

Rickettsial Diseases: A Study Evidenced by Weil-Felix Test in a Tertiary Care Hospital

K S Rashmi¹, Neetha S Murthy², K L Ravikumar³

¹Associate Professor, Department of Microbiology, Kempegowda Institute of Medical Sciences, Bengaluru, Karnataka, India, ²Postgraduate Student, Department of Microbiology, Kempegowda Institute of Medical Sciences, Bengaluru, Karnataka, India, ³Professor Emeritus, Department of Microbiology, Central Research Laboratory, Kempegowda Institute of Medical Sciences, Bengaluru, Karnataka, India

Abstract

Background: Rickettsial diseases are among the most obscured re-emerging arthropod borne zoonotic infections that are being increasingly recognized as one of the causes of pyrexia of unknown origin (PUO). Presenting with varied and non-specific symptoms, ignorance and low index of suspicion, they are often under diagnosed due to the unavailability of the reliable diagnostic test. Appropriate diagnosis in early stages is necessary to prevent the morbidity and mortality associated with these infections.

Objective: Present study attempts to understand the scenario of rickettsial infections causing acute febrile illness and to categorize rickettsial disease titers by Weil-Felix test (WFT) in our tertiary care hospital.

Materials and Methods: A total of 133 cases with acute undifferentiated fever were included in the study. These samples were subjected to qualitative slide agglutination and quantitative tube agglutination by WFT and interpreted along with clinical data.

Results: A total of 29 out of the 133 cases (21.8%) tested positive for rickettsial infections by the WFT. Out of 29 cases, 16 (55.17%) were seropositive for scrub typhus, 3 (10.34%) for spotted fever, and 2 (6.89%) for typhus fever. The remaining 8 (27.58%) samples showed mixed titers.

Conclusion: In view of its significance in timely diagnosis, treatment, and prevention of life-threatening complications in clinically compatible cases of PUO, WFT should not be disregarded as this test can be easily set up with a moderate level of infrastructure and expertise.

Key words: Rickettsial diseases, Scrub typhus, Spotted fever, Typhus fever, Weil-Felix test

INTRODUCTION

The family Rickettsiaceae encompasses obligate intracellular rods belonging to the subgroup Alphaproteobacteria. The members of this family are aerobic Gram-negative non-flagellate pleomorphic *Coccobacilli* adapted to parasitize ticks, lice, fleas, mites, chiggers, and mammals. Family Rickettsiaceae comprises of the following genera - *Rickettsiae*, *Ehrlichia*, and *Orientia*. These zoonotic pathogens cause infections that disseminate in the blood to many organs.¹ Rickettsial diseases have been documented

in India since the 1930s; many cases have been reported from Madhya Pradesh, Maharashtra, Karnataka, Tamil Nadu, Kerala, Jammu and Kashmir, Himachal Pradesh, Uttaranchal, Rajasthan, West Bengal, and Assam.²⁻⁴

Rickettsial diseases are veiled re-emerging infections, which pose a serious threat to public health if not meticulously diagnosed. Rickettsial infections are enlisted among the etiological factors attributed to cause pyrexia of unknown origin (PUO) and thereby require to be differentiated from other febrile illnesses such as enteric fever, malaria, dengue, leptospirosis, and infectious mononucleosis.⁵ The lack of data regarding geographical distribution and the low index of suspicion due to non-specific clinical manifestations poses a challenge to the physician and hinders the diagnosis.

Tests available to diagnose rickettsiosis are culture, serology including immunofluorescence, and molecular tests. Culture is extremely strenuous, hence impractical

Access this article online



www.ijss-sn.com

Month of Submission : 09-2015
 Month of Peer Review : 10-2015
 Month of Acceptance : 11-2015
 Month of Publishing : 11-2015

Corresponding Author: Dr. K S Rashmi, D1, Foot Prints, Good Earth Malahar, Doddabele Road, Kambipura, Bengaluru - 560 060, Karnataka, India. Phone: +91-9845269790. E-mail: rashminaga05@gmail.com

for routine diagnostic application. Except serological diagnosis, other tests are beyond the reach of most diagnostic laboratories.⁶ Serological tests, such as Weil-Felix test (WFT), latex agglutination, indirect hemagglutination, immunoperoxidase assay, Enzyme Linked Immunosorbent Assay (ELISA), and micro immunofluorescence (“gold standard”), can be used in laboratory evaluation of suspected rickettsial infections.⁷ Many cross-reactions are observed, and explicit species determination of the infecting agent is difficult. Western blot may be more specific in early sera. Cross-absorption may help to resolve the problem of cross-reaction, but it is technically demanding and expensive. Nested polymerase chain reaction (PCR) is the standard molecular diagnostic method for testing of blood and fresh tissue specimens at the centers for disease control and prevention.⁸ Real-time quantitative PCR and nested PCR are targeted at the gene encoding the major 56 Kda and/or 47 Kda surface antigen gene, which is valuable in serum testing of rickettsial diseases.¹

