



Original Research Article

Evaluation of serological tests in the diagnosis of scrub typhus

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ABSTRACT

Background: Scrub typhus is a leading cause of acute undifferentiated febrile illness in India. Overlapping clinical presentation and very low incidence of characteristic eschar makes serology the main stay of diagnosis. IgM ELISA is the most common test performed in tertiary care centres along with rapid diagnostic tests (RDT) and Weil Felix with varying sensitivity and specificity

Aims and Objectives: The present study was conducted to compare the RDT's and Weil Felix with IgM ELISA and analyse the demographic and clinical profile of scrub positive patients

Materials and Methods: Serum samples from clinically suspected scrub typhus patients admitted in a tertiary care hospital were tested by Weil Felix, RDT and IgM ELISA.

Results: Scrub typhus was confirmed in 38 of the 100 clinically suspected patients by IgM ELISA. Fever was the most common presenting symptom with significant proportion of patients being exposed to vegetation ($p=0.0001$). Sensitivity of Weil Felix and RDT was 42.1% and 84.2% respectively. Acute Kidney injury was the most common complication and mortality rate was 7.9%.

Conclusion: Weil Felix is not a reliable test for scrub typhus diagnosis due to low sensitivity. Results of positive RDT's can be used for early initiation of specific treatment and IgM ELISA can be used as a confirmatory test in highly suspected cases.

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1. Introduction

Scrub typhus is a vector-borne zoonotic infection caused by the bacteria *Orientia tsutsugamushi*. Scrub typhus has re-emerged as a major cause of acute undifferentiated febrile illnesses (AUI) with high morbidity and mortality.^{1,2}

Serological tests like Weil felix, rapid diagnostic tests (RDT) and IgM ELISA are the most commonly tests used for diagnosis of scrub typhus. Indirect Immunofluorescence assay (IFA) is considered as gold standard test but it is not feasible in routine clinical settings due to the technical

expertise in reading slides, subjective interpretation and requirement of paired samples for confirmation of results. Weil felix is a widely used test particularly due to its ease of use but the nonspecific nature and low sensitivity limit its use as standalone confirmatory test. RDT's are useful as point of care tests with sensitivity and specificity reported on par with ELISA and IFA.^{3,4} Polymerase chain reaction (PCR) is more reliable in the first week of illness due to high sensitivity and specificity but not feasible in routine clinical settings.

IgM ELISA is the most common test used for diagnosis of scrub typhus in hospital settings recommended by ICMR⁵ and was the confirmatory test used in 89% of hospital-based studies.⁶

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The present study attempts to compare the performance of serological tests in the diagnosis of scrub typhus and analyse the clinical profile of scrub typhus positive patients.

2. Aims and Objectives

1. To compare the diagnostic performance of serological tests in the diagnosis of scrub typhus.
2. To analyse the clinical, demographical features and risk factors for scrub typhus.

3. Material and Methods

This was a prospective observational study conducted over a period of eight months from September 2020 to April 2021 in a tertiary care hospital. Serum samples from inpatients with clinical features of scrub typhus were included in the study. All the samples were tested for scrub typhus antibodies by rapid diagnostic test (RDT), Weil Felix and IgM ELISA.

Samples received for scrub typhus testing from outpatient departments and samples insufficient for testing by three serological tests were excluded from the study.

ST Detect IgM ELISA kit (InBios International, Seattle, USA) was used which detects anti-56KDa protein antibody and an Optical density > 0.5 was considered positive.

Solid phase immuno-chromatographic assay (SD BioLine, Korea) which can detect IgG/IgM/IgA antibodies to scrub typhus was used as RDT.

Progen (Tulip diagnostics) kit was used for performing Weil Felix Test and a titre of $\geq 1:80$ for OX K was considered as positive for scrub typhus.

Statistical analysis was performed using the Graph pad prism statistical software Version 9.5.0 (730); categorical variables were compared using Fisher' Test. p value less than 0.05 was considered significant.

4. Results

A total of 100 patients with clinically suspected scrub typhus were tested during the study period and 38 patients were detected to be positive. Mean age of patients was 43.6 ± 15.5 years. There were more males in the study population with scrub typhus positivity being slightly more among females - 65% vs 60% as shown in Figure 1.

