Original Article

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Seroprevalence of Scrub Typhus among Pyrexia of Unknown Origin Patients: A Study from Tertiary Care Hospital in Eastern Odisha, India

Microbiology Section

SHREEKANT TIWARI[1], MONALISAH NANDA[2]

ABSTRACT

Introduction: Scrub typhus is a fatal rickettsial infection. It is caused by mite-borne bacterium *Orientia tsutsugamushi* which is transmitted by the bite of mite larvae called chiggers. It usually presents with acute febrile illness. Clinicians usually do not consider it as differential diagnosis as it is still an unknown entity.

Aim: To assess the seroprevalence of scrub typhus among the pyrexia of unknown cases in Eastern part of Odisha, India.

Materials and Methods: This was a cross-sectional study involving 260 serum samples obtained from clinically suspected cases of scrub typhus. Detection of antibodies was done on the samples by Immunochromatography (ICT) and IgM ELISA (Enzyme Linked Immunosorbent Assay). Samples were also processed for dengue fever, malaria, typhoid and leptospirosis which are the other causes of febrile illness in this region and excluded from the study once they come positive. Among these febrile cases which were excluded from study, malaria was predominant (44%) followed by typhoid (34.4%), dengue (15%) and leptospirosis (6.45%). Descriptive statistical analysis was applied to evaluate the results.

Results: Out of 260 clinical samples, 74 (28.46%) were positive by IgM ELISA. There was good correlation (98.6%) between ICT and IgM ELISA. Fever was the most common clinical symptom followed by abdominal pain. Pathognomonic feature such as eschar was seen in one patient only. Scrub typhus should be considered as one of the differential diagnosis of Pyrexia of Unknown Origin (PUO) cases, along with dengue fever, malaria typhoid and leptospirosis.

Conclusion: IgM ELISA is simple, rapid and economical test and should be used as reference diagnostic test for the diagnosis of scrub typhus.

Keywords: Eschar, Immunoglobulin M enzyme linked immunosorbent assay, *Orientia tsutsugamushi,* Rapid test

INTRODUCTION

Scrub typhus is a zoonotic acute febrile illness. It is caused by mite-borne bacterium *Orientia tsutsugamushi* which is transmitted by the bite of mite larvae called chiggers of the *Trombiculidae* family. *Orientia tsutsugamushi* is a gram negative, obligate intracellular slow growing bacteria. Crop fields are an important reservoir for transmission, hence often occurred by occupational or agricultural exposure [1]. It was first observed in 1899, in Japan; the disease was therefore called tsutsugamushi (*tsutsuga*=dangerous, and *mushi*=insect or mite). The term scrub is used because of the type of vegetation that harbours the vector [2]. Scrub typhus is endemic to a geographically distinct region, the term "tsutsugamushi triangle" includes Japan, Taiwan, China and South Korea. In India first case of scrub typhus was reported in 2003 from Southern India [3]. Though widely prevalent in Indian subcontinent, specific prevalence data are not available. Scrub typhus is under diagnosed in India because of lack of awareness and suspicion among clinicians, non-availability of confirmatory laboratory tests and clinical symptoms mimicking to other more prevalent disease such as dengue, malaria, typhoid and leptospirosis [4].

Although scrub typhus is a neglected disease in India, but in recent past, various cases has been reported from Tamil Nadu, Karnataka, Kerala, Maharashtra, Jammu and Kashmir, Uttarakhand, Himachal Pradesh, Rajasthan, Bihar, Assam, West Bengal and Meghalaya [5-15]. Human being gets infected by the bite of larval form of trombiculid mites. It has got four stages in its life cycle. The larval form also known as chiggers, transmit the disease to humans; that's why disease is also known as chigger-borne disease. The characteristic feature of these larval mites is that it leaves a black eschar, that is useful to the clinicians for making diagnosis [16]. This observation is often missed and other sign and symptoms of the disease are not characteristic thus making it a great challenge in timely diagnosis by the clinicians. Failure of timely diagnosis leads to significant morbidity and mortality which varies from 7%-30% (next only to malaria among infectious disease) [17].

There are several tests available with their own advantages and limitations. Indirect immunofluorescence test is considered to be the gold standard but it is costly and needs expertise for the interpretations. Weil-Felix test is the cheapest and easily available but it is an unreliable test. IgM ELISA after evaluation, found to be adequate in comparison to the above mentioned tests but the samples need to be pooled for the ELISA test. Rapid tests (ICT) are economic, quick and can be carried out in single test [18]. Hence, the present study was carried out to assess the seroprevalence of scrub typhus among clinically suspected patients and to compare the diagnostic utility of rapid tests with IgM ELISA for the detection of scrub typhus.

MATERIALS AND METHODS

This was a cross-sectional study carried out in a Tertiary Care Hospital in Eastern Odisha, over a period of four months from June 2019 to September 2019. A written informed consent was obtained from each patient.

Inclusion criteria:

1. Pyrexia for more than five days,
2. Age >18 years,
3. Positive serology for scrub typhus.

Exclusion criteria:

1. Patients with other causes of pyrexia in this region such as malaria, typhoid, dengue and leptospirosis,
2. Negative serology for scrub typhus,
3. If patients refused to participate in the study.

