DOI: 10.7860/NJLM/2021/45486:2458

Original Article

Microbiology Section

Seroprevalence and Clinical Profile of Scrub Typhus in Children in a Rural Tertiary Care Hospital, Tamil Nadu, India

R DEEPA[1], A VASUMATHI[2], S VASANTHI[3], M ARAVIND[4]

ABSTRACT

Introduction: Rickettsial disease in children has shown an increasing trend in several parts of India over recent years. The clinical features and seroprevalence vary in different geographic areas.

Aim: To determine the seroprevalence and clinical features of Scrub typhus in paediatric patients.

Materials and Methods: The prospective study was conducted for a period of six months included all paediatric patients <12 years of age with suspected scrub typhus infection. Serodiagnosis was done by performing Weil Felix (WF) on all serum samples. Clinical examination findings and other investigations were recorded from the medical case records. Statistical analysis of data was performed with Microsoft Excel. The significance of demographic and clinical features was determined by calculation of p-value by Fisher's-exact test using Statistical Package for the Social Sciences (SPSS) software version 17.0.

Results: Among 152 children satisfying the inclusion criteria, 60 (39.5%) had a titer ≥1:80 with OXK antigens in WF test and 92 children (60.5%) had a titre less than 1:80. Scrub typhus IgM Enzyme-linked immunosorbent assay (ELISA) was positive in 69 children (45.4%) and the rest were negative {n=83 (54.6%)} The sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and accuracy of WF as compared to the Scrub typhus IgM ELISA were 71.01%, 86.75%, 81.67%, 78.26% and 79.61%, respectively. The commonest age group of children with scrub typhus was 1-4 years {n=30 (43.5%)}. Fifty three children (76.8%) presented with high grade and intermittent fever. The commonest clinical signs were hepatomegaly {n=51 (73.9%)} splenomegaly {n=37 (53.6 %)} and eschar {n=21 (30.4%)}. The most common laboratory findings were anaemia {n=44 (63.8%)} and thrombocytopenia {n=21 (30.4%)}.

Conclusion: The seroprevalence of Scrub typhus in paediatric population was 45.4% (n=69). The eschar was present in 30% of the study group (n=21) and the most common clinical presentation was high grade intermittent fever with hepatosplenomegaly which are seen in other tropical infections as well. The laboratory plays a pivotal role with serodiagnosis by IgM ELISA, a necessity in confirming the diagnosis.

Keywords: 56 kDa Antigen, *Orientia tsutsugamushi*, Paediatric population, Rickettsial disease, Weil-Felix test

INTRODUCTION

Rickettsial diseases are a major group of zoonotic illnesses which have re-emerged in India and are often unrecognised cause of febrile illness [1]. Among these, Scrub typhus, caused by *Orientia tsutsugamushi* has become a widely prevalent rickettsial infection in many parts of South India. The burden of rickettsial diseases is high in children with a higher complication rate [2]. The genus *Orientia* belongs to the family Rickettsiaceae which comprises of small, non-flagellate, obligate intracellular gram negative pleomorphic coccobacilli transmitted by arthropod vectors. They infect the reticuloendothelial cells and vascular endothelium in the vertebrate hosts including humans. The bacteria are transmitted to humans by some species of larval trombiculid mites called as chiggers. The mites also carry the bacterium through transtadial and transovarial transmission. Rodents and shrews act as natural hosts [3].

These infections are incapacitating and difficult to diagnose; with case fatalities up to 30-45% in untreated cases due to multiple organ dysfunction. The variable and non-specific presentation often makes it difficult to diagnose clinically. Recognising the need of physicians at all levels of health care in our country, the task force constituted by the Indian Council of Medical Research and the Indian Academy of Paediatrics (IAP) published guidelines for diagnosis and management of Rickettsial diseases in India [4]. Scrub typhus is now recognised as a cause of febrile illness in Puducherry and bordering areas of Tamil Nadu. Confirmed cases are reported every year, especially during the cooler months (October-January) [5]. A significantly higher chigger index was reported in these areas especially during the cooler months as compared to other eco-epidemiological settings such as Meghalaya, Kerala and Kolkata. Other tertiary care centers have reported cases of paediatric Scrub typhus referred from Villupuram and Cuddalore districts of Tamilnadu [6]. In the past decade, the rural agrarian population of Villupuram and neighbouring districts has been seeking health care in the tertiary care hospital due to the easy accessibility of the hospital conveniently located on the National Highway. Therefore, this study was undertaken to obtain data which will reflect the burden of the disease among children seeking health care in Villupuram district. The aim was to study the prevalence of Scrub typhus among paediatric patients by determining the IgG antibodies by Weil-Felix (WF) test and Enzyme-Linked Immunosorbent Assay (ELISA) and to analyse the clinical and demographic profile of children with serologically confirmed Scrub typhus infection.

