



SERODIAGNOSIS OF SCRUB TYPHUS IN TERTIARY CARE HOSPITAL IN SOUTHERN RAJASTHAN

Microbiology

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ABSTRACT

Background: Scrub typhus is one of the most covert emerging and re-emerging Rickettsial infections. In developing countries, among the various laboratory tests to diagnose scrub typhus, Weil-Felix test is commonly performed despite its low sensitivity.

Objective: The present study was conducted to determine the seropositivity of IgM ELISA for scrub typhus in clinically suspected cases at tertiary care hospital.

Methods: The study was a hospital based prospective study where 1915 clinically suspected cases of scrub typhus were tested by IgM ELISA using commercially available kits.

Results: Out of 1915 sample tested, 633 (33.1%) samples were positive for Scrub typhus by IgM ELISA test. Out of total 633 seropositive samples 222 (35%) patients were male and 411 (65%) were female patients.

Conclusion: Scrub typhus should be included in the differential diagnosis of fever of unknown origin in Southern Part of Rajasthan by using IgM ELISA.

KEYWORDS

Scrub typhus, Seropositivity, IgM ELISA

INTRODUCTION

Rickettsial infections are reemerging 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]

Scrub typhus is a vector born zoonosis caused by *Orientia tsutsugamushi*, an obligate intracellular Gram negative bacterium which was first isolated in 1930. [2] The infection is transmitted by "chiggers" (i.e., the larvae of trombiculid mites of the genus *Leptotrombidium deliense*). The name scrub typhus is derived from the prevalence of mites in areas of heavy scrub vegetation. [3] Though it is rationally considered as disease found in rural areas, it is also well described from urban area like Delhi and Chennai infecting people, as the tiny mite island are seen in the vegetation around the dwellings. Therefore, the disease has been urbanized and the prevalence of the disease has broadened further. [3] The disease is endemic in the geographical region known as "tsutsugamushi triangle" which extends from northern Japan and far eastern Russian the north, to the territories around the Solomon Sea into northern Australia in the south, and to Pakistan and Afghanistan in the west. [1]

Recently, in India there are scrub typhus reports from Goa where the disease was not documented earlier indicating the occurrence of the disease in previously unidentified regions [4]. Also, there are reports from other states of India like Jammu and Kashmir, Himachal Pradesh, Uttaranchal, Rajasthan, West Bengal, Maharashtra, Kerala, Goa, Assam, Tamil Nadu and Pondicherry [4-9]. An estimated one billion people are at risk for scrub typhus and one million cases occur annually [8]. In Tamil Nadu, India a region where scrub typhus is endemic, the disease accounts for 50% of undifferentiated cases of fever presenting to hospital [9].

The early manifestations are an eschar, representing localized cutaneous necrosis at the site of mite feeding (which is not always present) and regional lymphadenopathy followed by fever, headache, myalgia, generalized lymphadenopathy, cough, gastrointestinal symptoms, rashes, transient hearing loss and conjunctival infection [10]. The eschar is seen at the site of chigger bite and is often found inguinal, axilla, genitalia and neck. [11]

Differentiating scrub typhus from other form of rickettsial infection, dengue, leptospirosis, typhoid fever, infectious mononucleosis, malaria and meningococcal infection may be difficult in early stages. The most common symptoms and signs are similar. The clinical features of organ dysfunction may vary depending on severity of illness. [12]

The exact diagnosis is made by Serological tests are main tools for diagnosis. Indirect immune fluorescence assay, latexagglutination, indirect haemagglutination, indirect immune peroxidase assay [IIP], and enzyme linked immune sorbent assay [ELISA] are various serological tests available. [12]

The most common diagnostic test in India is the Weil-Felix test. The test is widely in use as it is cheap, easily available and does not require technical expertise. However, it lacks specificity and sensitivity [13]. The current gold standard for serology is Indirect Immunofluorescence Antibody (IFA) test but it is imperfect as it is expensive requiring a fluorescence microscope. It also requires considerable training for both performing the test and interpretation of the result. The commercially available IFA kits cannot be afforded in resource poor settings. The IFA slide presents antigens from only 3 serotypes namely Karp, Kato and Gilliam, thus unable to detect other antigenically variable strains of *Orientia tsutsugamushi* affecting the sensitivity of the test [14]. In view of the disadvantages of both IFA and Weil-Felix tests, an alternate serological test, Enzyme Linked Immuno Sorbent Assay (ELISA) is currently preferred method. Present study was conducted to know the seropositivity in clinically suspected scrub typhus patients by ELISA.

SUBJECTS AND METHODS

Study design, settings and participants:

It was a hospital based prospective study conducted over a period of two years from October 16 to September 2018 in a tertiary care teaching hospital of Southern Rajasthan.

The patients attending this hospital as both inpatients and outpatients who were clinically diagnosed to have typhus fever were included. The patients who had a history of fever with or without eschar and rash and also had more than 2 symptoms such as head ache, myalgia, malaise, nausea, abdominal discomfort were included irrespective of age and sex were included.

Sera from patients which were positive for any of the following tests like, ELISA for dengue and leptospirosis, QBC for malaria, Widal

tests, Paul Bunnel tests, and IHA for filariasis were excluded from the study.

Specimen collection and processing

About 3-5ml of whole blood was collected in collecting vial without anticoagulant by vein puncture and then sample was left for 30 min. for blood coagulation and then it was centrifuged to get serum. If samples were not tested immediately, then samples were stored at 2-8°C for up to 48 hours or frozen at -20°C or lower for up to 30 days. Samples were brought to room temperature and mixed thoroughly by gentle swirling prior to use.

