International Journal of Contemporary Pediatrics
Kulkarni T et al. Int J Contemp Pediatr. 2018 Jul;5(4):1244-1248
http://www.ijpediatrics.com
pISSN 2349-3283 | eISSN 2349-3291

DOI: http://dx.doi.org/10.18203/2349-3291.ijcp20182059

Original Research Article

Prospective study with rapid and low cost serodiagnostic assay for rickettsial infections in children of Vijayapura district, Northern Karnataka, India

Trimal Kulkarni[1]*, Govind Benakatti[2], Laxman Bidari[3]

[1]Department of Pediatrics, Al-Ameen Medical College, Vijayapura, Karnataka, India
[2]Pediatric Intensive Care Unit, NMC Royal Hospital, Abu Dhabi, U.A.E.
[3]Department of Pediatrics, Ashwini Institute of Child Health and Research Center, Vijayapura, Karnataka, India

Received: 28 April 2018
Accepted: 08 May 2018

***Correspondence:**
Dr. Trimal Kulkarni,
E-mail: trimal.kulkarni@gmail.com

ABSTRACT

Background: Rickettsial infections do exist in study area posing diagnostic difficulties. The study is aimed to compare performance of serological assays for rapid and low-cost diagnosis for rickettsial infections in northern Karnataka, India.
Methods: Prospective study was done on 40 children upto 12 years old, hospitalized during 1-year period with fever and presence of one or more of the clinical features of rickettsial infections. Clinical and biochemical findings and serological assays such as indirect immunofluorescence assay (IFA), enzyme-linked immunosorbent assay-IgM (ELISA-IgM) and Weil-Felix were used to diagnose the disease. Statistical analysis was used to compare the results. Performance characteristics such as sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated using MedCalc for Windows.
Results: All 40 patients met the inclusion criteria (23 males and 17 females). Mortality was 2%. Predominant age group was 1-3 years (57.50%). Fever, rashes and hepatosplenomegaly was in all 40 patients (100%); whereas other clinical features showed mixed results. Biochemical findings: anemia in 90%, thrombocytopenia in 45% and elevated transaminases 57.50% and 55%. The ELI-SA-IgM assay showed a sensitivity of 95.00%, a specificity of 95.24% and 95.12% accuracy. The Weil-Felix assay showed a sensitivity of 77.50%, a specificity of 81.63% and 79.78% accuracy. ELISA-IgM test showed only 5% (p=0.5555) and Weil-Felix test showed 12.50% (p=0.3816) non-significant difference when both compared with IFA test. Whereas ELISA-IgM showed 17.50% more significant (p=0.0239) when compared with Weil-Felix.
Conclusions: ELISA-IgM may serve as rapid and low cost serodiagnostic assay over IFA for rickettsial infections.

Keywords: ELISA-IgM, IFA, Northern Karnataka, Rickettsial infections, Weil-Felix assay

INTRODUCTION

Rickettsial infections are very endemic in India and one of the emerging and reemerging infections of public health importance known since early 90s.[1,2] Rickettsial infections are very common cause of acute febrile illness in children with varied manifestations and severity and common cause of differentials among children with undifferentiated fever presenting to hospi-tals.[3] Rickettsial infections have been reported from various regions all over India over decades and first report was from Kumaon region as early as 1930.[4-7] The infecting sub-species, clinical syndrome and serological assays vary widely with geographical area.[8] Rickettsial

infections are classically divided into typhus fever group, spotted fever group and others (Q fever and Trench fever).

Scrub typhus is the predominant form reported from most regions of India, whereas murine typhus, Indian tick typhus and Q-fever are reported less often.[7] Since rickettsia infections are very sensitive to doxycycline antibiotics which is easily available, mostly they are treated empirically based on clinical profile before obtaining serological diagnosis/confirmation. Indirect immunofluorescence assay (IFA) is considered as gold standard for serological diagnosis and causative species identification.