MATERIALS AND METHODS

This cross-sectional study was conducted at the Department of Microbiology in association with the Department of Paediatrics for a period of six months from January 2019 to June 2019. The study was approved by the Institutional Ethics Committee (GVMC/IEC/2019/01) and consent obtained from guardians of the children.

Inclusion criteria: Children less than 12 years of age who had one or more of the following clinical scenario suggestive of rickettsial infection were included [3]:

a. Undifferentiated fever of more than five days;

b. Sepsis of unclear aetiology;

c. Fever with rash; oedema; headache and myalgia; hepatosplenomegaly and/or lymphadenopathy; acute kidney injury; acute gastrointestinal or hepatic involvement; cough and pulmonary infiltrates or community acquired pneumonia

Exclusion criteria: Children with immunocompromised condition such as primary or secondary immunodeficiencies and with specific diagnosis and laboratory confirmed non-rickettsial aetiologies were excluded.

A minimum sample size of 144 was derived by considering the expected prevalence of Scrub typhus among the paediatric age group as 60%, with 8% margin of error and 95% confidence level [7]. Three mL (3 mL) of whole blood was collected from the patient, serum separated and stored at -20°C for serological confirmation. Other investigations such as complete blood count, liver and renal function tests were done based on the clinical diagnosis. Patient's clinical history was noted.

The Weil-Felix test was performed on all the serum samples collected at the time of presentation (acute sample) by the tube agglutination method using coloured antigens against Proteus vulgaris OXK. A titre of 1:80 or more with Proteus mirabilis OXK antigens was considered significant for Scrub typhus. The serum was also subjected to ELISA for detection of IgM antibodies to *Orientia tsutsugamushi* derived recombinant antigens using the Scrub Typhus Detect kit (In Bios International, Inc., Seattle, WA, USA) [5]. Manufacturer's instructions were followed. The diluted sera were added to the microwells coated with 56 kDa antigen and incubated, followed by addition of polyclonal goat anti-human IgM antibodies labelled with horseradish peroxidase enzyme. Subsequently, liquid Tetramethyl-benzidine (TMB) substrate was added and the reaction read at 450 nm. The optimal cut-off value of scrub typhus IgM ELISA was determined using the mean OD +3 standard deviation value of 30 control sera from normal healthy volunteers. OD cut-off values of >0.525 for IgM were considered as positive.

STATISTICAL ANALYSIS

The data was entered on Microsoft Excel and analysed. The significance of demographic and clinical features was determined by calculation of p-value by Fisher's-exact test using SPSS software version 17.0. The p-value of <0.05 was considered statistically significant. The utility of WF test was analysed by comparison with the Scrub typhus IgM ELISA test results.

RESULTS

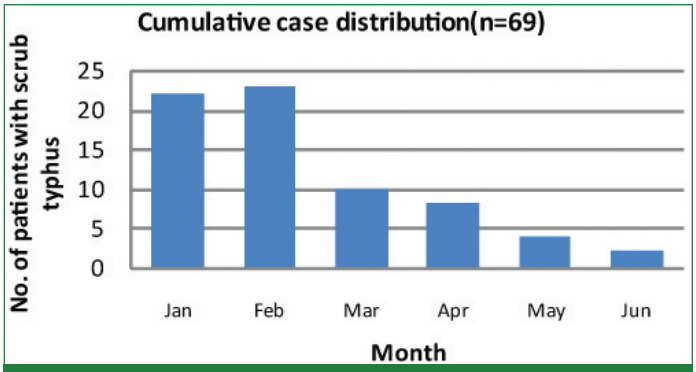
A total of 152 children with the inclusion criteria were included. The age of the children included ranged from 3 months to 12 years with a mean age of 5.7 years. The male female ratio was 1.9:1 with 101 males (66.4%) and 51 females (33.6%) [Table/Fig-1].

