

Original Research Article

## Comparison of Weil Felix Test and IgM ELISA in the diagnosis of Scrub Typhus in Kangra, Himachal Pradesh

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### ABSTRACT

**Introduction:** Rickettsial diseases are an important cause of fever of unknown origin. They pose a serious threat to public health if not diagnosed properly. Diagnosis of these diseases is a challenge to both clinicians and laboratorians. Kangra region of Himachal Pradesh is endemic for Scrub Typhus. Various serological tests are available, of which the Weil Felix Test (WFT) is most widely used. Recently, IgM ELISA for Scrub Typhus has been introduced in our set up for the diagnosis of Scrub Typhus.

**Objective:** To evaluate and compare results of Weil Felix Test and IgM ELISA in the diagnosis of Scrub Typhus.

**Materials and Methods:** A prospective study was conducted amongst the patients suspected of suffering from Scrub Typhus w.e.f. March 2013 through February 2014. IgM ELISA Scrub Typhus was carried out on sera of suspected patients. Those positive for Scrub Typhus were also evaluated by the Weil Felix Test. The WFT was carried out on the microtitre plate agglutination test, using doubling dilutions from 1:40 to 1:320. Sera having titres more than 320 were further screened till end titre dilution.

**Results:** 1653 patients suspected to be suffering from Scrub Typhus were enrolled for the study. The mean age of the patients was 30.93±17.02 years. Majority (56.87%) of patients were females. 487 patients were serologically positive for Scrub Typhus by IgM ELISA. Out of these 487 patients, 353 were also positive by Weil Felix OXK agglutination test. The diagnostic titre of Weil Felix considered positive was titre 160. Sensitivity and Specificity of WFT in relation to IgM ELISA at a titre of 160 was 72.5% and 91.4% respectively. As the titres increased, the WFT was more specific.

**Conclusion:** Though IgM ELISA for Scrub Typhus is more sensitive, rapid and specific for testing large number of sera in early phase of disease, the specificity of Weil Felix increases with increasing titre.

**Key Words:** Scrub typhus, Weil Felix Test, IgM ELISA

### INTRODUCTION

Rickettsial diseases are an important cause of fever of unknown origin. They pose a serious threat to public health if not diagnosed properly. Diagnosis of these diseases is a challenge to both clinicians and laboratorians. Scrub Typhus is a Rickettsial infection caused by *Orientia tsutsugamushi*.

It is a zoonotic infection caused by the bite of the larval form of trombiculid mite. The term scrub is used because of the type of vegetation that harbours the vectors of this disease. Scrub Typhus was first described in Japan in the year 1889. The distribution of Scrub Typhus is documented in a triangle limited by Japan and Russia in the north to

Australia in the south and Pakistan and Afghanistan in the west. [1] Scrub Typhus is a public health problem in Asia, where about 1 million new cases are identified annually and 1 billion may be at risk for this disease. [2]

In India, Scrub Typhus has occurred amongst the troops during the World War II in Assam, West Bengal and in Indo Pak war in 1965. [3] The disease is endemic in whole of the Shivalik range from Kashmir to Assam, Eastern and Western Ghats and Vindhyaachal and Satpura ranges in Central India. Outbreaks have also been reported from South India. [4] In 2003, 96 cases of fever with unknown origin were reported in Himachal Pradesh which were diagnosed as Scrub Typhus. [4] Since then, the disease is endemic in our state, cases occurring every year and more frequently in the months of July through November.

Clinically Scrub Typhus presents as an undifferentiated febrile illness with varied signs and symptoms like fever, headache, neurological manifestations, rash, lymphadenopathy, hepatosplenomegaly, renal insufficiency and gastro intestinal symptoms and a mortality rate of 1% to 30% in untreated patients. [5] Serology is the main stay of diagnosis and various tests that can be done are Weil Felix Test, Enzyme Linked Immuno Sorbent Assay (ELISA), Complement Fixation Test (CFT), Indirect Haemagglutination Test (IHA), Indirect Immuno fluorescence Antibody Test (IFA), Microimmunofluorescence, Latex Agglutination Test (LAT), Western Immunoblot and Line blot assay.