Due to its unavailability and being expensive test, Weil-Felix assay is still the widely used serological method in India; despite its poor sensitivity and specificity.[7] Instead, ELISA- IgM assay (enzyme-linked immunosorbent assay for IgM antibody) was found to be more sensitive than Weil-Felix test as also revealed in studies of Kulkarni et al and Rathi et al.[9,10]

Therefore, a more reliable, non-inferior and lowcost alternative is needed for resource limited countries like India. Hence, the aim of study is to compare the performance of ELISA-IgM and Weil-Felix assay with respect to IFA for rapid and low cost serological diagnosis of rickettsial infections in children of Vijayapura a backward district of northern Karnataka, India.

METHODS

This was a prospective observational study in 40 children admitted with clinical diagnosis of rickettsial infections of any severity at tertiary care children's hospitals from Vijayapura district, north Karnataka between November 2011 to October 2012.

Detailed informed consent was obtained from parents/legal guardians of all study patients before enrolment. Institutional ethics committee approved the study protocol before implementation.

Inclusion criteria

- All patients less than 12 years admitted with clinical diagnosis of rickettsial infections were included in the study

Exclusion criteria

- Patients older than 12 years.
- Patients who had acute febrile illness with or without rash and with alternative diagnosis.
- Patients with conditions those may interfere with kinetics of immunogenicity.

Clinical and biochemical investigations

Case records of children admitted in inclusion criteria were analyzed. Basic details of patients like age, sex and mortality were recorded. Symptoms like fever, rash, tick exposure history, myalgia, seizures, altered consciousness and vomiting were also entered. Clinical findings like hepatosplenomegaly, pedal and facial edema, lymphadenopathy and eschar were observed.

Laboratory investigations in all patients included; anemia, thrombocytopenia, Abnormal AST (aspartate transaminase), and abnormal ALT (alanine transaminase). Cerebrospinal fluid analysis and neuroimaging were done in patients with neurological manifestations. Two dimensional (2D)-echo was done in patients with myocarditis and shock to see for decreased ejection fraction. Organ dysfunction like acute kidney injury was also investigated in patients.

Serological diagnostic assays

The serological tests were performed more than 7 days after the onset of the disease. IFA as-say: Indirect immunofluorescence assay (IFA) (LMN LAB, France through Metropolis) was done on 10 random cases (every fourth patient due to its high cost and difficulty in availability) with titre of 1:64 and above was considered diagnostic of rickettsial infections.

This test was used as the gold standard test in present study. ELISA-IgM assay: Enzyme-linked immuno-sorbent assay for IgM antibody (ELISA-IgM) (Vircell Microbiologists, Spain) was done on all cases and antibody index (optical density of serum sample / cut off optical density of serum sample x 10) of more than 11 was considered as positive and less than 9 as negative for rickettsial infections as per manufacturer's recommendation. Weil-Felix assay: Weil-Felix test (Plasmatec, United Kingdom) was done in all patients and titre of at-least 1:160 and above was considered as diagnostic of rickettsial infections.

This was repeated at time of discharge or in second week of illness if initial titre was below 1:160 to see rise in titres. All patients for rickettsial infections were treated with doxycycline (4mg/kg/day) being the most frequently used therapy.

Statistical analysis

The results were entered into SPSS version 22.0 (SPSS Inc, Chicago, Ill) for statistical analy-sis. The proportional comparisons between the assays were calculated using Chi-square test for p value. P value <0.05 was taken as level of significance. The sensitivities and specificities of the serodiagnostic assays were calculated using MedCalc for Windows, version 13.3 (MedCalc Software, Ostend, Belgium).

RESULTS

During the study period, sera from 40 patients with suspected of having rickettsial infections were evaluated. All the 40 patients met the inclusion criteria. Table 1 depicts, male patients were predominant than female with a gender ratio of 23:17 (23 males and 17 females).

Majority rickettsial infections were diagnosed in age group of 1-3 years which was 57.50% followed by age groups of 4-6 years (20%), 7-12 years (17.50%) and <1 years (5%).

