ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 05 | May - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

TO STUDY THE ROLE OF SCRUB TYPHUS IN UNDIFFERENTIATED FEVERS AND TO EVALUATE THE SEROLOGICAL DIAGNOSTIC METHODS: WEIL FELIX AND RAPID CARD AGAINST ELISA AT TERTIARY CARE TEACHING HOSPITAL JHALAWAR, RAJASTHAN.

Microbiology

Dr Hemlata Dhawan* PG Resident, Department Of Microbiology, Jhalawar Medical College, Rajasthan *Corresponding Author

Dr Vinod Kumar HOD Department Of Paediatrics, Esic Hospital Udaipur, Rajasthan.

Dr Pawan Kumar SMO, District Hospital Dausa, Rajasthan.

ABSTRACT

Background: The diagnosis of Scrub Typhus is generally based on patients clinical presentation and history. The oldest test is the Weil-Felix OXK agglutination reaction which is inexpensive, easy to perform and results are available overnight. ELISA for the detection of IgM antibodies against Orientia tsutsugamushi offers advantages of being able to test large number of samples at a time and can be automated. **Objective:** 1. To study the role of scrub typhus in undifferentiated fevers and to evaluate the serological diagnostic methods: Weil Felix and rapid card against ELISA. 2.To detect the sensitivity, specificity, Negative Predictive Value and Positive Predictive Value of Weil- Felix as compared to ELISA. **Method:** 1.IgM-ELISA Will be performed by quantitative (Tube-test method) to detect the presence and titer of antibodies due to Rickettsial infections using proteus OXK antigen suspension. 2. Weil-Felix: IgM-ELISA will be performed for detection of IgM antibodies inpatient'ssera toderived recumbent antigen and results will be interpreted as per kit manufacturer's guidelines **Result:** In our study sensitivity, specificity, PPV, NPV and accuracy of rapid ICT as evaluated against IgM-ELISA were 41.28%, 95.86%, 90.00%, 64.44% and 72.89%, respectively. The agreement between the two tests was 72.89% In our study sensitivity, specificity, PPV, NPV and accuracy of rapid ICT as evaluated against IgM ELISA were 95.41%, 93.56%, 90.43%, 96.96, and 94.09%, respectively. The agreement between the two tests was 94.09% **Conclusion:** Although a non-specific test is still routinely used in third world countries. We have got WF positivity of 100 Proteus OXK agglutinin in WF as suggestive of acute ST by researchers from India and other countries. The sensitivity, specificity, PPV and NPV values were calculated for these three cut- off titres for OXK agglutinins. A positive WF test per-se is not a conclusive proof for ST infection and has to be correlated with clinical findings. In Bios Scrub typhus IgM-ELISA is affordable and reliable for use in resource poor laboratories. Results of WF test need to be interpreted with caution.

KEYWORDS

ELISA , Rapid card test, Scrub typhus, Weil- felix.

INTRODUCTION

Rickettsial diseases are considered as some of the most covert emerging and re-emerging diseases and are being increasingly recognized. Among these, Scrub typhus is the commonest occurring rickettsial infection in India. Scrub typhus or tsutsugamushi disease, is an acute febrile illness in humans caused by infection with Orientia tsutsugamushi following a bite of an infected mite vector of the genus Leptotrombidium[1]. The diagnosis of Scrub Typhus is generally based on patients clinical presentation and history. However differentiating ,Scrub Typhus from other acute trophical febrile illness, such as leptospirosis, murine typhus , malaria, dengue fever and viral hemorrhagic fevers, is difficult because their clinical sings and symptoms are very similar, presence of an eschar and history of travel an endemic area often help in clinicians the diagnosis. Escher not present in a large number of cases accurate diagnosis leading is a very important in shortening the duration of fever and presenting lethal complications. The main stay in scrub typhus diagnosis remains serology. The oldest test is the Weil-Felix OXK agglutination reaction which is inexpensive, easy to perform and results are available overnight [2]. ELISA for the detection of IgM antibodies against Orientia tsutsugamushi offers advantages of being able to test large number of samples at a time and can be automated[3]. Indirect fluorescent antibody assay is the gold standard assay for the serological detection of antibodies in scrub typhus [4,5].

