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Original Article

Microbiology Section

Serodiagnosis of Scrub Typhus at a Tertiary Care Hospital from Southern India

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ABSTRACT

Introduction: Scrub typhus, a zoonotic disease is one of the most covert emerging and re-emerging Rickettsial infections. There is an upsurge in the incidence of the disease worldwide with ever-changing habitat. Clinical diagnosis of scrub typhus is challenging as the signs and symptoms of scrub typhus are similar to other febrile illnesses. In developing countries, among the various laboratory tests to diagnose scrub typhus, Weil-Felix test is commonly performed despite its low sensitivity. The Immunofluorescence antibody (IFA) test has its limitations in terms of cost and expertise required. The present study was conducted to determine the seropositivity of IgM ELISA for scrub typhus in clinically suspected cases.

Materials and Methods: Weil-Felix test and IgM ELISA were performed using clinically suspected cases of scrub typhus using commercially available kits.

Results: Out of 482 samples tested, 109 were positive by both Weil-Felix test and IgM ELISA. One hundred and sixteen samples which were negative by Weil-Felix test reacted positive by IgM ELISA. Fourteen samples which were positive by Weil-Felix test were negative by ELISA.

Conclusion: Owing to the limitations of the Weil-Felix test and IFA, commercially available recombinant IgM ELISA which has a good sensitivity and specificity may be an alternative in laboratories with moderate set up.

Keywords: IgM ELISA, Weil-Felix test, *Orientia tsutsugamushi*

INTRODUCTION

Scrub typhus has been one of the most covert emerging and re-emerging Rickettsial infections with increasing trend in incidences of the disease worldwide including India. The tropical febrile vector borne disease also known as "tsutsugamushi disease" is caused by the organism *Orientia tsutsugamushi*, a gram negative obligate intracellular slow growing bacteria. The infection is transmitted by bite of larval stage (chiggers) of mites belonging to the family *Trombiculidae*. The mite acts as both vector and reservoir of the bacteria and efficiently transmits to its off springs through transovarian transmission. The disease is also transmitted from larval stage of mites to rats, where man is an accidental host.

The name scrub typhus is derived from the prevalence of the mites in areas of heavy scrub vegetation. Though it is rationally considered as disease found in rural areas, it is also well described from urban areas like Delhi and Chennai infecting people, as the tiny mite islands are seen in the vegetation around the dwellings. Therefore the disease has been urbanised and the prevalence of the disease has broadened further [1]. Scrub typhus is widespread, extending from Japan to Australia and from India to Pacific [2]. 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 [3]. Also, there are reports from other states of India like Jammu and Kashmir, Himachal Pradesh, Uttaranchal, Rajasthan, Sikkim, Goa, Assam, West Bengal, Maharashtra, Kerala, Tamil Nadu and Pondicherry [3-11]. An estimated one billion people are at risk for scrub typhus and one million cases occur annually [12]. In Tamil Nadu, India a region where scrub typhus is endemic, the disease accounts for 50% of undifferentiated cases of fever presenting to hospital [13].

The disease is usually underdiagnosed in India due to the non-specific clinical manifestations, lack of access to specific and sensitive diagnostic tests in most places and low index of suspicion among the clinicians.

The clinical symptoms are fever, headache, myalgia, malaise, rash and lymphadenopathy which are commonly seen in other acute febrile illness like malaria, enteric fever, leptospirosis, dengue etc. making the clinical diagnosis tough. The pathognomonic clinical sign is "eschar" (cigarette burn like appearance) which is a skin lesion at the site of mite bite and is inconspicuous as it is often present in the genital region and may go unnoticed until looked warily especially in the dark skinned people. Though presence of an eschar alone is sufficient to clinically distinguish scrub typhus from other febrile illness, its presence is highly variable ranging from 10% to 90% [14]. The severity of illness vary from mild to severe with multiorgan system involvement and cause deaths if not treated accurately at the early stage of illness, otherwise the treatment is affordable and mostly successful with dramatic clinical response to anti-rickettsial drugs within 48 hours.