Table 1: Gender, age and mortality details.

Investigations	No. of patients (n=40)	%
Gender		
Male	23	57.50
Female	17	42.50
Age		
<1 year	2	5
1-3 year	23	57.50
4-6 year	8	20
7-12 year	7	17.50
Mortality	2	5

Two out of the 40 patients (5%) under study expired during the study period. In both of these patients Weil-Felix and ELI-SA-IgM (enzyme-linked immunosorbent assay for IgM antibody) assay were positive.

The clinical features like fever, rashes and hepatosplenomegaly were observed in all the 40 patients (100%) followed by tick exposure 90%; whereas other clinical features were showing mixed results as shown in Table 2.

The biochemical findings in Table 3 depicts, there was anemia in 38 patients (90%), thrombo-cytopenia in 18 patients (45%), abnormal AST (elevated aspartate transaminase, above 100 IU/L) in 23 patients (57.50%) and abnormal ALT (elevated alanine transaminase, above100IU/L) in 22 patients (55%).

Table 2: Distribution of clinical feature.

Clinical features	No. of patients (n=40)	%
Symptoms		
Fever	40	100
Rash	40	100
Tick exposure	36	90
Myalgia	11	27.5
Seizures	8	20
Altered consciousness	6	15
Vomiting	5	12.5
Other findings		
Hepatosplenomegaly	40	100
Pedal and facial edema	11	27.5
Lymphadenopathy	5	12.5
Eschar	4	10
Cerebrospinal fluid pleocytosis	11	27.5
Decreased ejection fraction	7	17.5
Encephalitis	9	22.5
Myocarditis	7	17.5
Shock	3	7.5

Table 3: Distribution of biochemical findings.

Biochemical findings	No. of patients (n=40)	%
Anemia	38	90
Thrombocytopenia	18	45
Abnormal AST (aspartate transaminase) (AST > above 100IU/L)	23	57.50
Abnormal ALT (alanine transminase) (ALT > above 100IU/L)	22	55

Table 4: Performance of serodiagnostic assays in positive patients for rickettsial infections.

Assays	Sensitivity (%) (95%CI)	Specificity (%) (95%CI)	PPV (%) (95%CI)	NPV (%) (95%CI)	Accuracy (%)
ELISA-IgM assay in IFA positive patients	95.00 (83.08-99.39)	95.24 (83.84-99.42)	95.00 (83.06-98.66)	95.24 (83.80-98.72)	95.12
Weil-Felix assay in IFA positive patients	77.50 (61.55-89.16)	81.63 (67.98-91.24)	77.50 (65.10-86.42)	81.63 (71.12-88.91)	79.78

ELISA-IgM: Enzyme-linked immunosorbent assay for IgM antibody; IFA: Indirect immunofluorescence assay; CI: Confidence interval; PPV: Positive predictive value; NPV: Negative predictive value

In Table 4, the indirect immunofluorescence assay (IFA) was used as the gold standard test with which the other assays were compared. The Weil-Felix assay had a sensitivity of 77.50%, a specificity of 81.63%, a positive predictive value of 77.50%, and a negative predictive value of 81.63% and an accuracy value of 79.78% in the serum samples of all 40 patients. In Table 5, ELISA IgM assay showed only 5% non-significant proportional difference when compared with gold standard IFA assay (p=0.5555).

Weil-Felix assay showed 12.50% non-significant proportional difference as compared to gold standard IFA assay (p=0.3816). The 17.50% significant proportional difference was found when compared ELISA IgM assay with Weil-Felix assay (p=0.0239).

Table 5: Comparison of serodiagnostic assays in positive patients for rickettsial infections.