IgM ELISA for Scrub typhus (*O. tsutsugamushi*)

IgM antibodies detection by ELISA was carried out using commercial kit (In Bios International, Inc., United states) as per the manufacturer's instructions. The test was standardized with serum samples from healthy blood donors. The cut-off value was obtained by calculating the average of optical density (OD) plus three times of standard deviation (SD) from serum samples of healthy individuals. The OD \geq 0.5 were considered as positive. A set of positive and negative controls were included along with every test reaction.

Statistical analysis

Quantitative data was expressed in mean, standard deviation while qualitative data were expressed in percentage.

ETHICAL ISSUES

All participants were explained about the purpose of the study. Confidentiality was assured to them along with informed written consent. The study was approved by the Institutional Ethical Committee.

RESULTS

During the study period total 1915 blood samples were processed for Scrub typhus IgM ELISA test out of which 633 (33.1%) samples were positive by Scrub typhus IgM ELISA test. Out of total 633 seropositive samples 222 (35%) patients were male and 411(65%) were female patients. Age distribution of patients with their positivity for ELISA is shown in Table 1.

DISCUSSION

The present study was a hospital based prospective study conducted to know the seropositivity in clinically suspected scrub typhus patients attending tertiary care teaching hospital in Southern Rajasthan.

Scrub typhus being the re-emerging zoonosis is increasingly recognized in India. The diagnosis of Scrub typhus is generally made by the history and clinical presentation. The common clinical manifestations of the disease which is similar to other febrile illnesses makes the clinical diagnosis challenging. Though the presence of eschar is helpful in making a diagnostic clue for scrub typhus it is not always present. [3]

The disease treatment can be easily affordable with anti-rickettsial drugs, if accurate and precise diagnosis is made which can help in the speedy recovery of the patients. The mortality rate varies from 1% to 40% if left untreated, depending on the endemic area, patients condition and strain virulence of *Orientia tsutsugamushi* [15].

The battery of tests is available like Weil-Felix, Indirect immunofluorescence, 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. 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 sample need to be pooled for testing thus causing delayed diagnosis and treatment, which at time may be fatal. A rapid method which can provide the diagnosis on the same day and require less technical skill can really make a difference in the overall outcome. [1]

In the present study the seropositivity of Scrub typhus (by using the IgM ELISA test) was 33% while Saxena N. et al [16] (2015) reported 18.4% seropositivity from Kota Rajasthan, Sinha P. et al [17] (2014) reported 24.7% seropositivity from Jaipur Rajasthan, Ramyasree A. et al [1] (2015) reported 39% seropositivity from Andhra Pradesh and

Gupta N. et al [18] (2015) reported 14.4% in AIIMS New Delhi. In present study, higher seropositivity rate may be due to the fact that this part of southern Rajasthan is agriculture rich, green and also has rural and tribal area. In the peripheral region more scrub vegetations are present. Most of the persons are farmers and doing work in the fields therefore there are more chances of exposure to mites and rats.

The present study showed that seropositivity of Scrub typhus was higher in Females (65%) than males (35%). This finding was inconsonance with the study from Saxena N. et al [16] (2015) with females 58.33% and males 41.66%, Sinha P. et al [17] (2014) with 66.66% females and 33.33% males in Jaipur and Munilakshmi P. et al [19] (2015) with 58.06% females and 41.94% males in Andhra Pradesh. In rural and tribal area, generally females are doing household works and agricultural works. Females are also engaged in collection of fire woods and look after the cattle more than males that's why there are more chances of accidental exposure of females to the rats and mites and have more chances to get infected with the scrub typhus.

In present study, seropositivity for scrub typhus was higher in the 0-15 years (44.6%) of age group followed by the 31-45 years (38.4%) of age. Khan F. et al [2] (2015) reported most of the cases in 30-39 years of age group in Dehradun India, Munilakshmi P. et al [19] (2015) found that 40-49 years of age group of peoples were more affected in Andhra Pradesh, and Ramyasree A. et al [1] (2015) also reported that 40-49 years of age group were most commonly affected by scrub typhus infection.

CONCLUSION AND RECOMMENDATIONS

Scrub typhus is prevalent but an undiagnosed disease in Southern part of Rajasthan. It should be included in the differential diagnosis of fever of unknown origin along with dengue, malaria which are other endemic disease in Rajasthan. Scrub typhus is a potentially serious and easily treatable infection. Supplementation of serologic tests with sensitive detection of DNA or antigen of *O. tsutsugamushi* in clinical specimen would be appealing as an adjunctive diagnostic approach. An increase in the general awareness of scrub typhus is required to improve the management of illness in febrile patients living in or returning from region in which the disease is endemic. Treatment should be initiated early to reduce mortality and morbidity. The medical fraternity must include tests with better performance like IgM ELISA and nested PCR in the diagnostic algorithm of scrub typhus to reduce the burden of the disease.

Acknowledgement

The authors are grateful to all the participants for their support and contribution.

Table 1: Distribution of samples according to age of patients

Age (yrs)	No. of Tested			No. of Positive (%)		
	Male	Female	Total	Male	Female	Total
0-15	41	42	83	15 (36.6)	22 (52.4)	37 (44.6)
16-30	288	455	743	65 (22.5)	176 (38.7)	241 (32.4)
31-45	181	311	492	62 (34.2)	127 (40.8)	189 (38.4)
46-60	175	222	397	57 (32.6)	63 (28.4)	120 (30.2)
>60	95	105	200	23 (24.2)	23 (21.9)	46 (23)
TOTAL	780	1135	1915	222 (35.0)	411 (65.0)	633

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