



Research Article

Weil-Felix Test: Still a Useful Diagnostic Test for Scrub Typhus??

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Abstract

Background: *Scrub typhus is a mite born Rickettsial disease which is considered to be a re-emerging disease and one of the differential diagnosis of FUO. Often Rickettsial infection is undiagnosed because of its unusual presentation & lack of reliable as well as affordable diagnostic test .If the disease is undiagnosed it leads to complications with high mortality & morbidity.*

Materials & Methods: *Present study was done to evaluate the serological tests like Weil Felix, ELISA & IFA, using IFA as the gold standard. About 100 non replicative serum samples were collected from patients with PUO after excluding other causes of PUO. Serum samples were subjected to all the three tests WeilFelix (detect OXK antibodies),ELISA (detecting IgM antibodies against Orientia tsutsugamusghi) & IFA(detecting IgM antibodies to Orientia tsutsugamushi) & compared.*

Results: *27/100 were positive for IgM for scrub typhus using IFA, 22 were positive by Weil Felix, 26 were positive by IgM ELISA for Scrub typhus*

Sensitivity and Specificity of Weil-felix test in comparison with IFA was 33.3% & 84.68%, ELISA was 73% & 83.3%

Conclusion: *Our study shows Weil –Felix test as a less sensitive & less specific test instead ELISA test can be used since it as better sensitivity & specificity.*

Keywords: *Orientia tsutsugamushi, WeilFelix, ELISA, IFA.*

Introduction

Scrub typhus is an important cause of pyrexia of unknown origin (PUO) which accounts for upto50% of case fatality rates in untreated patients.¹ It is a bacterial zoonosis, caused by *Orientia tsutsugamushi* transmitted by larval stage of trombiculid mite, chiggers.^{2,3}

Among Rickettsial infection, Scrub typhus has been reported more frequently from India highest documented in Jammu and Kashmir, Himachal Pradesh, Uttaranchal (now Uttrakhand), Bihar, West

Bengal, Meghalaya, Rajasthan, Maharashtra, Karnataka, Tamil Nadu and Kerala.⁴ Recent past outbreaks reported in sub-Himalayan belt, Jammu to Nagaland during 2003-2004 and 2007 after which Scrub typhus has been considered as reemerging bacterial zoonotic disease in India.⁵ Since Scrub typhus usually present with non specific symptoms & unusual clinical presentation, frequency of diagnosis of Scrub typhus is less & also there is paucity of reliable diagnostic methods.

With all this existing facts, an attempt has been made to diagnose Scrub typhus with three different tests including Weil-Felix test detecting antibodies against OXK Antigen, ELISA detecting IgM antibodies against *Orientia tsutsugamushi*, IFA detecting IgM antibodies against *Orientia tsutsugamushi* considering IFA as gold standard.

Materials and Methods

This hospital based study was carried out in the Department of Microbiology, J.S.S. Medical College & Hospital Mysore, over a period of one year.

Patients with fever of more than 7 days with or without rash of all age groups were included in the study. Detailed clinical history was taken. Laboratory confirmed cases of Malaria, *Leptospira*, Dengue, Enteric fever, urinary tract infection were excluded from the study.

Non consecutive blood samples were collected from patients. Serum was separated & all serum samples were subjected to Weil-felix test (plasmatec), ELISA (Inbios Scrub Typhus detect IgM ELISA system) and Indirect Immunofluorescence (*Orientia tsutsugamushi*) IFA IgM antibody kit, (Fullerton California).

Manufacturer's test protocol was followed for testing and result interpretation.

Weil-felix test

Plasmatec kit was used to perform Weil-felix test which detects antibodies for OXK antibodies for Scrub typhus, Using 0.25% phenol saline as diluent, a series of tubes containing two fold dilutions of patient serum was made with a final volume of 1 ml. A drop of OX2, OX19 and OXK antigen suspension was added respectively to each tube and the mixture was incubated at 50-55° c for 4-6 hours. Positive tube showed visible flocculation or granulation which was accentuated when the tube was lightly agitated. Titre corresponding to the most dilute tube in series that still showed positivity was considered.

IgM Enzyme linked immunosorbent assay

Scrub typhus IgM ELISA was done to detect IgM antibodies to *O. tsutsugamushi* (OT). Serum samples were diluted to 1/100 using Inbios serum diluent, absorbance measurement was done at 450 nm, the absorbance measured was taken as directly proportional to IgM antibodies to *O. tsutsugamushi* present in test serum.