AIMS AND OBJECTIVES AIM OF THE STUDY

1. To study the role of scrub typhus in undifferentiated fevers and to evaluate the serological diagnostic methods: Weil Felix and rapid card against ELISA.
2. To detect the sensitivity, specificity, Negative Predictive Value and Positive Predictive Value of Weil- Felix as compared to the ELISA.
3. To detect the sensitivity, specificity Negative Predictive Value and Positive Predictive Value of RDT as compared to the ELISA.

MATERIAL AND METHODS STUDY

Site: Department of Microbiology medical college Jhalawar Rajasthan

Study Type: Prospective, cross sectional, hospital based.

Study Subjects: Consecutive Clinically suspected patients of Scrub typhus presenting with fever, fulfilling the inclusion & exclusion criteria, visiting the out-patient & inpatient of SRG Hospital and Medical College, Jhalawar will be included in this study.

Inclusion Criteria

Patients with Acute undifferentiated febrile illness of 7 days or more with or without eschar (If eschar is present, fever of less than 5 days duration will also be considered as scrub typhus suspects.) Other presenting features may be headache and rash (rash more often seen in fair persons), lymphadenopathy,multi-organ involvement like liver, lung and kidney involvement.

Exclusion Criteria

Samples that were hemolysed, icteric or turbid will be not included.

Ethical Approval

Approval from the institutional ethical committee were taken before commencement of the study.

Serological diagnose of scrub typhus: the serum samples will be tested by:-

1. RDT Test
2. IgM ELISA
3. Weil-Felix

Statistical Analysis

The sensitivity, specificity, Negative Predictive Value and Positive Predictive Value of Weil- Felix test and Rapid ICT against IgM ELISA.

OBSERVATIONS AND RESULTS

A prospective observational study was conducted at department of Microbiology at Government SRG Hospital, attached with Jhalawar Medical College, Jhalawar, a tertiary care center in south-east Rajasthan. Based on inclusion and exclusion criteria total 280 patients of acute febrile illness who admitted in hospital during july 2019 to june 2019, with IgM ELISA positive for Scrub Typhus were enrolled in the study as study participants.

Table 1: Distribution Of Patients According To Age And Gender.

Age in yr	Patient of scrub typhus		
	Male (%)	Female (%)	TOTAL (%)
1 -10	1(0.8)	3(1.8)	4(1.4)
11 – 20	18 (14.8)	20(12.5)	38 (13.5)
21 – 30	20 (16.5)	20 (12.5)	40 (14.2)
31 – 40	30 (24.7)	54(34)	84 (30)
41 – 50	16 (13.2)	20 (12.5)	36 (12.8)

51 – 60	24 (19.8)	22 (14)	46 (16.4)
61 – 70	10 (8.2)	18 (11.3)	28 (10)
>71	2 (1.6)	2 (1.2)	4 (1.4)
Total	121 (43.4)	159 (56.78)	280 (100)
	15.12±10.21	19.87±15.93	35 ±25.43

Table 1 depicts distribution of patients according to age and gender. Highest number of patients were seen in the age group of 31-40 years is 84 (30%) followed by 51-60 years is 46 (16.4%), 21-30 years is 40 (14.2%), <20years is 38(14.2%), 41-50 years is 36 (12.8%), 61-70 years is 28 (10%), >71 years 4 cases.

Study participants constitute 121(43.4%) males and 159(56.78.2%) females. Mean age of patients was 40.78±15.9 years. Mean age in males was 40.58±16.3 years while in females 40.93±15.7 years.

