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Original Article

Microbiology Section

Seroprevalence of Scrub Typhus among Febrile Patients from a Tertiary Care Hospital in Kerala

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ABSTRACT

Introduction: Scrub typhus or bush typhus is a form of typhus caused by the intracellular parasite *Orientia tsutsugamushi*. As the clinical features include fever, headache, vomiting, respiratory infections and rashes, this disease presents like typhoid, dengue, leptospirosis or malaria. Though Weil–Felix test is conventional, cheap and easy to perform, but IgM ELISA is a more sensitive test. There is no published data on this topic from this region.

Aim: To determine the seroprevalence of scrub typhus among febrile patients using Weil-Felix and IgM ELISA tests.

Materials and Methods: A cross-sectional study was carried out at the Government Medical College Hospital Thrissur, between the period of July 2015 to March 2016. Total 285 cases with febrile illness were screened for scrub typhus. Commercially available kits of Weil-Felix and IgM ELISA were used.

Results: Out of 285 samples 54 (18.95%) were positive by either Weil–Felix or Ig M ELISA or both. 21-30 years age groups showed maximum cases during the monsoon and post monsoon period and there was no gender difference. Rise in titer of OX2 and OX19 was also seen showing the presence of rickettsial disease other than scrub typhus.

Conclusion: Scrub typhus fever should be considered as one of the differential diagnosis of fever of unknown origin in this area.

Keywords: Chigger-borne disease, Eschar, IgM ELISA, *Orientia tsustsugamushi*, Weil–Felix

INTRODUCTION

Scrub typhus is a type of Rickettsial infection caused by a slow growing bacterium, *Orientia tsutsugamushi*. It is transmitted by the bite of larval form of trombiculid mites and is seen in the area bounded by Japan, Northern Australia, and the Arabian Peninsula [1]. The name is derived from the prevalence of the mites in areas of heavy scrub vegetation. The trombiculid mite has four life cycle stages; the larval forms or chiggers transmit the disease to humans accidentally following bite, so the disease is also known as 'Chigger-Borne disease' [2]. It is seen that around one million cases of scrub typhus occur every year resulting in more deaths than dengue fever if not treated properly [3]. Even though we are not much aware of this disease in India, there are reports from different states including Kerala in recent years [4-11]. There is no published data on this topic from this region as per our knowledge.

The incubation period of scrub typhus is 1-3 weeks and patients usually present with fever, eschar, headache, vomiting, myalgia, lymphadenopathy and maculopapular rash. The differential diagnosis of scrub typhus in our setting includes enteric fever, dengue, leptospirosis and malaria [5]. Even though the presence of an eschar is of diagnostic importance, it is not seen commonly [4]. As the mainstay in scrub-typhus diagnosis serology remains, the oldest test in current use is the Weil–Felix OX-K agglutination reaction, which is cheap, easy to perform, results are available overnight and specificity is up to 100%; but, it lacks sensitivity [1,8]. Even though the indirect immunofluorescence assay (IFA) is more sensitive and considered as the gold standard reference diagnostic method, it requires a level of technical expertise and more expensive equipment [3]. This test detects specific antibody from patient's serum by using fluorescent anti-human antibody [1,3]. Culture of this aetiological agent is difficult in rural areas as it requires BSL-III facility. Owing to the disadvantages of Weil-Felix and IFA, ELISA which detects IgM antibodies to *O. tsutsugamushi* in serum, is the currently used preferred method and satisfactory in comparison to gold standard [5]. It has a good sensitivity and specificity. PCR, although highly sensitive and specific, is an expensive procedure and make it impractical in developing countries [3]. In this study we used Weil–Felix and IgM ELISA tests to detect the presence of scrub typhus in the central part of Kerala.

MATERIALS AND METHODS

This was a cross-sectional study carried out on serum

samples of 285 patients who presented with acute febrile illness over a period of nine months extending from July 2015 to March 2016 in the Government Medical College Thrissur, Kerala.