Calculation of cut-off – minimum of 100 specimens from each categories –diseased (confirmed Scrub typhus), confirmed unrelated illness (e.g Brucellosis, enteric fever, etc), were recommended for determination of cut -off. (receiver operating characteristic (ROC) curve was used to determine cut-off value.

Based on these cut off value obtained was 0.216. Samples with spectrophotometric readings above cut off were considered as “Reactive” and samples below this criterion were considered to be “Non Reactive”

IgM immunofluorescence assay

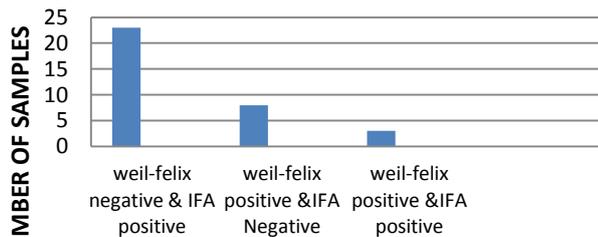
Patient sera was diluted using IgM serum diluent to 1:16 dilution, further dilution was done using wash buffer to obtain a final dilution of 1:64 which was referred to as screening dilution.

Positive reaction was interpreted with the presence of bright staining (atleast 1+) of short pleomorphic rod forms in any of the 4 antigen areas, size appearance, and density of each field is compared with positive and negative control. With no distinct and characteristic staining of the *Orientia* was considered negative. Patterns of reactivity different from the positive controls was considered non specific.

Results

100 blood samples were collected from patients as per inclusion criteria in which 27/100 were positive for IgM Scrub typhus using IFA. 26/100 showed positive by ELISA, 22/100 showed positive by Weil-felix test.

Comparison of Weil-Felix test with IFA



Comparison of ELISA with IFA

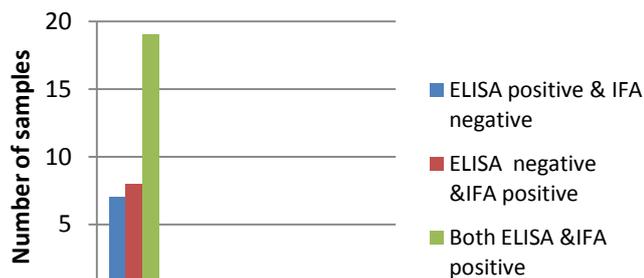
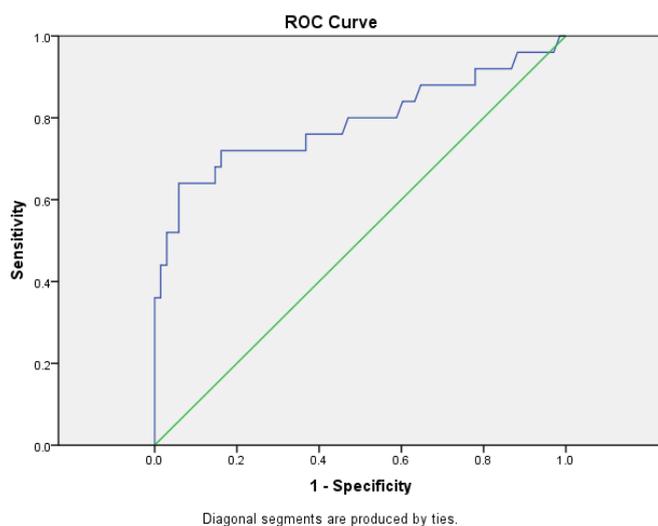


Table-showing -Sensitivity and specificity of Weil-Felix test in comparison with IFA IgM Scrub typhus

Statistic	Value	95% CI
Sensitivity	14.81%	4.19% to 33.73%
Specificity	75.34 %	63.86% to 84.68%

ROC curve to determine the cut-off value for ELISA results



Area Under the Curve

Test Result Variable(s): ELISA

Area
.789

Coordinates of the Curve Test Result Variable(s): ELISA

Positive if Greater Than or Equal To ^a	Sensitivity	Specificity
.204500	.720	0.824

.216500	.720	0.838
.245500	.680	0.838

0.216 is the best cut off value with sensitivity of **72%** and specificity of **83.8%**.