Scrub Typhus is an endemic disease in the Sub Himalayan region. Kangra region of Himachal Pradesh is endemic for Scrub Typhus. Various serological tests are available for diagnosis, of which the Weil Felix Test (WFT) is most widely used. Recently, IgM ELISA for Scrub Typhus has been introduced in our set up for the diagnosis of Scrub Typhus. Hence the present study was done to establish the diagnosis of scrub typhus by IgM ELISA and Weil Felix test and to compare both the

tests for diagnosis of Scrub Typhus.

## Objective

To evaluate and compare the results of Weil Felix test and IgM ELISA in the diagnosis of Scrub Typhus.

## MATERIALS AND METHODS

The study was conducted in the Department of Microbiology, DRPGMC & Hospital Kangra at Tanda. It was a prospective cross-sectional study of one year duration starting from March 2013 through February 2014. The study group comprised of clinically suspected patients of Scrub Typhus. Patients with signs and symptoms correlating with Scrub Typhus like fever, headache, myalgia, abdominal pain, altered sensorium, respiratory distress, jaundice, hepatosplenomegaly, lymphadenopathy, eschar and rash were included in the study. Patients who were suffering from Dengue, Malaria and other causes of PUO were not enrolled.

Five to ten ml of venous blood was collected under all aseptic conditions and centrifuged at 2500 rpm for 4-5 minutes for separation of serum. The separated sera were stored in two screw capped vials at 4°C and -20°C till the tests were performed. This sera was processed for IgM ELISA for Scrub Typhus and Weil Felix Test.

IgM ELISA was done on sera as per the standard manufacturers protocols. IgM ELISA kits manufactured by In Bios International Inc. were used. [6] Cut off value was calculated by determining the average optical density (OD) plus 3 times the Standard Deviation (SD) of normal human serum. The normal human serum was collected from healthy blood donors.

Weil Felix Test was performed on sera as per standard technique [7] using doubling dilutions from 1:40 till end titre dilution. The test was performed using the microtitre plate agglutination test method.

## Statistical Analysis

Data collected was entered into a computer in MS Excel Spreadsheet 2003. Statistical Analysis was done using MS Excel 2003 and Epi Info Version 3.5.1.

## RESULTS

A total of 1653 patients were tested serologically by IgM ELISA for Scrub Typhus and Weil Felix test. Out of these, 487 patients were IgM ELISA for Scrub Typhus positive, i.e. confirmed Scrub Typhus patients. The mean age of confirmed patients was  $30.93 \pm 17.02$  yrs. There were 145 males and 342 females. Maximum patients (402) had a rural back ground. The maximum patients reported in the months of September (27.32%) followed by October (22.58%) and August (16.64%) corresponding to monsoon and post monsoon season in the state. [Figure I & Table I]

Weil Felix test was performed on these patients. For the OXK antigen, antibody titre was less than 40, 40, 80, 160, 320, 640 and 1280 in 2, 43, 89, 180, 102, 30 and 41 respectively. At a diagnostic titre of  $OXK \geq 160$ , 353 patients were positive for Scrub Typhus by Weil Felix test. Among the patients negative for Scrub Typhus by IgM ELISA (1166), 100 were positive for OKK agglutination by Weil Felix test at the titre of  $\geq 160$ . [Table II] The sensitivity and

specificity of Weil Felix test at a titre of 160 was 72.5% and 91.4% respectively. Similarly, positive predictive value and negative predictive value at this titre was 77.9% and 88.8% respectively.