The present study was undertaken in our tertiary care institute with the intention to present the scenario of rickettsial infections as a cause of PUO. In the present study, we have used WFT in the diagnosis of rickettsial infections. WFT is a non-specific heterophile tube agglutination test in which antibodies against rickettsiae are detected using a heterophile *Proteus* antigen.

MATERIALS AND METHODS

A prospective hospital-based study was conducted in the Department of Microbiology of a teaching hospital between February 2014 and March 2015. The total of 133 patient samples were included in the study.

Inclusion Criteria

Patients hospitalized with undiagnosed fever; presenting with one or more of the following clinical features: Rash, edema, hepatosplenomegaly, lymphadenopathy, eschar, and tick or flea exposure were included in the study.

Exclusion Criteria

Patients treated on outpatient basis and patients with a known cause of fever at the time of admission were excluded from the study.

After following proper aseptic precautions, each patient's blood was collected in a sterile vacutainer by venipuncture in the laboratory. The patient's blood sample was centrifuged and the serum thus obtained was subjected to WFT (PROGEN, Tulip diagnostics Pvt. Ltd.) tested by qualitative slide agglutination and quantitative tube agglutination test according to standard protocols with doubling dilution

of 1:20-1:160, for initial screening followed by further dilutions to achieve end titer.⁸ Positive samples were also correlated with other tests like Widal test, Dengue IgM ELISA and the presence of *Proteus* infection by urine culture of the patients. Statistical calculations were done using percentage analysis and Chi-square test.⁹

RESULTS

Based on the baseline titer of rickettsial diseases in this geographical area, OX-K, OX-19, and OX-2 titers of 160 and above were considered significant.⁵

Out of 133 samples, 32 blood samples showed slide agglutination. 3 samples tested positive qualitatively by slide agglutination but were negative by tube agglutination; hence, they were considered equivocal, and the test was repeated using fresh serum samples from the respective patients after 1 week and confirmed to be negative. The 29 (90.62%) of 32 samples were regarded significant based on titers obtained by tube agglutination test. Out of 29 significant titers, 16 (61.53%) samples were significant for OX-K antigen and thus for scrub typhus.

The significant titers for OX-2 and OX-19 were 12 (41.37%) and 5 (17.24%), respectively (Table 1). Based on OX-2 and OX-19 titers 3 samples were suggestive of spotted fever (9.3%), 2 samples suggestive of typhus fever (6.25%), and 8 samples yielded mixed titers (25%) making it difficult to interpret the results (Table 2).

Age-wise analysis of the positive cases evidenced 8 cases in pediatric age group (0-12 years), 2 cases in adolescent (13-20 years), 16 cases in adult (21-64 years), and 3 cases in elderly (>65 years) age group with $P = 0.0007$ (Figure 1).¹⁰ Among 29 samples, 20 (68.96%) were males and 09 (31.03%) were females with $P = 0.041$ (Figure 2).

Table 1: Titers obtained by Weil-Felix tube agglutination

Titers	1:640	1:320	1:160
OX-K	2	7	7
OX-2	-	4	8
OX-19	2	2	1

Table 2: Split up of the titers obtained

OX 19	OX 2	OX K	Number	Diagnosis
-	-	Positive	16	Scrub typhus
Positive	Positive	-	3	Spotted fever
Positive	Positive/negative	-	2	Typhus fever
Positive/negative	Positive/negative	-	8	Mixed titers

All the seropositive patients presented with fever among whom 55% had rashes; 27% had hepatomegaly, and 20% had splenomegaly. Table 3 explains the relationship between clinical presentation and test positivity.

Among the samples showing mixed titers, only one sample was positive for Salmonella, and two samples were positive for dengue (Table 4). Dengue IgM was detected by ELISA. Dengue PCR to confirm the diagnosis could not be done due to lack of availability. The Widal positive sample showed titers of 1:160 for Typhi O and H and <1:80 for paratyphoid AH and BH. Blood culture for isolation of typhoid bacilli was performed on this Widal positive sample by using automated BACTEC 9050, and it turned out to be negative. All the patients tested negative for urine Proteus.