Age	Male	Female	Total
0-12 (months)	7	3	10
1-4 (years)	39	20	59
5-8 (years)	32	15	47
9-12 (years)	23	13	36
Total	101	51	152

[Table/Fig-1]: Age and Sex distribution of the study population (n=152).

Among the 152 serum samples tested, 69 (45.4%) were positive for Scrub typhus IgM antibodies by ELISA. The month wise case distribution was found to be as follows: January (n=22), February (n=23), March (n=10), April (n=8), May (n=4) and June (n=2) [Table/Fig-2].

WF test performed on 152 single samples showed a positivity (titer ≥1:80) of 39.5% (n=60) with OXK antigens. Out of the 60 patients with the positive WF test, the titres were as follows: 1 in 320 or more in 18 patients (30%), 1 in 160 in 23 patients (38.3%) and 1 in 80 in 19 patients (31.7%).



[Table/Fig-2]: Cumulative case distribution of patients with Scrub typhus (n=69).

At a titer of ≥1:80, the sensitivity, specificity, PPV, NPV and accuracy of WF test OXK antigen suggestive of Scrub typhus as compared to the Scrub typhus IgM ELISA were 71.01%, 86.75%, 81.67%, 78.6% and 79.61%, respectively patients [Table/Fig-3].

	Scrub typhus IgM		
OXK (≥1:80)	Positive	Negative	Total
Positive	49	11	60
Negative	20	72	92
Total	69	83	152

[Table/Fig-3]: Comparison of Weil Felix (WF) and IgM ELISA for diagnosis of Scrub typhus.
Sensitivity: 71.01%; Specificity: 86.75%; PPV: 81.67%; NPV: 78.26%; Accuracy: 79.61%

Among the 69 children with a positive Scrub typhus IgM antibodies, the commonest age group was 1-4 years {n=30 (43.5%)} followed by 5-8 years, {n=16 (23.2%)} [Table/Fig-4]. The frequency of disease was more in males {n=45 (65.2%)} as compared to females. Among the patients with scrub typhus, all patients presented with fever {n=69 (100%)}. The fever was high grade in 53 patients (76.8%) and of intermittent type in 60 patients (87%). Sixty four patients (92.8%) had duration of fever of 5-10 days. The fever was associated with myalgia in 7 (10.1%) and headache in 2 (2.9%) patients, respectively. The other associated symptoms were cough in 43 (62.3%), vomiting in 34 (49.3%), abdominal pain in 11 (15.9%), constipation in 2 (2.9%) and loose stools in 4 (5.8%) patients. The presence of common symptoms such as cough (p=0.47), vomiting (p=0.98), and abdominal pain (p=0.87) in patients with scrub typhus were not statistically significant [Table/Fig-4].

	Scrub typhus IgM ELISA		p-value
Parameters	Positive (n=69)	Negative (n=83)	
Age			
0-12 months	3 (4.3%)	7 (8.4%)	0.13
1-4 years	30 (43.5%)	29 (35 %)	
5-8 years	16 (23.2%)	31 (37.3%)	
9-12 years	20 (29%)	16 (19.3%)	
Sex			
Males	45 (65.2%)	56 (67.5%)	0.76
Females	24 (34.8%)	27 (32.5%)	
Symptoms			
Fever			
Present	69 (100%)	83 (100%)	1
Absent	0	0	
Duration of fever			
5-10 days	64 (92.8%)	75 (90.4%)	0.59
>10 days	5 (7.2%)	8 (9.6%)	