Mean age of scrub positive patients was 43.6 years and 55.2 % of them were seen in 40 to 60 years age group as seen in Figure 2.

Fever was the most common and presenting symptom in all scrub positive patients followed by myalgia, shortness of breath, hepatomegaly, and vomiting (Table 1). Eschar was not documented in any of the patients.

IgM ELISA was positive in 38 of the clinically suspected patients. RDT and Weil Felix test were negative in 6 and 22 of IgM ELISA positive patients respectively (Table 2)

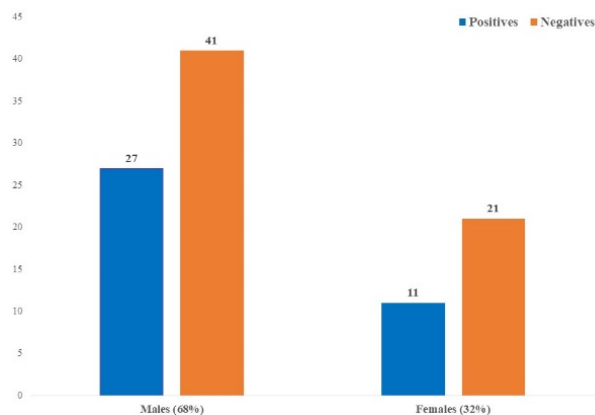


Figure 1: Gender distribution among study population

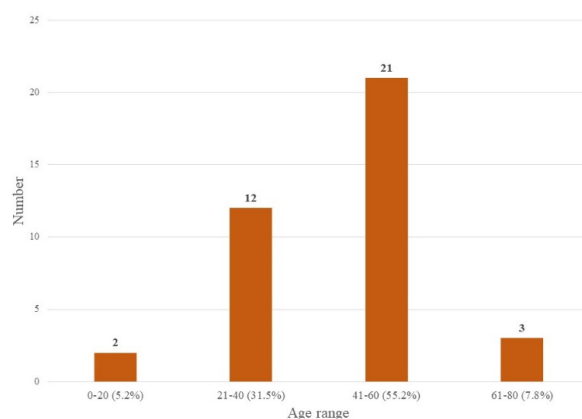


Figure 2: Age wise distribution of scrub typhus positive patients

Table 1: Clinical features of scrub positive patients

Clinical features	No. of patients (%)
Fever	38 (100)
Myalgia	22 (57.8)
Shortness of breath	17 (44.7)
Hepatomegaly	15 (39.4)
Vomiting	12 (31.5)
Rash	8 (21)
Splenomegaly	5 (13.1)
Diarrhoea	5 (13.1)
Lymphadenopathy	1 (2.6)

Table 2: Diagnostic performance of serological tests

Diagnostic assay	Positives (no.)	Negatives (no.)
Rapid ICT	32	68
Weil Felix	16	84
IgM ELISA	38	62

Table 3: Statistical comparison of serological tests in scrub typhus diagnosis

Test	Sensitivity (95CI*) (%)	Specificity (95CI) (%)	PPV‡ (%)	NPV [§] (%)	Accuracy (%)
Rapid test	84.2(68.7 to 93.98)	100(94.2 to 100.00)	100	91.2	94
Weil Felix	42.1(26.3 to 59.2)	100(94.2 to 100)	100	73.8	78

*Confidence interval, †-Positive predictive value, ‡-Negative predictive value

Table 4: Serological tests positivity in relation to duration of illness

Duration of symptoms -days(total patients)	Positives (No.)			Negatives
	ICT	Weil Felix	IgM ELISA	
0-7 (52)	16	7	19	33
8-14 (31)	13	7	15	16
15-21 (13)	3	2	4	9
22-28 (2)	0	0	0	2
29-35 (2)	0	0	0	2

Table 5: Complications in Scrub positive patients

Complication	Number (%)
Acute kidney injury	21(55.2%)
Septic shock	11 (29%)
Acute respiratory distress syndrome	10 (26.3%)
CNS symptoms	8 (21%)