DISCUSSION

In recent years, Rickettsial diseases are one among the re-emerging radices of acute undifferentiated febrile illness in several parts India.¹¹⁻¹³ India being a developing country; there is scarce data and insufficient research on rickettsial diseases. The clinical diagnosis of Rickettsial

diseases is intricate and cumbersome. Laboratory culture of this organism is not endorsed due to high chances of laboratory-acquired infections to the handling personnel. Therefore, the diagnosis of rickettsial infections depends on serological and molecular diagnostic techniques. Serological tests include latex agglutination, immunofluorescence, indirect hemagglutination, ELISA, and WFT.⁴ Molecular diagnostic techniques, such as the PCR for detection of rickettsial DNA although available, are not realistic due to cost related issues.

WFT depends on a fortuitous similarity of certain carbohydrate antigenic determinants, which occur in most species of pathogenic rickettsia species and in the OX-19, OX-2 and OX-K strains of *Proteus vulgaris* and *Proteus mirabilis*.⁹ The test has too many stumbling blocks, and irrelevant results can be seen in many other conditions. False positive results have been obtained in cases with Salmonellae, *Streptococcus pyogenes*, and Proteus infection.¹⁴ The importance of this test, though being not a standard test, is still reasonably good when it comes to evaluating pyrexia of unknown origin (PUO). In the present study, the agglutination of OX-2 and OX-19 were seen in 12 (41.37%) and 05 (17.24%) patients, respectively. Agglutination of OX-K was seen in 16 (55.17%) patients.

WFT, when performed in conjunction with other tests, such as Widal, Dengue IgM ELISA, and urine culture for Proteus infection, can eliminate the false positive cases. In the present study, out of 29 positive samples, only three were positive for typhoid and dengue; however, false positivity could not be entirely ruled out due to the absence of a specific test.

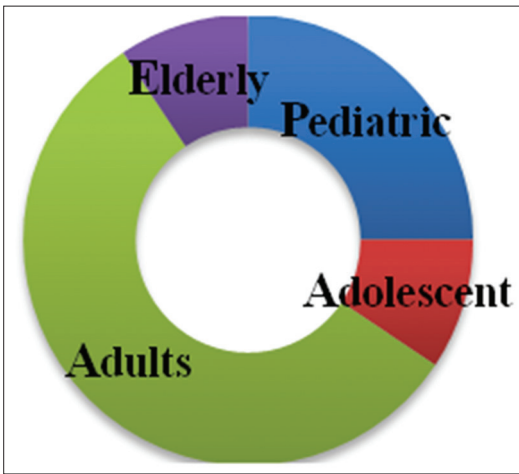


Figure 1: Age-wise split up of patients with significant titers (P = 0.0007)

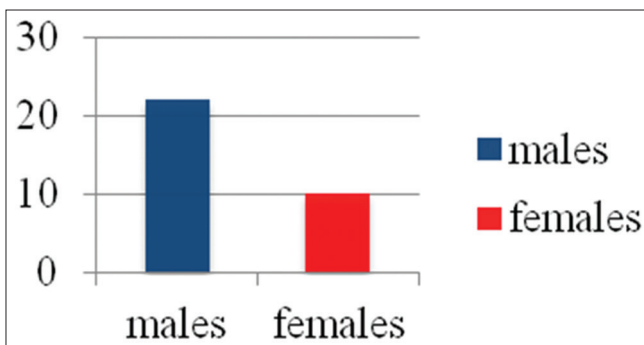


Figure 2: Male:female ratio of patients with significant titres (P value = 0.041)

Table 3: Clinical features in patients with positive WFT

Clinical features	Number of cases
Fever	29
Hepatomegaly	9
Splenomegaly	6
Rash/eschar	16

WFT: Weil-Felix test

Table 4: Tests done to rule out other causes and check the correlation with WFT

Tests	Number of samples (n=29)	
	Positive	Negative
Widal	01	28
Dengue IgM test	02	27
Proteus isolation	00	29
Chikungunya	00	29
Leptospiira	00	29
Malaria	00	29

WFT: Weil-Felix test



Figure 3: Eschar in a scrub typhus patient

Undiagnosed fever was the major inclusion criteria for the study. The other symptoms associated with fever also played a role in clinical suspicion towards a diagnosis of rickettsial infections. Among the 133 patients tested as part of the study, rashes and eschars were seen in 16 of the 29 Weil-Felix positive cases (55.17%). Figure 3 shows a typical eschar which was found on the cheek of a 48 year old female patient with OX-K titre of 1:320 and OX-2, OX-19 titres less than 1:80. However, all patients showing a positive WFT did not show a characteristic rash. We noticed that 09 patients who had fever and rash showed the negative result with WFT. A study by Udayan *et al.* showed 61.7% association⁷ and a study by Mittal *et al.* showed 51.7% association of rashes with positive WFT.⁵ Hepatomegaly was evidenced by 9 patients who showed OX-K levels >1:320. This was in correlation with the study by Udayan *et al.*⁷ However, only 6 of these patients showed splenomegaly.