The culturing of the organism is not only inappropriate as the time duration of the same is not effective enough for the early diagnosis of scrub typhus. The organism is also extremely dangerous requiring BSL–III facility for its culture which is impractical in most laboratories in India [15]. 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 [15]. The current gold standard for serology is Indirect Immunofluorescence Antibody (IFA) test which uses a fluorescence labelled anti-human immunoglobulin to detect antibodies in patients serum that have bound to immobilised bacterial antigen that is coated on the IFA slide. The current gold standard 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. In this study an attempt has been made to know the seropositivity in clinically suspected scrub typhus patients.

MATERIALS AND METHODS

It is a prospective study which was carried out between January 2012 to June 2015 (3 years 6 months), in a tertiary care hospital in Puducherry, South India. The approval for the study was taken from JIPMER scientific advisory committee and JIPMER Institute Ethics committee. The patients attending this hospital as both inpatients and outpatients who were clinically diagnosed to have typhus fever were included. The patients age varied from 10 months to 80 years were taken for the study. 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.

Sera from patients which were positive for any of the following tests like Widal tests, Paul Bunnel tests, ELISA for dengue and leptospirosis, QBC for malaria and IHA for filariasis were excluded.

Specimen collection and processing

The blood samples were collected from all febrile patients who visited JIPMER hospital during the study period and were clinically suspected as typhus fever. Five millilitre of venous blood sample was collected in plain tube from each patient for both Weil-Felix test and IgM ELISA. The study was conducted only after obtaining written informed consent from the patients.

Weil-Felix tube agglutination test

The test was performed for all samples using the commercial kit (Plasmatech, UK) as per the manufacturer's instructions. Agglutination titres of ≥160 to OX K antigen were considered as positive for scrub typhus.

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

Detection of IgM antibodies by ELISA was carried out using commercial kit (In Bios International, Inc., United states) as per the manufacturer's instructions. The test was standardised 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≥ 0.5 were considered as positive. A set of positive and negative controls were included along with every test reaction.

RESULTS

After screening 545 patients with acute febrile illness with a battery of diagnostic tests such as Para F for malaria, Widal tests, ICT for dengue and Ig M ELISA for leptospirosis, 63 samples reacted positively for above mentioned tests and were excluded. Out of 482 samples tested for scrub typhus, 109 (23%) were positive by both Weil-Felix test and ELISA. One hundred and sixteen samples (24%) showed negative by Weil-Felix test but positive by ELISA. Fourteen samples (3%) that showed positive by Weil-Felix test were negative by ELISA. Negative by both the tests were observed in 243(50%) samples. Among 482 samples 270 (56%) were from male patients and 212 (44%) from female patients. The results of Weil-Felix test and IgM ELISA for scrub typhus is shown in [Table/Fig-1]. Age distribution of patients with their positivity for ELISA is shown in [Table/Fig-2].

	Weil-Felix test (reactive to OX K antigen)	Weil-Felix test (non-reactive to OX K antigen)
Reactive to IgM ELISA	109	116
Non-reactive to IgM ELISA	14	243
Total no. of samples	482	

[Table/Fig-1]: Results of Weil-Felix tests and IgM ELISA in scrub typhus diagnosis (n=482)

Age (yr.)	No.tested			No. (%) positive		
	Male	Female	Total	Male	Female	Total
0-15	104	72	176	66 (63)	42(58)	108(61)
16 - 30	30	39	69	9(30)	21(53)	30(44)
31 - 45	87	77	164	22(25)	30(39)	52(32)
46 - 60	40	20	60	18(45)	11(55)	29(48)
>60	9	4	13	4(44)	2(50)	6(46)
Total	270	212	482	119(44)	106(50)	225(47)

[Table/Fig-2]: IgM ELISA positivity for scrub typhus among clinically suspected cases

DISCUSSION

Scrub typhus being the re-emerging zoonosis is increasingly recognised in India. The diagnosis of scrub typhus is generally made by the history and clinical presentation. The vast variability and 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. The preferential site of mite bite where the eschar is formed mostly in the intertriginous surfaces (axilla, scrotum, perianal region) which can be easily overlooked by the doctors [16].