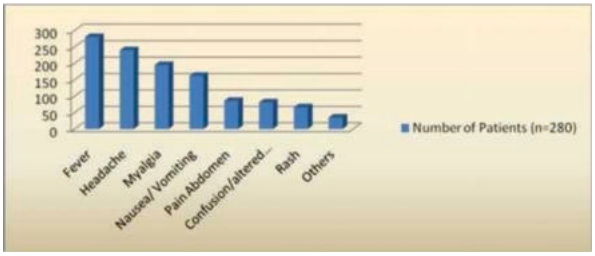

Figure 1: Clinical Symptoms Of Patients

Figure 1 depicts various symptoms among scrub typhus patients. Fever was the main presenting symptom, found in almost all 280(100%) patients, followed by headache 240 (85.7%), myalgia 195(69.64%),nausea/vomiting 162(57.8%), pain abdomen 86 (30.7%), confusion/alteredmental status 81(28.9%), rash 66(23.5%), others 35 (12.5%) generalized body swelling and diarrhea patients had various other symptoms which include generalized tonic clonic body movement, hematuria, burning micturition, vertigo, limb weakness, chest pain etc.

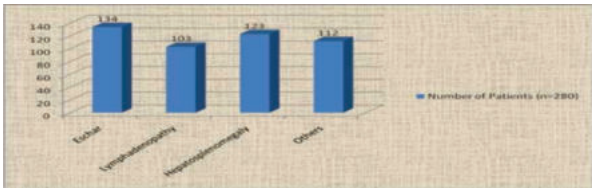

Figure 2: Clinical Sign In Patients

Figure 2 depicts various clinical sign seen in patients of scrub typhus. Eschar which is characteristic of scrub typhus was found in 134 (47.85%) patients. Majority of patients had hepatosplenomegaly 123(43.92%) followed by lymphadenopathy 103(36.78%). A total of 24(17.91%) patients out of 134 had blood pressure <90mmHg systolic for at least 1 hour despite of adequate fluid resuscitation.

Table 2: Performance Of Rapid ICT Against IgM ELISA.

	IgM ELISA +ve (n=218)	IgM ELISA - ve (n=342)
Rapid ICT +ve (n=230)	208	22
Rapid ICT- ve (n=330)	10	320

Sensitivity	Specificity	PPV	NPV	Accuracy
95.41%	93.56%	90.43%	96.96%	94.09%

The sensitivity, specificity, PPV, NPV and accuracy of rapid ICT as evaluated against IgM ELISA were 95.41%, 93.56%, 90.43%, 96.96, and 94.09%, respectively. The agreement between the two tests was 94.09%

Table 3: Performance Of Weil Felix Against IgM ELISA.

	IgM ELISA +ve (n=218)	IgM ELISA - ve (n=342)
Weil felix +ve (n=100)	90	10
Weil felix-ve (n=460)	128	232

Sensitivity	Specificity	PPV	NPV	Accuracy
41.28%	95.86%	90.00%	64.44%	72.89%

The sensitivity, specificity, PPV, NPV and accuracy of rapid ICT as evaluated against IgM ELISA were 41.28%, 95.86%, 90.00%, 64.44% and 72.89%, respectively. The agreement between the two tests was 72.89%.

DISCUSSION

This study was carried out in the Department of Microbiology, SRG Hospital attached with Jhalawar Medical College, Jhalawar in Rajasthan state. Patients of scrub typhus serology were categorized further into ICT, weil felix test method and compare with as our study gold standard method ELISA. Based on inclusion and exclusion criteria total 280 patients of acute febrile illness who admitted in hospital during july 2019 to june 2020, with IgM ELISA positive for Scrub Typhus were enrolled in the study as study participants.