Discussion

Scrub typhus is considered to be a reemerging bacterial zoonosis, it may lead to increase in morbidity and mortality if timely diagnosis is not done This may be attributed to deficiency in a reproducible and quantifiable assay, generally IFA and IIP(indirect immunoperoxidase) which are considered to be used as gold standard serological assays .

Patients with fever more than 7 days with or without rash, IFA positive for orientia tsusugamushi was in 27 (27/100), Weil-felix (OXK) test positive was in 22(22/100) & ELISA IgM 26(26/100) in our study group .

In Comparison of Weil-Felix with IFA, 23 (23/27,85.18%) samples which showed IFA positive showed negative with Weil Felix test &8(29.62%) samples of Weil- Felix positive showed IFA negative. A sensitivity of 33.73% and specificity of 84.68% was derived for Weil Felix test in comparison with IFA. These values were in concordance to the study done by S.A.M Kularathe et al where sensitivity was 33% and relatively low specificity.⁶ Weil-felix test has low sensitivity, i.e. high percentage of false negative results which are more common in cases of Scrub typhus & although this test is not very sensitive but test when positive is rather specific. One of the major drawback of serology is the cross-reactivity that exists among antigens of pathogen within the same genus and

occasionally also in different genera. This difference can be solved by collecting paired serum samples (acute and convalescent sample).⁷

The poor sensitivity of Weil-felix test is well demonstrated by many studies but people use this as a screening test because when positive it is reasonably specific. In spite of all its drawbacks WF test is useful & a cheap diagnostic tool for lab diagnosis of Rickettsial diseases. In comparison of IgM ELISA with IFA, 8 (8/27, 29.62%) serum samples were IgM ELISA negative but IFA positive. 19 (19/27, 70.37%) serum samples were both ELISA & IFA positive showing sensitivity of 72% and specificity 83.3%. These values of sensitivity and specificity were less in comparison to the study conducted by Stuart D Blackshell et al where their study showed sensitivity of 84% and specificity 98%.⁸ Also another study done by Won Jong Jang et al showed sensitivity and specificity of IgM ELISA as 96.3% and 99%.⁹ Our study has showed less values of sensitivity and specificity of IgM ELISA which may be attributed to various factors. False positive test may be obtained by presence of Rheumatoid factor or false negative test due to increase in IgG level which occurs in case of secondary infection occurring during that period might have interfered with the sensitivity and specificity results.¹⁰ In the study performed by Bernard LA Scola et al showed that diagnostic approach by IgM ELISA determined the optimal diagnostic cutoffs which had strong correlation with IFA results.¹¹

In comparison of Weil-Felix test with ELISA, Weil-Felix negative but ELISA positive was in 20 (20/26 76.92%) samples. Out of these 20 samples, 11 samples (11/26 42.30%) were Weil-Felix negative, ELISA positive & IFA positive which explains that Weil-Felix test had missed these 11 samples which were positive for Scrub typhus & this missed positivity was picked up by ELISA and showed that Weil-Felix is less sensitive when compared to ELISA. Weil-Felix test positive but ELISA & IFA negative were in 15 samples which proved that Weil-Felix test is also less specific when compared to ELISA.

With all these comparisons, our study showed that Weil-Felix test is highly non-specific test & in India it is used as preliminary test for screening of Rickettsial disease. The usage of this test should be discouraged because we will be missing out many positive cases of Scrub typhus. Instead we have to think of other alternative tests like ELISA, IFA, immunoperoxidase assays or PCR for Scrub typhus. Although various serological methods of diagnosis for Rickettsial diseases are available, only Weil-Felix test is easily available in India. So there should be reconsideration to choose other tests especially when clinician is suspecting Scrub typhus in particular. Our study includes comparison of only these three tests for Scrub typhus although PCR was not done for confirmation and sample size was also less to conclude that absolute necessity to say a particular test as standard for Scrub typhus.

Conclusion

In this study it was found that Weil-felix test is less sensitive & less specific. Instead ELISA is a better alternative with good sensitivity & specificity. IFA is a gold standard test for diagnosing Scrub typhus & it has the ability to evaluate large number of samples rapidly. So IFA is a better tool for diagnosing Scrub typhus but it is subjective & expensive when compared to ELISA. With this we conclude that ELISA can be a better alternative to Weil-felix test for diagnosing Scrub typhus in our country.

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