Grade of fever			
High grade	53 (76.8%)	67 (80.7%)	0.55
Low grade	16 (23.2%)	16 (19.3%)	
Pattern of fever			
Continuous	9 (13%)	9 (10.8%)	0.67
Intermittent	60 (87%)	74 (89.2%)	
Headache			
Present	2 (2.9%)	4 (4.8%)	0.54
Absent	67 (97.1%)	79 (95.2%)	
Myalgia			
Present	7 (10.1%)	8 (9.6%)	0.91
Absent	62 (89.9%)	75(90.4%)	
Abdominal pain			
Present	11 (15.9%)	14 (16.9%)	0.87
Absent	58 (84.1%)	69 (83.1%)	
Cough			
Present	43 (62.3%)	47 (56.6%)	0.47
Absent	26 (37.7%)	36 (43.4%)	
Vomiting			
Present	34 (49.3%)	41 (49.4%)	0.98
Absent	35 (60.7%)	42 (50.6%)	
Constipation			
Present	2 (2.9%)	4 (4.8%)	0.54
Absent	67 (97.1%)	79 (95.2%)	
Loose stools			
Present	4 (5.8%)	7 (8.4%)	0.53
Absent	65 (94.2%)	76 (91.6%)	
Clinical signs			
Eschar			
Present	21 (30.4%)	0	<0.001 (significant)
Absent	48 (69.6%)	83 (100%)	
Hepatomegaly			
Present	51 (73.9%)	22 (26.5%)	0.63
Absent	18 (26.1%)	61 (73.5%)	
Splenomegaly			
Present	37 (53.6%)	19 (22.9%)	0.40
Absent	32 (46.4%)	64 (77.1%)	
Pleural effusion			
Present	2 (2.9%)	3 (3.6%)	0.80
Absent	67 (97.1%)	80 (96.4%)	
Encephalitis			
Present	2 (2.9%)	1 (1.2%)	0.59
Absent	67 (97.1%)	82 (98.8%)	
Laboratory investigations			
Anaemia			
Present	44 (63.8%)	54 (65.1%)	0.86
Absent	25 (36.2%)	29 (34.9%)	
Leucocytosis			
Present	16 (23.2%)	20 (24.1%)	0.98
Absent	53 (76.8%)	63 (75.9%)	
Leukopenia			
Present	3 (4.3%)	7 (8.4%)	0.31
Absent	66 (95.7%)	76 (91.6%)	
Thrombocytopenia			
Present	21 (30.4%)	21 (25.3%)	0.48
Absent	48 (69.6%)	62 (74.7%)	
Elevated AST			
Present	17 (24.6%)	14 (16.9%)	0.31
Absent	52 (75.4%)	69 (83.1%)	
Ultrasonogram findings			
Gall bladder oedema			
Present	6 (8.7%)	8 (9.6%)	0.84
Absent	63 (91.3%)	75 (90.4%)	
Mesenteric adenitis			
Present	2 (2.9%)	4 (4.8%)	0.54
Absent	67 (97.1%)	79 (95.2%)	

[Table/Fig-4]: Demographic and clinical features of patients with study population (n=152).
Fisher's-exact test was used to test statistical significance; p-value of <0.05 was considered statistically significant; ELISA: Enzyme-linked immunosorbent assay; AST: Aspartate aminotransferase

The common clinical signs observed were hepatomegaly in 51 (73.9%), splenomegaly in 37 (53.6%), eschar in 21 (30.4%) and pleural effusion in 2 (2.9%) patients. Encephalitis was noted in 2 patients (2.9%). The presence of eschar in patients with scrub typhus was found to be statistically significant (p<0.001). The association of hepatomegaly (p=0.63), splenomegaly (p=0.40), pleural effusion (p=0.80) and encephalitis (p=0.59) were not statistically significant [Table/Fig-4].

Laboratory investigations revealed anaemia in 44 patients (63.8%), thrombocytopenia in 21 (30.4%) patients, leucocytosis in 16 patients (23.2%), elevated, Aspartate aminotransferase (AST) in 17 patients (24.6%) and leukopenia in 3 (4.3%) patients. Ultrasonogram (USG) showed gall bladder oedema in six patients (8.7%), mesenteric adenitis in 2 patients (2.9%). No statistical significance was found for anaemia (p=0.86), thrombocytopenia (p=0.48), leucocytosis (p=0.98), leukopenia (p=0.31), elevated AST (p=0.31), the USG findings of gall bladder oedema (p=0.84) and mesenteric adenitis (p=0.54) in patients with scrub typhus [Table/Fig-4].

