Comparison of Weil Felix Test and ELISA with the Gold Standard IFA for the Diagnosis of Scrub Typhus in Clinically Suspected Cases of Scrub Typhus at a Tertiary Care Hospital in Tamil Nadu

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ABSTRACT

BACKGROUND

Scrub typhus is a febrile disease, the clinical diagnosis of which is difficult due to its vague symptoms. Scrub typhus is diagnosed mainly via serological tests. Sensitivity of Weil Felix was found to be poor, but the specificity of Weil Felix was variable in different studies. Enzyme linked immunosorbent assay (ELISA) IgM is known to be a sensitive test, but its specificity was variable in different studies. Therefore, in this study we have compared these two tests with the gold standard IFA.

METHODS

The study was performed as a cross-sectional study. Among the suspected scrub typhus cases, fifty consecutive IFA scrub typhus positive and fifty consecutive IFA negative samples were taken for comparison of Weil Felix and ELISA tests. The indirect fluorescent antibody test (IFA) was taken as the gold standard.

RESULTS

The sensitivity of ELISA and Weil Felix at a titre of 1 : 160 was found to be 96 % and 40 % respectively. The specificity of ELISA and Weil Felix at 1 : 160 and was found to be 88 % and 94 % respectively.

CONCLUSIONS

Scrub typhus should be kept in mind as a possible diagnosis in acute febrile illnesses. Weil - Felix was found to have a good specificity but low sensitivity. Hence, if the results by Weil Felix are negative, scrub typhus cannot be ruled out and may require further testing. ELISA showed good sensitivity making it a preferable screening test but is not highly specific and confirmation with IFA may be required when feasible.

KEYWORDS

Scrub Typhus, ELISA, Weil Felix, IFA, Laboratory Diagnosis

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BACKGROUND

Scrub typhus presents as an acute undifferentiated febrile illness and could become life threatening. It is caused by an obligate intracellular bacterium called *Orientia tsutsugamushi* that is transmitted by the bite of the larval trombiculid mite, which is the reservoir of the agent and the only life stage that feeds on a vertebrate host.[1,2] Recent reports from several parts of India, including South India, indicate that there has been a resurgence of the disease.[3]

The vague clinical symptoms and lack of a definitive protocol for its diagnosis often results in scrub typhus going undiagnosed. The diagnosis of scrub typhus depends largely on the laboratory diagnosis of which serology is the main stay. The oldest test is the Weil-Felix OX K agglutination reaction which is inexpensive, easy to perform and results are available overnight.[4] In Weil Felix test agglutinating antibodies are detectable 5 – 10 days following the onset of symptoms and the antibodies detected are mainly of IgM type.[5] 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. Indirect fluorescent antibody assay is the gold standard for the serological detection of antibodies in scrub typhus.[5,6]

All the serological tests that are currently available for scrub typhus have its limitations of which the clinician needs to be aware. Generally, a $\geq$ fourfold increase in antibody titre between two consecutive samples taken with a gap of 10 – 14 days is diagnostic, but this is usually retrospective and cannot guide in treatment. Serological diagnosis based on a single acute serum sample requires a cut off antibody titre depending on the endemic titre and could vary from 1 : 10 to 1 : 400.[6]

Diagnosis and surveillance of scrub typhus is challenging particularly in the absence of advanced diagnostic techniques. The availability and cost of other serological methods are a major problem in India.[7]

The purpose of this study was to evaluate the serological diagnostic methods: Weil Felix and ELISA against Indirect Fluorescent antibody assay (IFA) which is the gold standard for the diagnosis of scrub typhus.

METHODS

At the start of the study, approval was obtained from the Institutional Ethics committee and was renewed periodically during the study period which took place over a span of 2 years from October 2012 to August 2014. The study was performed as a cross sectional study. Considering the specificity of ELISA to be 94 % from previous studies with an absolute precision of 5 %, the minimum sample size was calculated to be 90. We tested Weil Felix, ELISA and IFA on a total of 100 samples collected from clinically suspected cases of scrub typhus during the period of study i.e., patients with acute undifferentiated illness of 5 days or more with or without eschar.

Weil Felix test was done using the PROGEN antigen suspension kit (Tulip Diagnostics (p) LTD, Goa). Titres of $\geq$ 1 : 160 was taken as significant. The ELISA test was done using the scrub typhus detect™ IgM ELISA kit (In Bios International Inc., USA). Samples showing values above the cut off titre according to manufacturer's instructions was considered as positive. *Orientia tsutsugamushi* IFA IgM antibody kit (Fuller Labs, California, USA) was used for IFA testing. Bright staining (at least 1+) of short pleomorphic rod forms in any of the 4 antigen areas was taken as a positive reaction. Each field was compared with the positive and negative control reactions with respect to the size, appearance, and density. If the reactivity pattern was different from the positive controls, it was considered non-specific. If the slides show positivity at a dilution of 1 : 64, it was considered positive and if negative at 1 : 64 dilution, it is reported as negative.

RESULTS

The results of Weil Felix, ELISA and IFA were entered via excel sheet and the data was analysed using the IBM SPSS software. The test parameters and McNemar's test were calculated with IFA as gold standard. Sensitivity of a test is defined as the ability to correctly identify the infected individuals and specificity is the ability to correctly identify the uninfected individuals.

