North Eastern Himalayan region-Sikkim: An emerging threat. *Indian J Med Microbiol* 2013;31:72-4.
- Dass R, Deka NM, Guwarah GS, Barman H, Hoque R, Mili D, *et al.* Characteristics of pediatric scrub typhus during an outbreak in the North Eastern region of India: Peculiarities in clinical presentation, laboratory findings and complications. *Indian J Pediatr* 2011;78:1365-70.
- Palanivel S, Nedunchelian K, Poovazhagi V, Raghunadan R, Ramachandran P. Clinical profile of scrub typhus in children. *Indian J Pediatr* 2012;79:1459-62.
- Kumar M, Krishnamurthy S, Delhi Kumar CG, Narayanan P, Biswal N, Srinivasan S. Scrub typhus in children at a tertiary hospital in southern India: Clinical profile and complications. *J Infect Public Health* 2012;5:82-8.
- Tsay RW, Chang FY. Serious complications in scrub typhus. *J Microbiol Immunol Infect* 1998;31:240-4.
- Kammili N, Swathi A, Devara SM, Anuradha PR. Prevalence of scrub typhus among acute undifferentiated febrile illness cases provisionally diagnosed as Dengue fever. *J Evol Med Dent Sci* 2013;2:2661-64.
- Boorugu H, Dinaker M, Roy ND, Jude JA. Reporting a case of scrub typhus from Andhra Pradesh. *J Assoc Physicians India* 2010;58:519.
- Prabhakaran A, Lal S, Biswas S, Vinoth S, Asraf AS, Mittal V. Serological study of rickettsial diseases in human and rodent population in chittoor dist. (A.P.). *J Commun Dis* 2010;42:209-13.
- Subbalaxmi MV, Chandra N, Teja VD, Lakshmi V, Rao MN, Raju YS. Scrub typhus-experience from a South Indian tertiary care hospital. *BMC Infectious Diseases* 2012 12(Suppl 1):p77.
- Ogawa M, Hagiwara T, Kishimoto T, Shiga S, Yoshida Y, Furuya Y, *et al.* Scrub typhus in Japan: Epidemiology and clinical features of cases reported in 1998. *Am J Trop Med Hyg* 2002;67:162-5.
- Kim DM, Lee YM, Back JH, Yang TY, Lee JH, Song HJ, *et al.* A serosurvey of *Orientia tsutsugamushi* from patients with scrub typhus. *Clin Microbiol Infect* 2010;16:447-51.
- Prakash JA, Abraham OC, Mathai E. Evaluation of tests for serological diagnosis of scrub typhus. *Trop Doct* 2006;36:212-3.

27. Chinprasatsak S, Wilairatana P, Looareesuwan S, Chappuis F, Akkhavong K, Laferl H, *et al.* Evaluation of a newly developed dipstick test for the rapid diagnosis of scrub typhus in febrile patients. *Southeast Asian J Trop Med Public Health* 2001;32:132-6.
28. Jang WJ, Huh MS, Park KH, Choi MS, Kim IS. Evaluation of an immunoglobulin M capture enzyme-linked immunosorbent assay for diagnosis of *Orientia tsutsugamushi* infection. *Clin Diagn Lab Immunol* 2003;10:394-8.

How to cite this article: Ramyasree A, Kalawat U, Rani ND, Chaudhury A. Seroprevalence of Scrub typhus at a tertiary care hospital in Andhra Pradesh. *Indian J Med Microbiol* 2015;33:68-72.

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