DISCUSSION

Scrub typhus has been reported extensively in Tamilnadu especially in and around Vellore and the state border with Puducherry [5-8]. This could possibly be due to denser vegetation in these areas consequent to increased agriculture land holdings thereby, leading to increase exposure to the trombiculid mites that harbor *O. tsutsugamushi* [8]. A spike in cases during the post monsoon season and in the cooler months has been documented earlier. Kumar M et al., studied the clinico-epidemiological profile of Scrub typhus in children and observed that the majority of infections occurred between September and February [6]. Varghese GM et al., studied the epidemiology and geographical factors of Scrub typhus in South India and found a rising Incidence Rate Ratio (IRR) in the cooler months September (IRR=23.8) to January (IRR=30.7) followed by drastic reduction in the cases between February to June [8]. A five year analysis of the rickettsial fevers in South Indian children revealed a high burden of cases (74%) between September and January [9]. The present study found a decline in the number of cases from March to June.

Rickettsial diseases in children have been reported from several parts of India over recent years [10-12]. Reports from South India including Tamilnadu also show an increasing frequency of the disease over the past decade [1,6,7,13-15]. The seroprevalence of the disease among children in the present study was 45.4% during the study period from January to June, with the highest frequency in the age group 1-4 years and a male preponderance (65.2%). The seroprevalence of the disease in children have varied from 0 to 61% as reported in various studies in different regions in India [Table/Fig-5] [1,7,10,12,14-18]. In the present study, fever was the commonest clinical presentation in patients with Scrub typhus, which was consistent with other studies [4,12]. Most of the patients

Author of Study (Year) [Ref no.]	Region	Year of study	Sample size	Study population	Method of testing	Seroprevalence of scrub typhus among children
Rajagopal V et al., (2014) [18]	Vellore, India	April to June 2012	80	Adult and paediatric patients with recent history of febrile illness	IgM ELISA	0
Bhattar S et al., (2015) [17]	Delhi, India	February 2013 to March 2014	100	Paediatric patients presenting with febrile illness	IgM ELISA, Immunochromatographic test	10%
Roopa KS et al., (2015 [7]	Puducherry, India	January 2012 to June 2015	482	clinically suspected cases	Weil Felix test and IgM ELISA	61%
Kalal BS et al (2016)[1]	Bengaluru, Karnataka, India	January 2010 to October 2012	103	Hospitalised children with clinical features suggestive of rickettsial infection	IgM ELISA	60.2%
Jakharia A et al., (2016) [16]	Arunachal Pradesh, India	2016	300	General population	IgG ELISA	5.6%
Lalrinkima H et al., (2017) [10]	Mizoram, India	October 2014 to December 2016	4081	Clinically suspected adult and paediatric patients with scrub typhus	Rapid Immunochromatography test	9.19%
Jacob SM et al., (2018) [15]	Chennai, India	November 2015 to February 2016	100	Adult and paediatric Febrile patients	IgM ELISA	27% in 4 to 20 age group
Bal M et al., (2019) [12]	Odisha, India	June –November 2017	413	Children with acute fever >5 days	IgM ELISA & PCR	48.7%
Present study	Villupuram, Tamil Nadu, India	January-June 2019	152	Children with clinical features suggestive of rickettsial infection	Weil Felix test,IgM ELISA	45.4%

[Table/Fig-5]: Seroprevalence of Scrub Typhus among children reported in studies across India in the last decade [1,7,10,12,15-18].
ELISA: Enzyme-linked immunosorbent assay; Ig: Immunoglobulin; PCR: Polymerase chain reaction

presented with a high grade fever intermittent type with duration of 5-10 days. Masand R et al., studied the clinical profile of Scrub typhus in children and reported fever in 100% of the cases [11]. Bal M et al., investigated the clinical profile of children with Scrub typhus and observed high grade fever in 88.1% of patients [12].