Present study was conducted on 280 patients of scrub typhus. Majority of patients were in the age group of 31 to 40 years84(30%) followed by 51 to 60 years 23(16.4%), 40(14.2%) patients in 21 to 30 age group and 36(12.8%) patients in 41 to 50 year age group. This trend was probably because the age group 20 to 50 years people were mainly workers in agricultural field. Similar to our study, a study done by Md. Jamil et al[6] (2014) also reported highest incidence of scrub typhus 25(42.37%) in age group of 18 to 30 year and 17(23.7%) in age group of 31 to 40 years. In another study by RAJENDRA PRASAD TAKHAR et al[7] (2017) reported similar incidence 48(72.7%) in age group of 20 to 50 year. In our study, out of 280, males were 121(43.4%) and females were 159(56.78%) with mean age in male was 40.58±16.3 years and in female 40.93±15.7 years though this difference was not significant. In our study, female were affected more, this could be probably because of females in this region were more engaged in agriculture work and animal husbandry. Similar to our result a study done by RAJENDRA PRASAD TAKHAR et al [7](2017) reported that females 42(63.6%) were affected more than males 24(36.4%). While a study by Md. Jamil et al[6] (2014) reported that males 35(59.3%) were affected more than females 24(40.7%).

In our study fever was main presenting symptom in all patients of scrub typhus 280(100%), followed by globalheadache 240(85.7%), myalgia 195(69.64%), nausea or vomiting 162(57.8%), confusion or altered mental status 81(30.7%), abdominal pain 86(30.7%), Rash 66(23.5%), other35(12.5%) patients and other symptoms like generalized tonic clonic body movement, hematuria, burning micturiton, vertigo, limb weakness and chest pain were present in 17(12.68%) patients. In a study by Md. Jamil et al [6] (2014) reported similar presenting symptoms with fever as most common 59(100%) followed by headache 56(94.91%) and cough 29(49.15%) in patients. Similar result obtained in a study done by S.P. Singh et al[9] (2014) reported fever as most common presentation 47 (100%) followed by myalgia 38(80.9%) and headache 35 (74.5%).

In our study, necrotic Eschar which is considered most useful diagnostic clue for scrub typhus was found in 134 (47.85%) patients. Majority of patients had hepato-splenomegaly 123(43.92%) followed by lymphadenopathy 103(36.78%). a study by James Philomena et al [8](2016) found eschar in 46(45%) patients, hepatosplenomegaly in 42(42%) patients, lymphadenopathy in 24(24%) patients. Similar result obtained in a study by Anurag Bhargava et al [10](2015) that eschar was present in 49(17.3%) patients, lymphadenopathy in71(25%) patients, hepatosplenomegaly in 138(48%) patients and blood pressure <90mmHg in 31(10.9%) patients.

In our study Rapid ICT showed the sensitivity, specificity, PPV, NPV and accuracy of rapid ICT as evaluated against IgM ELISA were 95.41%, 93.56%, 90.43%, 96.96, and 94.09%, respectively. The agreement between the two tests was 94.09% good sensitivity and specificity in our study and is also demonstrated in previous studies in India [11, 12] and abroad [13, 14]. This was probably due to the fact that rapid ICT detected all three IgM, IgA, IgG whereas ELISA targeted only IgM. Besides this rapid ICT has a very less turnaround time for yielding results whereas ELISA is comparatively time consuming and requires pooling of sample

WF test, showed The sensitivity, specificity, PPV, NPV and accuracy of rapid ICT as evaluated against IgM ELISA were 41.28%, 95.86%, 90.00%, 64.44% and 72.89%, respectively. The agreement between the two tests was 72.89%. Similar result were obtained in a study done by In a prospective study by Dashora et al[15] (2017) A total of 216 consecutive ST. 2017. The sensitivity, specificity, PPV, NPV and accuracy of rapid ICT as evaluated against IgM ELISA were 90.43%,