The minimum sample size was calculated as 240, anticipating a prevalence rate of 39% based on previous studies [4]. The patients were in the age group of 1-80 years. Out of 285, 168 were males and 117 females. . The samples were subjected to the detection of IgM antibodies by ELISA and Weil–Felix tests for the diagnosis of scrub typhus [2]. The samples that reported negative for dengue, leptospirosis, malaria or enteric fever were excluded from the study. The patients were diagnosed to have scrub typhus if the serum was positive for Weil-Felix OX K or IgM ELISA or both. Approval from Institution Ethics committee was obtained.

The Qualitative detection of IgM antibodies to *O. tsutsugamushi* derived recombinant antigen by ELISA was done using InBios International TM IgM ELISA kit. The Weil-Felix assay was done by using antigenic kits manufactured by FAR srl. A doubling dilution of 1:20 to 1:320 was used and a titer ≥ 1:80 or four fold rise in titer was considered positive. All the tests were done according to the manufacturer's guidelines. Descriptive statistics was applied to evaluate the results.

RESULTS

Out of 285, 54(18.95%) serum samples were positive either by ELISA or Weil-Felix method of which 31(57%) were males and 23(43%) females [Table/Fig-1]. 15 cases (28%) were in the young age group [Table/Fig-2]. Headache was the common clinical feature seen in febrile patients (46.3%) followed by oliguria (40.7%), myalgia (39%), respiratory infections (31.4%) and rashes (20.3%). Eschar was seen in a single patient only [Table/Fig-3]. We got more cases in October-January period [Table/Fig-4]. Another important finding in our study was positive titer of OX19 (3 cases) and OX2 (1 case) antibodies.

Sex	No. of positives (%)
Male	31 (57%)
Female	23 (43%)
Total	54 (100%)

[Table/Fig-1]: Sex wise distribution of scrub typhus patients.

Age group	Positive cases	Percentage (%)
1-12	9	17
13-20	4	7
21-30	15	28
31-40	8	15
41-50	6	11
>50	12	22

[Table/Fig-2]: Age wise distribution of scrub typhus patients.

Clinical feature	No. of patients	Percentage (%)
Headache	25	46.3
Oliguria	22	40.7
Myalgia	21	39
Resp.infections	17	31.4
Rashes	11	20.3
Vomiting	10	18.5
Lymph nodes	9	16.6
Conjunctival congestion	7	13
Eschar	1	1.85

[Table/Fig-3]: Clinical presentations and the percentage of patients with scrub typhus.

Months	No. of cases	Percentage (%)
July	9	16.6
August	2	3.7
September	4	7.4
October	13	24
November	7	13
December	8	15
January	8	15
February	1	1.8
March	2	3.7

[Table/Fig-4]: Month wise distribution of scrub typhus.

DISCUSSION

Out of 285 febrile patients that were screened 54 were positive for scrub typhus. So the prevalence with 95% C.I. was 18.95(14.5 ±24). The prevalence of scrub typhus varies up to 60% in different countries [1]. It was found that the prevalence in the Andhra Pradesh was 56% and 39% in the year 2011 and 2013 [4,8] . But from the South part of Kerala, Thiruvananthapuram Medical College Hospital, out of 1268 patients, 217(17.1%) were positive for scrub typhus [5]. The two neighboring states of Kerala, Puducherry and Tamil Nadu, have a higher rate of prevalence, 24% and 37.5% respectively [11,12]. When Garima Mittal et al., studied the aetiologies of acute undifferentiated febrile illness in 2547 adult patients in Dehradun, it was found that 14.42% were diagnosed to have scrub typhus [13]. From the two data available from Kerala including this study, we could find that prevalence of scrub typhus is low when compared to the nearby states like Andhra Pradesh, Tamil Nadu and Puducherry [4,5,8,11,12]. But this disease should be included in the differential diagnosis of a case of fever of unknown origin.