Eschar, a painless, non-pruritic crusty necrotic lesion was found in 30.4% of the patients in the present study ($p<0.001$). The presence of eschar supports the diagnosis, but its presence is variable. Eschars are about 1 cm in diameter with or without surrounding erythematous halo, suggesting the location of the bite of the chiggers and is usually seen in the moist areas of the body. The frequency of eschar reported by other studies has varied from 3.3-48%. [6,11,12,14,19]. Rose W et al., reported eschar in 40.8% of children with scrub typhus which enabled early diagnosis and treatment. A strong evidence of association was found between presence of eschar and breathing difficulty and thrombocytopenia [19].

Other features to clinically distinguish Scrub typhus from other co-endemic diseases such as Typhoid, Leptospirosis and Dengue are few. Abdominal pain and vomiting are common symptoms associated with fever in scrub typhus. Liver and spleen may be enlarged in a significant proportion of those infected. The major signs observed in the present study are hepatomegaly (73.9%) and splenomegaly (53.6%,). Pleural effusion and encephalopathy were also observed. Maculopapular rash which may be associated with Scrub typhus was not observed in present study patients. Kumar M et al., reported vomiting and abdominal pain in 49% and 34% of patients and hepatomegaly, splenomegaly, pleural effusion and encephalopathy in 91%, 60%, 14% and 17% of patients, respectively in their study at Puducherry, while maculopapular rash was noted in 14% of patients [6]. Rash in scrub typhus is usually maculopapular. No rash was observed in the present study. A similar observation was noted by Mahajan SK et al., [20]. Masand R et al., observed the presence of rash in 10% of cases [11]. Palanivel S et al., noted that more than 80% of the children had hepatosplenomegaly and pallor, while rash was observed in in 35% of the children [21].

The common signs and symptoms in scrub typhus are fever, headache, body pain, eschar, enlarged lymph nodes and sometimes rashes. However, in the absence of specific symptoms and signs specific to Scrub typhus, the laboratory plays a supportive role in providing the diagnosis. In this study, routine blood investigations revealed thrombocytopenia, leukocytosis, and leucopenia and elevated AST of patients. These findings were consistent with the observation by Kumar M et al., who reported thrombocytopenia in 31%, leukocytosis in 37%, leukopenia in 3%, elevated AST in 31% of their study population [6]. Palanivel S et al., observed thrombocytopenia, elevated liver enzymes and leukocytosis in 77%, 64% and 49%, respectively [21].

The laboratory diagnosis of Scrub typhus is largely based on serological tests such as WF test, ELISA and Indirect Immunoflourescenceasssay (IFA) and less commonly on Polymerase Chain Reaction (PCR) of blood and eschar samples [2,4]. WF, a heterophile antibody test based on sharing of the antigens between rickettsia and proteus demonstrates agglutinins to Proteus vulgaris strain OX19, OX2 and Proteus mirabilis OXK. This test still serves as a useful and inexpensive diagnostic tool for rickettsial disease, the disadvantage being lack of high sensitivity and specificity. In present study, a titre of ≥1:80 were considered as indicative of possible infection. Criteria suggested for the diagnosis of scrub typhus is a single titre of 1:320 or greater, or a fourfold rise in titre starting from 1:80 for OXK [4]. The present study demonstrated a sensitivity and specificity of 71.01% and 86.75% for WF test at a titre of ≥1:80 respectively in comparison with IgM ELISA. Rani S et al., and Anitharaj V et al., reported a sensitivity and specificity of the test of 72.4%; 91.4% and 81.34%; 70.93%, respectively [22,23]. Due to the disadvantages of the IFA which is considered as the reference test, such as cost and inter observer variations, non-availability in resource limited settings, the ELISA based estimation of IgM is preferred for detection of acute/primary infection [24].

The ELISA method detects IgM antibodies against the 56-kDa antigen, the major immunodominant protein located on the outer membrane of the bacteria, using a recombinant antigen. Validation of a new antibody detection diagnostic test in a local setting is required to determine an appropriate diagnostic cut-off by the end user, using geographically relevant serum samples especially in settings where scrub typhus is endemic. Overlooking this step and application of a generic cut-off leads to inaccurate laboratory results, and high background levels of antibody may lead to false positivity [25,26]. In the present study, the diagnosis of Scrub typhus was performed by IgM detection through ELISA and the diagnostic cut-off was 0.525 by calculation as per manufacturer's instructions. The various cut-offs for ELISA IgM reported are 0.6 (Kalal BS et al., Karnataka, India), 0.5 (DHR-ICMR), 0.6 (Blacksell SD et al.,

Thailand) and 0.8 (Varghese GM et al., Vellore India) [1,4,25,26]. Thus, the present study found a significant proportion of children infected with Scrub typhus presenting with a spectrum of clinical features which was confirmed by IgM ELISA.