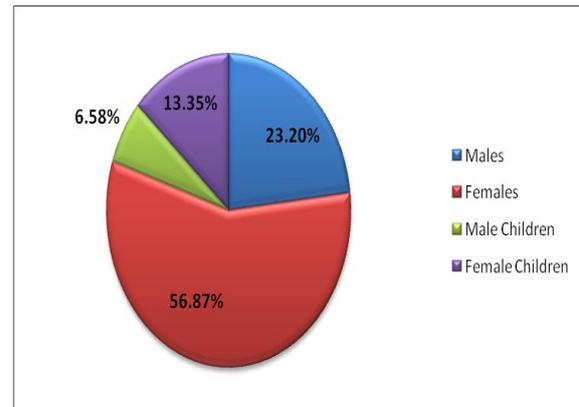


Figure I: Sex Distribution of Patients of Scrub Typhus

TABLE I: Age and Sex Composition of Scrub Typhus Patients

Age Group (years)	Males N (%)	Females N (%)	Total
≤ 10	14 (9.65)	31 (9.07)	45 (9.25)
11 – 20	33 (22.75)	72 (21.06)	105 (21.57)
21 – 30	25 (17.24)	95 (27.78)	120 (24.65)
31 – 40	26 (17.94)	68 (19.88)	94 (19.3)
41 – 50	14 (9.65)	37 (10.82)	51 (10.47)
51 – 60	12 (8.28)	27 (7.89)	39 (8)
61 – 70	16 (11.04)	12 (3.5)	28 (5.74)
≥ 71	5 (3.45)	0	5 (1.02)
<b>Total</b>	<b>145 (100)</b>	<b>342 (100)</b>	<b>487 (100)</b>

TABLE II: Comparison of IgM ELISA and Weil Felix test at a titre of 160

	IgM ELISA + ve	IgM ELISA - ve	Total
Weil Felix + ve	353 (a)	100 (b)	453 (a + b)
Weil Felix - ve	134 (c)	1066 (d)	1200 (c + d)
<b>Total</b>	<b>487 (a + c)</b>	<b>1166 (b + d)</b>	<b>1653 (a + b + c + d)</b>

Table III: Comparison of IgM ELISA and Weil Felix test at a titre of 320

	IgM ELISA + ve	IgM ELISA - ve	Total
Weil Felix + ve	173 (a)	35 (b)	208 (a + b)
Weil Felix - ve	314 (c)	1131 (d)	1445 (c + d)
<b>Total</b>	<b>487 (a + c)</b>	<b>1166 (b + d)</b>	<b>1653 (a + b + c + d)</b>

Table IV: Comparison of IgM ELISA and Weil Felix test at a titre of 640

	IgM ELISA + ve	IgM ELISA - ve	Total
Weil Felix + ve	71 (a)	0 (b)	71 (a + b)
Weil Felix - ve	416 (c)	1166 (d)	1582 (c + d)
<b>Total</b>	<b>487 (a + c)</b>	<b>1166 (b + d)</b>	<b>1653 (a + b + c + d)</b>

Table V: Comparison of IgM ELISA and Weil Felix test at a titre of 1280

	IgM ELISA + ve	IgM ELISA - ve	Total
Weil Felix + ve	41 (a)	0 (b)	41 (a + b)
Weil Felix - ve	446 (c)	1166 (d)	1612 (c + d)
<b>Total</b>	<b>487 (a + c)</b>	<b>1166 (b + d)</b>	<b>1653 (a + b + c + d)</b>

On comparison of IgM ELISA for Scrub Typhus and Weil Felix test it was seen that the Specificity increased from 72.5% at a diagnostic titre of  $OXK = 160$  to 100% at a titre of 640 where as Sensitivity decreased from 72.5% at a titre of 160 to

14.6% at a titre of 640. Similarly, positive predictive value of Weil Felix test increased from 77.9% at a titre of 160 to 100% at a titre of 1280 and negative predictive value of this test decreased from 88.8% to 72.3% as the titre reached 1280. The percentage of

false negatives and the false positives at the titre of 160 was 27.5% and 8.6% respectively. The percentage of false negatives increased to 91.6 % and false positives reached 0% at a titre of 1280. [Tables III, IV & V]

## DISCUSSION

The present study was done to evaluate and compare Weil Felix test in relation to IgM ELISA in the diagnosis of Scrub Typhus patients. In the study the mean age of the patients was  $30.93 \pm 17.02$  years and majority of the patients were in the age group of 11-40 years. This correlates well with the active years of life when people are mostly engaged in outdoor activities like farming and visits to forests and is in concordance to the study done by PK Sharma et al [8] which had the highest incidence in the age group of 30 – 44 years. Most of the patients were females (70%) in our study with a male: female ratio of 1:2.3 our findings were similar to the findings in the studies done by PK Sharma et al [8] and SS Kweon et al [9] which showed female preponderance of 54.8% and 64.5% respectively. The present study had 82.5% patients from rural background which goes well with other studies.