88.52%, 85.86%, 92.31% and 89.35%, respectively. The agreement between the two tests was 89.35% (good) Similar result were obtained in a study done by In a study by Velmurugan Anitharaj et al[16] (2016) Among 220 patients with acute febrile illness and clinical suspicion of ST, 140 were seropositive for O. tsutsugamushi IgM antibody in InBios Rapid ELISA and/or ImmuneMed Rapid/InBios ELISA. However, only 134 patients were positive in reference standard InBios IgM ELISA and among these patients, 127 were positive in all three kits, viz., both rapid kits and InBios ELISA. InBios Rapid kit has given highest positivity of 139. Regarding WF test, only 66 patients had single OXK. Against the reference standard IgM InBios ELISA test, the Immunochromatography tests InBios and ImmuneMed had shown commendable levels of sensitivity and specificity of 99.25%, 93.02% and 94.87%, 94.19% respectively. WF test had a low sensitivity of 50.38%, but a high specificity of 95.51%.

SUMMARY AND CONCULISION

Present study was conducted in 280 patients of scrub typhus at microbiology department of Jhalawar Medical College, Jhalawar during the time period from July 2019 to June 2020.

The following results were drawn from the study

- In our study total number of patients was 280 out of which 121 were male and 159 were females.
- In this study out of 280 patients most of the patients were in the age group of 21 to 40 years (44.28%).
- In our study most common presenting symptom was fever(100%) followed by Headache(85.7%) and myalgia(69.64%) while Rash was present in only 23.5%.
- In our study majority of patients had eschar which is pathognomic of scrub typhus was found in 47.85% patients. Among eschar positive patients most common site was inguinal area (41%) followed by Axillary(28.3%).
- In our study Fever is a hallmark of rickettsial diseases and it is often accompanied with rash (visible particularly in fair skinned population).
- In our study it has been observed that presence of eschar is also a useful diagnostic clue in ST with a varying frequency of 7–97% .
- In our study We observed rash and eschar in only 23.5 % and 47.85% of patients, respectively but its presence was highly suggestive of seropositivity.
- In our study The sensitivity, specificity, PPV, NPV and accuracy of rapid ICT as evaluated against IgM ELISA were 41.28%, 95.86%, 90.00%, 64.44% and 72.89%, respectively. The agreement between the two tests was 72.89%
- In our study The sensitivity, specificity, PPV, NPV and accuracy of rapid ICT as evaluated against IgM ELISA were 95.41%, 93.56%, 90.43%, 96.96, and 94.09%, respectively. The agreement between the two tests was 94.09%

CONCLUSION

- Scrub typhus is an important cause of acute febrile illness with multisystem involvement in south eastern belt of Rajasthan.
- A high index of suspicion is needed in patients presenting with fever especially during monsoon and post monsoon season.
- Fever , headache , myalgia, rash with high WBC, low platelet are usual features.
- Though eschar is pathognomic of the disease, it may not be seen, and its absence does not rule out scrub typhus.
- Although a non-specific test is still routinely used in third world countries. We have got WF positivity of 100 Proteus OXK agglutinin in WF as suggestive of acute ST by researchers from India and other countries. The sensitivity, specificity, PPV and NPV values were calculated for these three cut- off titres for OXK agglutinins. A positive WF test per-se is not a conclusive proof for ST infection and has to be correlated with clinical findings. There is difference of opinion regarding the significant of Proteus OXK agglutinin in WF as suggestive of acute ST by researchers from India and other countries. recommended [1,17,18,19]
- InBios Scrub typhus IgM ELISA is affordable and reliable for use in resource poor laboratories. Until ST IFA and molecular diagnostic tests are standardized and readily available in India, this rapid ICT kit could serve the purpose of early diagnosis of acute scrub typhus. Results of WF test need to be interpreted with caution.
- In resource limited settings and pending laboratory confirmation we should implement a 'suspect and treat' strategy and initiate prompt treatment with Doxycycline or Azithromycin, to prevent serious morbidity and fatality in this potentially treatable and curable disease.
- More widespread access to medical care, coupled with the increased use of affordable and accurate rapid test, is required to improve diagnosis and treatment of this easily treatable disease.