Negative predictive value is the proportion of those with a negative test result who are uninfected and positive predictive value as the proportion of those with a positive test result who are infected.

		IFA +ve	IFA -ve	Total
WF 1 : 80 positive		27	4	**31**
WF 1 : 80 negative		23	46	**69**
Total		**50**	**50**	**100**
Chi- Square Tests			Value	Exact Sig.
	McNemar Test			<.001a
	N of valid cases		100	

Table 1.1 Comparison of Results of Weil Felix at a Titre of 1 : 80 and IFA

		IFA +ve	IFA -ve	Total
Weil Felix titre 1 : 160 +ve		20	3	**23**
Weil Felix titre 1 : 160 -ve		30	47	**77**
Total		**50**	**50**	**100**
Chi- Square Tests			Value	Exact Sig.
	McNemar Test			<.001a
	N of valid cases		100	

Table 1.2 Comparison of Results of Weil Felix at a Titre of 1 : 160 and IFA

	IFA +ve	IFA -ve	Total
ELISA +ve	48	6	**54**
ELISA -ve	2	44	**46**
Total	**50**	**50**	**100**
		Value	Exact Sig.
McNemar Test			<.289a
N of valid cases		100	

Table 1.3. Comparison of Results of ELISA and IFA

Of the 50 IFA positive cases, 20 were Weil Felix positive (at a titre of 1 : 160) and 48 ELISA positive, and 20 were both Weil Felix (at a titre of 1 : 160) and ELISA positive. Of the 50 IFA negative cases, 47 were Weil Felix (titre less than 1 : 160) negative and 44 were ELISA negative and 40 were both together negative. The parameters of the test obtained are given in the table 2.

	Weil Felix at 1:160	Weil Felix at 1:80	ELISA
Sensitivity	40.0 %	54.0 %	96.0 %
Specificity	94.0 %	92.0 %	88.0 %
PPV	87.0 %	87.1%	88.9 %
NPV	61.0 %	66.1%	95.7 %

Table 2. Parameters of Weil Felix and ELISA

Out of the six ELISA tests which were false positives, two were positive for dengue, one had raised Widal titres of 1 : 160 for *S. typhi* H antigen. More details are given in table 3.

Sl. No. of Cases	Coinfection / Infectious Focus
1	*Salmonella typhi* H 1:160 others less than 1:20
2	Secondary dengue positive hepatitis A positive urine culture – Pseudomonas aeruginosa (10^5)
3	Urine culture – Escherichia coli (10^5)
4	Blood culture - Staphylococcus aureus
5	Nil
6	Secondary dengue positive

Table 3. Co Infections Observed in ELISA Positive and IFA Negative Cases (6 Cases)

DISCUSSION

Diagnosis of scrub typhus is challenging specially in the presence of co-endemic diseases like dengue, leptospirosis, and typhoid. The Weil Felix test is the oldest serological test for the diagnosis of Scrub typhus and non rickettsial antigens are used. Our study has reported good specificity for the diagnosis of scrub typhus as is reported in other studies[8,9] though in the latter study[9] a titre 1 : 80 was taken as the cut off. In the study by Pradutkanchana J et al.[8] the Weil Felix test at a break point of 1 : 160 gave a sensitivity of 52.1 % when compared to the gold standard IFA. A study from Sri Lanka reported low specificity with Weil Felix and a high Weil Felix titre of 1 : 320 in 54 % of healthy volunteers and 62 % of non-rickettsial fever patients.[10] In contrast in an unpublished data from our hospital a titre of $\leq$ 1 : 20 was recorded in healthy volunteers. The grossly insensitive results for Weil Felix in this study are in concordance with the other studies[8,9] and is attributed to the late appearance of antibody, suppression of their production by antibiotic treatment or due to its absence in re-infections that occurs frequently in endemic areas.[8]

Earlier ELISA with native *O. tsutsugamushi* were used either individually for Karp, Kato, Gilliam strains or pooled but now due to safety issues associated with the culturing of live rickettsia, the recombinant 56- kDa immunodominant protein from *O. tsutsugamsuhi* is used to develop serological ELISA for scrub typhus. Large quantities of this can be prepared using *E.coli* without needing BSL 3 facilities.[11] The r56 ELISA when compared with indirect immunoperoxidase showed a sensitivity of 86 % in one study[11] and 93 % in another study,[12] whereas in our study a sensitivity of 96 % was obtained with IFA taken as the gold standard. The specificity in our study was 84 % and it showed good correlation with one study[11] but showed 94 % specificity in another study.[12]

Our study observed false positive reaction for IgM ELISA in cases of dengue, typhoid fever and one case of blood culture positive for *Staphylococcus aureus*. False - positive reactions were observed in patients with falciparum malaria, pulmonary tuberculosis, *S. viridans* septicaemia and typhoid fever using IgM ELISA in other studies.[13]

CONCLUSIONS

We have observed good specificity but a low sensitivity with Weil Felix test. Therefore, if the results by Weil Felix are negative, scrub typhus cannot be ruled out and further testing may be needed. The good specificity we have observed could also be because of a low endemic titre in the local population. ELISA showed good sensitivity thus is the preferable screening test but is not highly specific and may require confirmation with IFA when feasible.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
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