In our study 57% of the patients were males and 43% females [Table/Fig-1]. The gender difference was not significant. The age profile data suggested maximum positive cases (28%)

	R. Jyothi et al., 2015 [5]	K. Usha et al., 2011 [8]	K. Kamarasu et al., 2004 [12]	V. Rajagopal et al., 2014 [14]	Ramyasree et al., 2013 [4]	G. Mittal et al., 2012 [13]	Current Study
Prevalence (%)	17.11	56.42	37.5	8.8	39	14.42	18.95
Age Group	40-60 years	25-65 years	>14 years	16-25 years	16-30 years	Not given	21-30 years
Common Symptoms	Headache & vomiting	Vomiting & Headache	Headache & Myalgia	Myalgia	Vomiting & Headache	Icterus	Headache & Oliguria
Seasonal Preponderence	Sept- Dec	Oct- Feb	Aug- Jan	Not given	Not given	Aug-Oct	Oct-Jan

[Table/Fig-5]: Data from different studies about scrub typhus - India.

in 21-30 years age group followed by 22% in >50 age group [Table/Fig-2]. In a study by V Rajagopal et al., (Vellore) the highest percentage was in 16-25 years [14]. In studies from Thiruvananthapuram and Shandong province of China showed more cases in the 40 above age group [5,15]. Ragini et al., found maximum cases of 31-40 age group in a study on febrile illness in Uttharakhand [6]. In our study younger age groups were most affected. Youths were exposed to the bites of mites and ticks due to their occupational exposure to the scrub vegetation.

Headache was the most common clinical feature of scrub typhus in this area (46.3%), followed by oliguria, myalgia, respiratory infections and rashes. Eschar was seen in a single patient only [Table/Fig-3]. Headache and vomiting were common symptoms found in a study from North Eastern region of India [16]. Vomiting, myalgia and respiratory infections were commonly reported from many studies including different states of India and Northern China [Table/Fig-5]. Even though eschar was identified as an important sign of scrub typhus, we could find it in only a single patient. Ramyasree et al., from Andhra Pradesh and Kamarasu et al., from Vellore could not find a single case of eschar in scrub typhus cases [4,12]. V. Rajgopal et al., (Vellore) did not see a characteristic eschar in a single case when they studied 80 cases of scrub typhus [14]. Since, eschar is very small and without any abnormal sensation, it is difficult to detect especially in dark skinned people [8,15,17].

In this study 13/54 (24%) of cases of scrub typhus reported in the month of October [Table/Fig-4]. Scrub typhus cases were maximum in monsoon and post monsoon days than summer days. Studies from Thiruvavnathapuram Medical College (Kerala), Tamil Nadu and Uttarakhand found more cases during September to December and maximum cases in October [5,6,12]. Garima Mittal et al., (Dehradun) and Vivek Kumar et al., (Chandigarh) found the maximum occurrence of acute undifferentiated febrile illness including scrub typhus following rainy season [13,18]. This may be due to breading of mites during rainy days.

Another important finding in our study was the presence of rise in titer of Weil–Felix OX2 antibody in three cases and OX 19 antibody in one case. This suggests the presence of Indian tick typhus and Epidemic typhus in this area. In a study from Tirupathi, eight cases were positive for OX2 and four cases for OX19 when tested 280 healthy volunteers [8]. Rickettsial infections are emerging in our area. All the cases of fever of unknown origin should be checked for OXK, OX2 and OX19 along with other conditions like dengue, leptospirosis, typhoid fever or malaria.

LIMITATION

We could not perform Ig M ELISA for the rickettsial disease other than scrub typhus due to financial limitation.

CONCLUSION

Rickettsial diseases, especially scrub typhus should be included in the differential diagnosis of fever of unknown origin in this area. By doing the Weil–Felix test alone we get preliminary information about the rickettsial infections which can be established by other methods like IgM ELISA.

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