REFERENCES

1. Traub R, Wisseman Jr CL. The ecology of chigger-borne rickettsiosis (scrub typhus). J Med Entomol 1974; 11: 237-303.
2. SK Mahajan, R Kashyap, A Kanga, V Sharma, BS Prasher, LS Pal. Relevance of Weil Felix in the diagnosis of Scrub typhus in India. J Assoc Physicians India: August 2006; 54: 619 – 621
3. Stephen Gillespie, Peter M. Hawkey.Principle and practices of clinical microbiology: John Wiley & Sons, 12-May-2006.
4. LaScola B, Raout D. Laboratory diagnosi s of Rickettsiosis: current approaches to diagnosis of old and new Rickettsial diseases. J ClinMicrobiol Nov 1997; 35 (11): 2715 – 2727.
5. Jensenius M, Fournier PE, Raoult D. Rickettsiosis and the international traveler. Clin Infec Dis Nov 2004; 39(10):1493 – 1499.
6. Md. Jamil, KG Lyngrah. Clinical Manifestations and Complications of Scrub Typhus : A Hospital Based Study from North Eastern India. Journal of the association of physicians of india Dec 2014; 62 : 19-23.
7. Rajendra Prasad Takhar et al. Scrub Typhus: A prospective observational study during an outbreak in Rajasthan, India. National medical journal if india Feb 2017 ; 30: 69-72.
8. Philomena J et al. Int J Adv Med. 2016 Aug;3(3):586-590.
9. S. P. Singh et al A study of complications of scrub typhus in a tertiary health care institute of Uttarakhand, India. Int J Res Med Sci. 2014 Feb;2(1):246-249
10. Anurag Bhargava et al. Scrub typhus in Uttarakhand & adjoining Uttar Pradesh: Seasonality,clinical presentations & predictors of mortality. Indian J Med Res 144, December 2016, pp 901-909.
11. Browning JS, Raphael M, Klein EF, Coblenz A. Scrub typhus. Amer J Trop Med 1945; 25:481–92.
12. Daryl J.kelly, Paul A.Fuerst, Wei- Mei Ching and Allen L.Richards. Scrub typhus: The geographical distribution of phenotypic and Genotypic variants of Orientiat-sutsugamushi .Clin. Infect. Dis.2009; 48(3); 203 – 230.
13. Jeong YJ, Kim S, Wook YD, Lee JW, Kim KI, Lee SH. Scrub typhus: Clinical, pathologic and imaging findings .Radiographics. Jan-Feb 2007;27(1):161-72.
14. Tamura A, Ohashi N,Urakami H,Miyamura S. Classification of Rickettsia tsutsugamushi in a new genus,Orientiagen.nov., as Orientiatsutsugamushi comb. nov .Int J SystBacteriol 1995; 45:589– 591.
15. Shital Adhikari et al. Predictors of Mortality in Scrub Typhus Infection Requiring Intensive Care Admission in Tertiary Healthcare Centre of Nepal. Interdisciplinary Perspectives on Infectious Diseases. June 2018;2018: https://doi.org/10.1155/ 2018/ 4867958.
16. Ritesh Kumar et al. Factors for Severe Outcome in Scrub Typhus: A Hospital Based Study in Sub Himalayan Region. Journal of The Association of Physicians of India. May 2018;66:36-38.
17. Bang HA, Lee MJ, Lee WC. Comparative research on epidemiological aspects of tsutsugamushi disease (scrub typhus) between Korea and Japan. Jpn J Infect Dis 2008; 61: 148-150.
18. Saah, A.J. 2000. Orientia tsutsugamushi (scrub typhus). In: Mandell, G.L., Bennett, J.E. and Dolin, R. eds. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Disease, 5th edition. Philadelphia: Churchill Livingstone, pp: 2056–20577.
19. V Ramasubramanian, P SenthurNambi. Scrub Typhus. In: A.Muruganathan, T.Geetha edit. Medicine Update 2013; 23: 29 – 22.