In our study the cases started to rise in the month of August and maximum cases (73%) were reported in the months of August to November corresponding to the monsoon season in our area. This type of seasonal trend was also seen by MVS Subhalaxmi et al [10] and in their studies where the confirmed cases peaked in autumn.

IgM ELISA for Scrub Typhus was positive in 29.46% (487/1653) patients. This finding is in concordance to the findings by P. Sinha et al [11] and KPS Narvencar et al [12] who reported 24.7% and 34% positivity in their respective studies. A. Mahajan et al [13] in an outbreak in 2009 in Jammu reported 74% patients positive by IgM ELISA for Scrub Typhus which was very much higher than our study. The Weil Felix test had positivity of 27.4% (453/ 1653) for

OXK antigen at a titre of  $\geq 160$ . The positivity rate of Weil Felix test is less than positivity rate detected by IgM ELISA for scrub Typhus whereas K. Usha et al [14] reported the prevalence of antibodies to Scrub Typhus by Weil Felix test and IgM ELISA as 56.42% and 58.21%. Among the IgM ELISA for Scrub Typhus positive patients, Weil Felix test for OXK antigen was positive ( OXK  $\geq 160$ ) in 353 patients and in IgM ELISA for Scrub Typhus negative patients, 100 patients were positive by Weil Felix test at a diagnostic titre of  $\geq 160$ .

Considering IgM ELISA for Scrub Typhus as gold standard and a titre of  $\geq 160$  diagnostic for Scrub Typhus, the sensitivity and specificity of Weil Felix test in relation to IgM ELISA is 72.5% and 91.4%. With an increase in titre of Weil Felix test, sensitivity of the test decreased and specificity increased. With a single serum sample available for diagnosis, the Weil Felix test is suggestive of infection only at high cut off titre of 320. In our study also, at a cut off titre of 320 the specificity of Weil Felix test positive predictive value was 97% and 83.2% respectively. Prakash et al [15] reported a sensitivity and specificity of 43% and 98% respectively for titres of 80 or more. The sensitivity of IgM ELISA reported by this study was 86.5%.

A good correlation exists between the results of Weil Felix test and IgM ELISA. [14] According to Isaac et al [16] the specificity of the Weil Felix test is high even at low titres and they suggested that the patients should be evaluated for Scrub Typhus even at low titres. However, the present study showed that higher the antibody titre for OXK antigen, the more is the specificity of the Weil Felix test.

With a single serum sample available for diagnosis, the Weil Felix test is suggestive of infection only at a high cut off titre of 320. [3,17] At high cut off titres, the positive predictive value and specificity are reliable. [17] In the present study also, at a cut off titre of 320, the specificity of Weil Felix test and positive predictive value was

97% 83.2% respectively.

Thus, though the Weil Felix test is not a sensitive test, but when positive in high titres, it is found to be very specific test. Its use is acceptable in resource constrained areas where more sensitive and specific tests like IFA, IgM ELISA are not available. The Weil Felix test needs to be interpreted in the light of correct clinical context and antibody titres.

## CONCLUSION

The present study used Weil Felix test and IgM ELISA for diagnosing Scrub Typhus. On evaluating Weil Felix test against IgM ELISA for Scrub Typhus, it was found that at high titres of antibodies against OXK antigen, the specificity and the positive predictive value of Weil Felix test is very high. Hence, Weil Felix test can be used in peripheral institutions for initial screening where tests like IFA, IgM ELISA for Scrub Typhus, PCR etc are not available. This will decrease the cost in diagnosis and treatment of the disease.

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