Table 6: Comparison of clinical features of previous studies with present study

Study variable	Study	Prevalence
Scrub typhus infection	Anitha Raj et al 2016 ⁷	63.6
	Kularatne SA et al 2003 ⁸	36.7
	Present study	38
Male gender	Subbalaxmi et al 2014 ⁹	59.3
	Vivekanandan et al 2010 ¹⁰	44
Eschar	Present study	68
	Varghese et al 2013 ¹¹	55
	Sivarajan S et al 2016 ¹²	11.1
Mortality	Present study	0
	Su TH et al 2013 ¹³	0
	Varghese GM et al 2014 ²	9
	Present study	7.9

Considering IgM ELISA as gold standard, sensitivity and specificity of ICT and Weil Felix were calculated. Sensitivity of ICT and Weil Felix tests were 84.2% and 42.1% respectively and there were no false positives with either of these tests as shown in Table 3.

Most of the cases were detected within 2 weeks of illness (34/38; 89%) and sensitivity of ICT in first and second week was 84.2 and 86.6% respectively. Sensitivity of Weil Felix test was slightly better in the second week -36.8% vs 46.6%.(Table 4)

57.8% (22/38) of the scrub positive patients were either farmers or agricultural labourers and was a significant risk factor for the disease (p=0.0001). Acute kidney injury was the most common complication seen in 55.2% of scrub

positive patients. At least one complication was seen in 24 patients and mortality was seen in 3 patients with multiple complications (Table 5).

5. Discussion

Scrub typhus is emerging as a major cause of morbidity and mortality due to AUFI in India. The prevalence of scrub typhus in the present study was 38%. A systematic review of hospital-based studies in 2021 estimated a prevalence rate of 23.5% among patients with AUFI with median age of 28.1 years compared to 45.5 years in the present study.⁶ The most common presenting symptoms in descending order of frequency were fever, myalgia, and shortness of

breath. Eschar was not documented in any of scrub typhus positive patients; eschar incidence varies from 7% to 97% in endemic areas.¹⁴ Comparison of prevalence, demographic features, and mortality of previous studies with present study is presented in Table 6.

In present study, 57.7% of scrub typhus patients were farmers or agricultural labourers similar to the data from a systematic review in India (53.3%)⁶ and was a significant risk factor for scrub typhus positivity ($p=0.0001$)

Weil Felix tests had a low sensitivity of 42.1% missing 22/38 scrub positive patients in the present study. Weil-Felix test has low sensitivity and specificity and shows false negative results in the early stage of disease as the agglutinating antibodies can be detected only in the second week of illness.¹⁵ 24% of the patient's samples positive by IgM ELISA were non-reactive by Weil-Felix test in another study.¹⁶

Sensitivity of RDT in the present study was 84.2% and was negative in six of the 38 IgM ELISA positive patients. Evaluation of the SD Bioline ICT in Thailand patients in 2012 had shown that it is more sensitive than IFA with a specificity as high as 98.4% in diagnosing acute phase samples.¹⁷ SD Bioline ICT has a high sensitivity (99%), specificity (96%) and serological agreement (97.5%) with immunofluorescent assay.³ A correlation of 97 %, between IgM ELISA and SD Bioline Tsutsugamushi rapid diagnostic test, was reported in another study from Andhra Pradesh, South India among 100 suspected cases of scrub typhus in India.⁴

Weil Félix with very low sensitivity cannot be relied as a confirmatory test for scrub typhus and all negatives have to be retested with a confirmatory ELISA and or RDT. IgM ELISA has been documented to perform satisfactorily and can be used as an alternative to the “gold standard” IFA.^{18–20} RDT with acceptable sensitivity can be used as point of care tests for early initiation of specific Doxycycline therapy but the possibility of false negatives necessitates a confirmatory test before issuing final result.

Case fatality rate was 7.9% with mortality reaching 42.8% in patients with three or more complications which correlates with a systematic review of 138 hospital-based studies where the overall case-fatality rate was 6.3%, and the mortality among those with multi-organ dysfunction syndrome was 38.9%.⁶

6. Conclusion

Scrub typhus needs to be included in the differential diagnosis of acute undifferentiated febrile illness. Weil Felix though a widely used test cannot be relied as a single confirmatory test due to low sensitivity and has to be supplemented with RDT and or IgM ELISA based on clinical suspicion and patient response.

7. Source of Funding

None.

8. Conflict of Interest

None.

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