The clearance was taken by the Institutional Ethical Committee of our Institute. Study was carried out on serum samples obtained from clinically suspected cases of scrub typhus and pyrexia of unknown cases [4]. For the detection of IgM antibodies of scrub typhus, ELISA and rapid test was done on the samples. Detection of IgM antibodies by ELISA was done using In Bios International Scrub Typhus Detect™ IgM ELISA [16]. Similarly, detection of IgM antibodies by ICT test was done using SD Bioline *tsutsugamushi* one step Scrub Typhus antibody test kit [16]. Samples were also tested for dengue fever, typhoid fever, malaria and leptospirosis. Clinical features of the patients were also taken from hospital records.

STATISTICAL ANALYSIS

Descriptive statistical analysis was applied to evaluate the results and significant values were obtained.

Percentage of correlation=No. of samples positive by rapid tests/ No. of samples positive by IgM ELISA×100.

RESULTS

Out of 260 samples, 74 (28.46%) were positive by IgM ELISA [Table/Fig-1]. Amongst 74 positive samples, 48 (64.86%) were males and 26 (35.14%) were females. Maximum cases were in middle age group (31-40 years) [Table/Fig-2]. There was 98.6% correlation between IgM ELISA and rapid test. Out of 74 positive cases, all the 74 cases were showing positive result by IgM ELISA but only 73 cases were showing positive result by ICT. One test was positive by IgM ELISA but showing negative result with ICT. Fever (100%) was the most common symptom followed by abdominal pain (89.1%) and headache (56.7%) [Table/Fig-3]. Eschar was seen in 1 (1.35%) patient only. Maximum numbers of cases were seen in rainy season [Table/Fig-4]. Sensitivity of ICT was 99% and specificity 96%. Similarly for IgM ELISA sensitivity was 100% and specificity 99%.

Tests	Number of positive cases (%)	Correlation between IgM ELISA and ICT
IgM ELISA	74 (28.46)	98.6%
ICT	73 (28)	

[Table/Fig-1]: Serodiagnosis of Scrub typhus by different tests (n=260).

Age group (in years)	Positive cases	Percentage (%)
18-30	17	22.97
31-40	22	29.73
41-50	15	20.27
51-60	9	12.17
>60	11	14.86

[Table/Fig-2]: Age wise distribution of Scrub typhus patients (n=74).

Clinical features	Number of patients n=74 (%)
Fever	74 (100%)
Nausea/Vomiting	39 (52.7%)
Headache	42 (56.7%)
Cough	36 (48.6%)
Skin rash	26 (35.1%)
Pain abdomen	66 (89.1%)
Myalgia	29 (39.1%)
Diarrhoea	11 (14.86%)
Hepatosplenomegaly	30 (40.5%)
Eschar	1 (1.35%)
Lymphadenopathy	12 (16.2%)
Convulsion	6 (8.1%)

[Table/Fig-3]: Clinical features of the patients and their percentage.

Months	Number of cases	Percentage
June	10	13.51
July	28	37.84
August	21	28.38
September	15	20.27

[Table/Fig-4]: Months wise distribution of scrub typhus cases (n=74).

DISCUSSION

Scrub typhus is the most common rickettsial infection in the Indian subcontinents. It is a zoonotic disease and an important cause of PUO. These infections are threat to public health if not diagnosed in time. Evidence of exposure to vector, knowledge of geographical distribution and clinical features such as fever, headache, myalgia, rash, eschar along with high index of suspicion are crucial factors for early diagnosis [19].

The prevalence of scrub typhus varies from 8 to 60% in different countries [20]. In present study 260 samples were tested, out of which 74 (28.46%) were positive, which was similar to study done by Gurung S et al., where 30.8% samples were positive [21]. Of these 74 positive patients, 48 (64.86%) were males and 26 (35.14%) were females. Highest seropositivity was seen in middle age (31-40 years) group. Fever (100%) was the most common symptoms in this study which was similar to study done by Ramyasree A et al., from Andhra Pradesh, India [16]. Hepatosplenomegaly (40.5%) were other major clinical presentation in this study which makes it something different from others [7,16,19]. The salient differentiating feature of scrub typhus from other causes of infective gastrointestinal involvement is anicteric hepatitis [10]. The clinical manifestations of the disease vary from minimal disease to severe fatal illness. People working outdoors tend to be affected more often. In this study, the patients also presented with similar clinical manifestations and these have been reported in another study from Indian subcontinents [6].

Eschar is a tan, brown or blackish necrotic lesion resembling cigarette burn, commonly seen in areas where skin is thin and moist. Presence of eschar at the site of bite of larval mite is considered pathognomonic of scrub typhus. The eschar forms within few days (median five days) after the bite, and may take several weeks to heal completely. Presence of scar varies in different studies [22]. Its presence confirms the diagnosis of scrub typhus but its absence does not exclude the possibility of scrub typhus. It can be readily seen in fair skinned people as compared to dark-skinned patients [4].