Assays	No. of positive patients (%)	Difference (%)	Chi-square test	p value
ELISA-IgM assay verses IFA assay	38 (95%) verses 9 (90%)	5	0.348	0.5555 [Non-significant]
Weil-Felix assay verses IFA assay	31 (77.50%) verses 9 (90%)	12.50	0.766	0.3816 [Non-significant]
ELISA-IgM assay verses Weil-Felix assay	38 (95%) verses 31 (77.50%)	17.50	5.100	0.0239* [Significant]

ELISA-IgM: Enzyme-linked immunosorbent assay for IgM antibody; IFA: Indirect immunofluorescence assay. The p value of <0.05 was considered statistically significant difference and represented by asterisk '*'

DISCUSSION

Rickettsial fever is an under-diagnosed zoonotic disease in north Karnataka due to lack of awareness among physicians and unavailability of specific laboratory tests. Rickettsial diseases are reported in various parts of India, this is the first study adding its existence in Vijayapura district a backward region of north Karnataka, south India.[4,5,7,10]

Spotted fever patients have peculiar clinical presentation like fever with maculopapular rash, hepatosplenomegaly, edema.[11,12] Almost all patients were diagnosed to have rickettsia conorii infections as against most of the other studies like Kamasaru et al which documented scrub ty-phus in south India.[13] In present study, age group of 1-3 years showed maximum prevalence. Rickett-sia conorii was the species identified in all. Cause of death in first patient of present study was myo-carditis and the other patient died due to pulmonary edema even after being ventilated. Majority of patients presented with fever, maculopapular, erythematous rash even involving palms and soles and edema which was more in comparison to Colombo et al Heptosplenomegaly was seen in 100% of patients, which was far more in comparison to Mahajan SK et al (43%).[7,14]

Seizures were seen in 20% in comparison to Mahajan SK et al (19%). Complications seen like encephalitis (22.5%), myocarditis (17.50%), and shock (7.50%) were more in comparison to Rathi et al conducted in Maharashtra, India.[7,11] Although, some patients deteriorated and got seizures, encephalitis, myocarditis but majority improved with oral doxcycline and in some patients with parentral chloramphenicolwas used for 7-10 days. Anemia, thrombocytopenia and elevated transaminases were the common abnormalities noted which were in comparison to Rathi et al and other important findings were of reduced ejection fraction on echo and lymphocytic pleocytosis.[8]

Until now, laboratory diagnosis of Rickettsial infections was largely relied on serological as say using immunofluorescence detecting specific antibodies from acute and convalescent phase serum samples. Indirect immunofluorescence assay (IFA) is rarely available in India, is expensive, and can take more than a week to get the results. However, there is a trend toward the use of enzyme-linked immunosorbent assay-IgM (ELISA-IgM) to replace IFA due to its low cost, rapid, high sensitivity, specificity and simplicity. In present study, ELISA-IgM assay method revealed almost similar performance characteristics (Table 4) and only 5% comparison difference (Table 5) with IFA assay.

Therefore, analysis of specific IgM antibodies based on ELISA may be the new standard assay for the detection and differentiation of rickettsial infections in patients of north Karnataka region of south India. Further, present study revealed that Weil-Felix assay was not a specific diagnostic test and other studies also proposed the similar outcomes.[15,16] In present study ELISA-IgM assay showed statistically significant high sensitivity, specificity, accuracy (Table 4) and pick up rate more 17.50% of patients positive when compared to Weil Felix with p=0.0239 than Weil-Felix assay (Table 5). The studies of Kulkarni A et al and Rathi et al also support our findings when compared to the gold standard IFA.[9,15]

We recognize several limitations of the cur-rent study report. Although this study is a prospective performed at a single center pediatric tertiary care hospital reporting data from patients who were hospitalized with fever of undetermined source and other clinical features commonly seen with rickettsial infections.

More studies and larger multi centric trials will be required to know the prevalence of rickettsiosis in the entire north Karnataka region of south India. IFA is the most commonly used technique, but it can be replaced by ELISA-IgM assay with similar sensitivity and specificity

results. Therefore, we pro-pose ELISA-IgM assay is a non-inferior low-cost alternative to IFA in resource limited countries and it should be widely used.