Limitation(s)

The limitation of the study was the short study period done on period of six months from January to June. Hence, the prevalence of the disease throughout the year could not be determined.

CONCLUSION(S)

The study reveals that Scrub typhus is a significant infection in the paediatric age group with a seroprevalence of 45.4%. The disease has a seasonal trend and a tendency to occur during the post monsoon season with a declining incidence from March to June. Most common clinical presentation was high grade intermittent fever with hepatomegaly and presence of eschar supports the clinical diagnosis. As other clinical manifestations are seen in other tropical infections as well, the laboratory plays a significant role in confirming the diagnosis. The utility of the WF test is limited because of lower sensitivity and specificity. The detection of IgM antibodies by ELISA can be of immense use in the diagnosis.

Acknowledgement

The study was conducted with support of financial grant for the year 2018-19 by the Tamilnadu State Research Committee, King Institute of Preventive Medicine, Guindy, Chennai-600032.

REFERENCES

[1] Kalal BS, Puranik P, Nagaraj S, Rego S, Shet A. Scrub typhus and spotted fever among hospitalised children in South India: Clinical profile and serological epidemiology. Indian J Med Microbiol. 2016;34:293-98.
[2] Rathi N, Kulkarni A, Yewale V. IAP guidelines on rickettsial diseases in children. Indian paediatrics. 2017;54(3):223-29.
[3] Rathi N, Rathi A. Rickettsial infections: Indian perspective. Indian Paediatr. 2010;47:157-64.
[4] DHR-ICMR Guidelines for Diagnosis & Management of Rickettsial Diseases in India. February 2015.
[5] Stephen S, Sangeetha B, Ambroise S, Sarangapani K, Gunasekaran D, Hanifah M, et al. Outbreak of scrub typhus in Puducherry & Tamil Nadu during cooler months.. Indian J Med Res. 2015;142(5):591-97.
[6] Kumar M, Krishnamurthy S, Delhikumar 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(1):82-88.
[7] Roopa KS, Karthika K, Sugumar M, Bammigatti C, Shamanna SB, Harish BN. Serodiagnosis of scrub typhus at a tertiary care hospital from Southern India. J Clin Diagn Res. 2015;9(11):DC05.
[8] Varghese GM, Raj D, Francis MR, Sarkar R, Trowbridge P, Muliyil J. Epidemiology & risk factors of scrub typhus in south India. Indian J Med Res. 2016;144(1):76-81.
[9] Thomas R, Puranik P, Kalal B, Britto C, Kamalesh S, Rego S, et al. Five-year analysis of Rickettsial fevers in children in South India: Clinical manifestations and complications. J Infect Dev Ctries. 2016;10(6):657-61.
[10] Lalrinkima H, Lalremruata R, Lalchhandama C, Khiangte L, Siamthara FH, Lalnunpuia C, et al. Scrub typhus in Mizoram, India. J Vector Borne Dis. 2017;54:369-67.
[11] Masand R, Yadav R, Purohit A, Tomar BS. Scrub typhus in rural Rajasthan and a review of other Indian studies. Paediatrics and International Child Health. 2016;36(2):148-53.
[12] Bal M, Mohanta MP, Sahu S, Dwibedi B, Pati S, Ranjit M. Profile of paediatric scrub typhus in Odisha, India. Indian Paediatrics. 2019;56(4):304-06.
[13] Vivekanandan M, Mani A, Priya YS, Singh AP, Jayakumar S, Purty S. Outbreak of scrub typhus in Pondicherry. J Assoc Physicians India. 2010;58:24-28.
[14] Krishnan R, Pillai RK, Elizabeth KE, Shanvas A, Bindusha S. Paediatric scrub typhus in Southern Kerala: An emerging public health problem. Clinical Epidemiology and Glob al Health.2016;4(2):89-94.