Failure in early diagnosis is associated with significant mortality and morbidity. The fulminant course of rickettsial infections can lead to life-threatening manifestations such as disseminated intravascular coagulation, meningoencephalitis syndrome, acute renal failure, hepatic failure, non-cardiogenic pulmonary edema, interstitial pneumonitis, and myocarditis.⁴ The therapy is affordable and yields dramatic results when diagnosed early in the course of the disease. Epidemiological features, history of exposure to vector, and a high index of suspicion are crucial aids toward accurate diagnosis.^{4,5}

CONCLUSION

Persistence of fever even after 48 h, the presence of rash and tick exposure with altered biochemical parameters

should alert the clinician toward rickettsial diseases. Rickettsial diseases can cause severe illness and even death in otherwise healthy adults and children; however, if timely treatment with doxycycline or third generation cephalosporins is instituted the adverse consequences can be well averted.

Thus, when proper precautions are taken with respect to the use of standardized antigen and inclusion of positive serum controls, the WFT can help in establishing the presumptive diagnosis of rickettsial disease. When this is teamed alongside with clinical correlation of patient's signs and symptoms, there can be successful, cost-effective diagnosis and treatment.

REFERENCES

1. Didier R. Introduction to rickettsioses and ehrlichioses. In: Gerald M, John B, Raphael D, editors. Mandell, Douglas, and Bennett's: Principles and Practice of Infectious Diseases. 6th ed., Vol. 2. Philadelphia, PA: Churchill Livingstone, Elsevier; 2005. p. 2284-9.
2. Blewitt B. Fevers of the typhus group in the Bhimtal area of Kumaon Hills. J Royal Armed Corps 1938;70:241-5.
3. Kalra SL, Rao KN. Typhus fevers in Kashmir State. Part II. Murine typhus. Indian J Med Res 1951;39:297-302.
4. Rathi N, Rathi A. Rickettsial infections: Indian perspective. Indian Pediatr 2010;47:157-64.
5. Mittal V, Gupta N, Bhattacharya D, Kumar K, Ichhpujani RL, Singh S, *et al.* Serological evidence of Rickettsial infections in Delhi. Indian J Med Res 2012;135:538-41.
6. Sharma A, Mahajan S, Gupta ML, Kanga A, Sharma V. Investigation of an outbreak of scrub typhus in the Himalayan region of India. Jpn J Infect Dis 2005;58:208-10.
7. Udayan U, Dias M, Machado S. A hospital based study of Rickettsial diseases evidenced by Weil Felix test in a tertiary care hospital. CHRISMED J Health Res 2014;1:150-3.
8. Marmion BP, Worswick DA. *Coxiella burnetii* and other medically important member of the family Rickettsiace. In: Colle JG, Mermion MP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology. 14th ed. New York: Churchill Livingstone; 1996.
9. Stangroom J. Social Science Statistics, 2015. Available from: <http://www.socscistatistics.com/pvalues>. [Last cited on 2015 Oct 08].
10. Tortorice J. Age Specific Guidelines, 2007. Available from: <http://www.ceufast.com>. [Last cited on 2015 Oct 08].
11. Dass R, Deka NM, Duwarah SG, Barman H, Hoque R, Mili D, *et al.* Characteristics of pediatric scrub typhus during an outbreak in the North Eastern region of India: Peculiarities in clinical presentation, laboratory findings and complications. Indian J Pediatr 2011;78:1365-70.
12. Ahmad S, Srivastava S, Verma SK, Puri P, Shirazi N. Scrub typhus in Uttarakhand, India: A common rickettsial disease in an uncommon geographical region. Trop Doct 2010;40:188-90.
13. Chaudhry D, Garg A, Singh I, Tandon C, Saini R. Rickettsial diseases in Haryana: Not an uncommon entity. J Assoc Physicians India 2009;57:334-7.
14. Cruickshank R. The Weil-Felix reaction in typhus fever. J Hyg (Lond) 1927;27:64-9.

How to cite this article: Rashmi KS, Murthy NS, Ravikumar KL. Rickettsial Diseases: A Study Evidenced by Weil-Felix Test in a Tertiary Care Hospital. Int J Sci Stud 2015;3(8):128-131.

Source of Support: Nil, **Conflict of Interest:** None declared.