CONCLUSION

Enzyme-linked immunosorbent assay for IgM antibody (ELISA-IgM) is similar like indirect immunofluorescence assay (IFA) and more sensitive and specific than Weil-Felix assay in diag-nosis of rickettsial infections. The ELISA-IgM assay may be used as rapid and low cost serodi-agnostic assay for early detection, treatment and prevention of mortality and morbidity from rickettsial infections in children of Vijayapura district, northern Karnataka, India.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Rathi N, Rathi A. Rickettsial infections: Indian perspective. Indian Pediatr. 2010;47:157-64.
2. Parola P, Paddock C, Raoult D: Tickborne rickettsioses around the world: emerging diseases challenging old concepts. Clin Microbiol Rev. 2005;18:719-56.
3. Phongmany S, Rolain J-M, Phetsouvanh R, Blacksell SD, Soukkhaseum V, Rasachack B, et al. Rickettsial Infections and Fever, Vientiane, Laos. Emerg Infect Dis. 2006;12:256-262.
4. Batra HV. Spotted fevers and typhus fever in TamilNadu-commentary. Indian J Med Res. 2007;126:101-3.
5. Padbidri VS, Rodrigues JJ, Shetty PS. Tick-bornerickettsiosis in Pune district, Maharashtra, India. Int J Zoonoses. 1984;11:45-52.
6. Sirisanthana V, Puthanakit T, Sirisanthana T. Epidemiologic, clinical and laboratory features of scrub typhus in thirty Thai children. Pediatr Infect Dis. J 2003;22:341-5.
7. Mahajan SK, Kashyap R, Kanga A, Sharma V, Prasher BS, Pal LS. Relevance of Weil-Felix test in diagnosis of scrub typhus in India. J Assoc Physicians India. 2006;54:619-21.
8. Parola P, Paddock CD, Socolovschi C, Labruna MB, Mediannikov O, Kernif T, et al. Update on Tick-Borne Rickettsioses around the World: a Geographic Approach. Clin Microbiol Rev. 2013;26:657-702.
9. Kulkarni A. Childhood rickettsiosis. Indian pediatr. 2011;78:81-7.
10. Rathi M, Gupte MD, Bhargava A, Varghese GM, Arora R. DHR-ICMR Guidelines for diagnosis & management of rickettsial diseases in India. Indian J Med Res. 2015;141:417-22.
11. Anton E, Font B, Munoz T, Sanfeliu I, Sequra F. Clinical and laboratory characteristics of 144 patients with mediteranean spotted fever. Euro J Clin Microbial Infect Dis. 2003; 22:126-8.
12. Siberry GK, Dumler JS. Rickettsial infections. In: Kliegman RM, Behrman RE, Jenson HB, Stanton BF, eds. Nelson Textbook of Pediatrics. 18th ed. Pennsylvania: Saunders; 2007:1289-301.
13. Kamarasu K, Malathi M, Rajagopal V, Subramani K, Jagadeeshramasamy D, Mathai E. Serological evidence for wide distribution of spotted fevers & typhus fever in Tamil Nadu. Indian J Med Res. 2007;126:128-30.
14. Colombo C, Lawrence I, Saparito I, Polara V, Rubino IR, Titone L, et al. Mediterranean Spotted Fever : Clinical and laboratory characteristics of Sicilian children.BMC infect Dis. 2006;6:60.
15. Rathi NB, Rathi AN, Goodman MH, Aghai ZH. Rickettsial diseases in central India. Indian Pediatr. 2011;48:867-72.
16. Amano K, Hatakeyama H, Okuta M, Suto T, Mahara F. Serological studies of antigenic similarity between Japanese spotted fever rickettsiae and Weil-Felix test antigens. J Clin Microbiol. 1992;30:2441-6.

Cite this article as: Kulkarni T, Benakatti G, Bidari L. Prospective study with rapid and low cost serodiagnostic assay for rickettsial infections in children of Vijayapura district, northern Karnataka, India. Int J Contemp Pediatr 2018;5:1244-8.