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. With either delay in diagnosis or administration of inappropriate antimicrobial therapy can lead to severe complications such as Acute Respiratory Distress Syndrome (ARDS), septic shock and multisystem organ failure often causing death in 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* [17].

The most widely performed laboratory test in India is Weil-Felix test which has low sensitivity and specificity. The test shows false negative results in the early stage of disease as the agglutinating antibodies can be detected only in the second week of illness [18]. IFA being the gold standard for serology of scrub typhus is considered as imperfect with its own limitations [15]. The IgM ELISA with good sensitivity and specificity, ease to perform, swift results and also suitable for testing large number of specimens may be considered as good replacement for Weil-Felix test and IFA test in diagnosis of scrub typhus. In the 2008, a study carried out in JIPMER by Prabagaravarthanan et al., 6 out of 29 clinically suspected cases were tested positive by Weil-Felix test and were successfully treated with doxycycline or chloromycetin [8].

Out of a total of 44 patients with undiagnosed fever, 15 (34%) showed positive by IgM ELISA and male preponderance was observed with 34 (77%) cases being positive [3]. Out of 204 samples with pyrexia of unknown origin, scrub typhus was positive for 51 patients with a titre of >320 against OX K antigen and IgM ELISA were positive for 63 patients [5]. In a study by Sharma et al., 52 cases were found positive for OX K antigen by Weil-Felix test among 150 PUO cases [10]. A study carried out by Kamarasu et al., showed that 38% (115 samples) of positivity by Weil-Felix test. Out of a total 306, male preponderance was observed with 70% being males. In 2005, same group conducted a study in 964 patients, out of which 9% (89) of the samples were reactive by Weil-Felix test [6]. A study carried out in Rajasthan showed 49% (133/271) of the patients sample were reactive by IgM ELISA for scrub typhus [18]. In a study by Prakash et al., sensitivity of 44% and 87% were observed with Weil-Felix test and IgM ELISA respectively [19]. In 1993, Kim et al., developed recombinant antigen for IgM ELISA detection by using 56 kDa polypeptide which showed sensitivity and specificity of 97% and 100% respectively as compared to Immunofluorescence test [20]. A study was conducted in Delhi among the febrile paediatric patients which did not have definitive diagnosis. The serum samples were tested for antibodies to *Orientia tsutsugamushi* employing Immunochromatographic tests and IgM ELISA. Ten percentage (3/30) seropositivity to both the tests were observed [1].

In the present study, 47% of the patients were reactive by IgM ELISA for scrub typhus whereas Weil-Felix test against OX K antigen showed positivity for 26%. Twenty four percentage of the patient's sample which showed positive by IgM ELISA was non-reactive by Weil-Felix test.

The classic serologic diagnosis of Rickettsial diseases is based on a ≥4-fold rise in the titre between paired acute and convalescent sera determined by a specific test [21]. In our study single time point estimation is done, which is a major drawback. The drawback of serology test is that the advantage of testing directly the presence of organism is not possible [14]. All serological tests are retrospective where diagnosis is done at the end of one week. Therefore it is recommended to perform both pathogen and antibody based tests.

Molecular tests like nested PCR for diagnosis acts as a viable, early and accurate diagnostic tool as compared with that of serological tests. Nested PCR for detection of 56 kDa gene specific for *Orientia tsutsugamushi* with good sensitivity of and specificity may be recommended in laboratories with molecular lab facility [14]. In a previous study carried out in this centre, nested PCR was performed for 406 clinically suspected cases of scrub typhus, out of which 91(22%) were found to be positive by nested PCR targeting 56kDa gene which is specific for *Orientia tsutsugamushi* whereas only 51(13%) cases were positive by Weil-Felix test against OX-K antigen [22]. This again emphasises the fact that Weil-Felix test is not a sensitive test for diagnosis of scrub typhus. This is hospital based study which has its own limitations, therefore future research can be carried out with community-based study to get more data on seroprevalance of scrub typhus in Puducherry.

CONCLUSION

The medical fraternity needs to understand the pitfalls of currently available Weil-Felix test which is still common in India and 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.

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