[15] Jacob SM, Sekkizhar G, Kanagasabai S, Gopal P, Gopal T, Elumalai S. Seroprevalence and clinical manifestations of scrub typhus infection in Chennai city: A cross-sectional study. International Journal of Health & Allied Sciences. 2018;7(3):201.
[16] Jakharia A, Borkakoty B, Biswas D, Yadav K, Mahanta J. Seroprevalence of scrub typhus infection in Arunachal Pradesh, India. Vector-Borne and Zoonotic Diseases. 2016;16(10):659-63.
[17] Bhattar S, Mehra B, Singh I, Bhalla P. Is scrub typhus a cause of febrile illness among paediatric population of Delhi? J Clin Diagn Res. 2015;9(3):DL02.
[18] Rajagopal V, Bhaskar M, Devi RR, Rajkumar P. Serological diagnosis of scrub typhus in patients attending a government hospital at Vellore, Tamil Nadu. Indian J Med Res. 2014;140(5):686.
[19] Rose W, Rajan RJ, Punnen A, Ghosh U. Distribution of eschar in paediatric scrub typhus. Journal of Tropical Paediatrics. 2016;62(5):415-20.
[20] Mahajan SK, Rolain JM, Sankhyan N, Kaushal RK, Raoult D. Paediatric scrub typhus in Indian Himalayas. Indian J Paediatr. 2008;75:947-49.
[21] Palanivel S, Nedunchelian K, Poovazhagi V, Raghunadan R, Ramachandran P. Clinical profile of scrub typhus in children. Indian J Paediatr. 2012;79(11):1459-62.
[22] Rani S, Thakur K, Sood A, Chauhan V, Jaryal SC, Sood A. Comparison of Weil Felix test and IgM ELISA in the diagnosis of scrub typhus in Kangra, Himachal Pradesh. Int J Health Sci Res. 2016;6(12):28-32.
[23] Anitharaj.V, Stephen S, Pradeep J, Park S, Kim SH, Kim YJ, et al. Serological diagnosis of acute Scrub typhus in Southern India: Evaluation of InbiosScrub typhus detect IgM Rapid test and comparison with other serological tests. J Clin Diagn Res. 2016;10(11):DC07.
[24] Phetsouvanh R, Thojaikong T, Phoumin P, Sibounheuang B, Phommasone K, Chansamouth V, et al. Inter-and intra-operator variability in the reading of indirect immunofluorescence assays for the serological diagnosis of scrub typhus and murine typhus. The American Journal of Tropical Medicine and Hygiene. 2013;88(5):932-36.
[25] Blacksell SD, Tanganuchitcharnchai A, Nawtaisong P, Kantipong P, Laongnualpanich A, Day NP, et al. Diagnostic accuracy of the InBios scrub typhus detect enzyme-linked immunoassay for the detection of IgM antibodies in Northern Thailand. Clin Vaccine Immunol. 2016;23(2):148-54.
[26] Varghese GM, Rajagopal VM, Trowbridge P, Purushothaman D, Martin SJ. Kinetics of IgM and IgG antibodies after scrub typhus infection and the clinical implications. International Journal of Infectious Diseases. 2018;71:53-55.

PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Microbiology, Government Villupuram Medical College, Villupuram, Tamil Nadu, India.
2. Associate Professor, Department of Microbiology, Government Villupuram Medical College, Villupuram, Tamil Nadu, India.
3. Assistant Professor, Department of Microbiology, Government Villupuram Medical College, Villupuram, Tamil Nadu, India.
4. Third Year MBBS Undergraduate Student, Government Villupuram Medical College, Villupuram, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
R Deepa,
The Metrozone, N902, 44 Pillaiyar Koil Street, J.N.Road, Annanagar West,
Chennai-600040, Tamil Nadu, India.
E-mail: DEEPARSN@YAHOO.CO.IN

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jun 15, 2020
- Manual Googling: Nov 04, 2020
- iThenticate Software: Jan 08, 2021 (13%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: As declared above
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jun 14, 2020**
Date of Peer Review: **Jul 22, 2020**
Date of Acceptance: **Nov 04, 2020**
Date of Publishing: **Apr 01, 2021**