Kamarasu K et al., from Vellore and Abdul Kader JK from Kerala and Ramyasree A et al., from Andhra Pradesh, could not find a single case of eschar in scrub typhus cases [5,7,16]. Eschar was reported 4-12% by different Indian studies whereas incidence was higher in studies from Vietnam, Taiwan and Korea [23-25], most probably due to fair skinned population of these studies which increases the chance of finding eschar. Premaratna R et al., also commented that early or very small eschar could be easily overlooked in dark skinned patients [26]. The prevalence of rash has been reported varying from 9 to 90% by different authors [27] whereas in this study it was 35%. The seasonal variation of scrub typhus varies with the climate of the particular place. In India peak incidence of scrub typhus was observed during the highest rainfall. In the present study also maximum cases were observed in the month of July followed by August and September [Table/Fig-5] [4,5,16,28,29]. This may be due to breading of mites during rainy days.

Due to lack of diagnostic facilities in India, low index of suspicion, absence of specific presentation of an eschar and presence of non-specific sign and symptoms, the diagnosis of scrub typhus poses a great challenge. The available tests have their own limitations (WeilFelix, Indirect immunofluorescence, PCR, ICT and ELISA). In resource setting countries like India, some of these diagnostic tests

Autor, place of study and year of Publication	Kamarasu K et al., Tamil Nadu, 2007 [5]	Mittal G et al., North India, 2015 [28]	Ramyasree A et al., Andhra Pradesh, 2015 [16]	Jyothi R et al., Kerala, 2015 [29]	Takhar RP et al., Rajasthan, 2017 [4]	Current study, Odisha, 2020
Seroprevalence (%)	37.5	14.42	39	17.11	22.8	28.46
Seasonal preponderance	Aug-Jan	Aug-Oct	Not given	Sep-Dec	July-Oct	July-Sep

[Table/Fig-5]: Seasonal trends and seroprevalence of scrub typhus in different studies from India [4,5,16,28,29].

which provide accurate and specific diagnosis like PCR and indirect immunofluorescence are either not available or they are much expensive. Weil-Felix is a commonly used serological test but it has got low sensitivity and specificity [30]. Now, there is need of a simple, rapid and economical test. ELISA is an example of such test but its result may not be available on the same day because it requires pooled samples to be tested. This causes delayed in diagnosis and treatment which sometimes may be fatal.

In present study rapid test (ICT) was compared with IgM ELISA for the diagnosis of scrub typhus. Prakash JA et al., evaluated Weil-Felix, IgM ELISA and Dot Enzyme Immunoassay (EIA) for the diagnosis of scrub typhus and reported sensitivity of 43.5%, 86.5% and 100% in the tests respectively [31]. Dot EIA and IgM ELISA showed false positive reactions in patients with malaria, pulmonary tuberculosis, typhoid fever and septicaemia [31,27]. Mund K et al., compared IgM ELISA with rapid test for the diagnosis of scrub typhus and found a good correlation between the result of rapid test and IgM ELISA [2]. In their study out of 253 positive samples 7 (1%) samples negative by rapid test were positive by IgM ELISA. Gurung S et al., used rapid test and IgM ELISA for the diagnosis of scrub typhus and found one sample was positive by rapid method and the same sample found negative by ELISA [21]. In the present study IgM ELISA and rapid test (ICT) were used for the diagnosis of scrub typhus. There was 98.6% correlation between these two tests. One sample (Out of 74) positive by ICT but the same sample was negative by ELISA. The above studies indicated the advantage of ELISA over rapid tests. Therefore, it can be concluded that diagnostic centres should use ELISA test but with less number of samples and for early diagnosis of scrub typhus, rapid method may be used.

Diagnosis of scrub typhus is difficult in India because of its varied clinical presentation and absence of characteristic feature i.e. eschar in many patients as eschar is considered to be pathognomonic of the disease but its absence does not exclude the possibility of the infection. It is recommended that the study should be based on large number of samples which may throw better light on the seroprevalence and clinical presentation of scrub typhus.

Limitation(s)

Single serum specimen was collected for the detection of IgM antibodies. An antibody titre in paired sera for detection in the rise of antibodies could not be detected. Studies with large samples which may give a better idea about seroprevalence and clinical presentations of scrub typhus can be taken up further.

CONCLUSION(S)

Scrub typhus should be included in the differential diagnosis of PUO along with the other infections like dengue fever, malaria and leptospirosis, which are endemic specially in India. This will help in proper and timely management of the cases and avoid unnecessary complications which are associated with high mortality. Present study concluded that all cases of PUO should be screened for scrub typhus by ICT and the positive results can be confirmed by IgM ELISA.

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PARTICULARS OF CONTRIBUTORS:
1. Professor and Head, Department of Microbiology, Hi-Tech Medical College and Hospital, Rourkela, Odisha, India.
2. Associate Professor, Department of Skin and V.D, S.C.B. Medical College and Hospital, Odisha, Cuttack, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Shreekant Tiwari,
Quarter No. 5R/1, S.C.B. Campus, Multistoried Building, Ranihat Medical Road,
Cuttack, Odisha, India.
E-mail: drshreekant@rediffmail.com

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