



Original Research Article

A prospective study on Rickettsia infections in Central India

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ARTICLE INFO

Article history:

Received 10-02-2024

Accepted 13-04-2024

Available online 09-05-2024

Keywords:

Rickettsia

Central India

Real time PCR

Molecular diagnosis

ABSTRACT

Background: Rickettsial infections are under-reported in India. The actual disease burden has been underestimated as non-specific serological tests are mostly used to diagnose these infections.

Aim & Objective: The aim of this study was the detection of Rickettsia infections in clinical specimens by Real-time Polymerase Chain Reaction.

Materials and Methods: A study was established to analyse blood samples taken from pyrexia of unknown origin (PUO) or undifferentiated fever cases. A total of 69 such patients, were referred/walked-in to our diagnostic centre, Indore, India between October 2021 and May 2023. The blood samples were collected in EDTA lavender (purple) top tube.

Results: Of the tested samples, 14 patients (20.3%) were positive for Rickettsia infection. Gender wise distribution revealed higher incidence (57%) of Rickettsial infections in male patients. Children were majorly infected (50% of cases); multiple infection was also noted in them, though severity was not high. Severe clinical manifestation including hospitalization was also noted in some patients.

Conclusion: Few reports are available from India on molecular diagnosis of Rickettsia infection. Early-stage molecular diagnosis will help to stave off and prevent serious complications.

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1. Introduction

Rickettsia is a group of vector-borne organisms, spread by eukaryotic vectors like ticks, mites, fleas and lice that cause acute febrile illnesses throughout the world. Rickettsial infections are caused by a variety of obligate intracellular bacteria of the genus Rickettsia and are grouped into one of four categories: spotted fever group, typhus group, ancestral group, and transitional group. Geographical variations are observed in epidemiology and causative species; however, the clinical presentation of rickettsial infection is similar.¹

Rickettsia has been reported from many parts of the world.¹ In India, they are reported from Maharashtra, Tamil

Nadu, Karnataka, Kerala, Jammu and Kashmir, Uttaranchal, Himachal Pradesh, Rajasthan, Assam and West Bengal.²⁻⁴ Symptoms include fever, headache, muscle pain, cough and gastrointestinal problems.⁵ Rash, eschar, splenomegaly and lymphadenopathies are also common. Diagnosis is difficult in this infection due to the low index of suspicion, non-specificity of signs and symptoms.^{6,7} Serological tests for diagnosis become positive around a week after onset of fever whereas early diagnostic tests like polymerase chain reaction (PCR) are not freely available thus, making the therapy dubious even if suspected by the clinicians.

The purpose of the study was to identify Rickettsial infections from referred pyrexia of unknown origin (PUO) patients in Madhya Pradesh, India through molecular diagnosis.

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2. Materials and Methods

2.1. Study design

This present prospective study of Rickettsial cases was diagnosed through Real time PCR at our Molecular Biology Department during October 01, 2021, to May 31, 2023. During this period, the Molecular Biology laboratory received 69 samples from patients with fever $\geq 99^{\circ}\text{F}$ and fever duration ≥ 5 and ≤ 15 days. For Fever panel, patients were referred from both rural and urban areas of Madhya Pradesh, India. Standard investigations for common pathogens causing AFI in India (malaria, dengue, and enteric fever) were also carried out.

2.2. Sample collection and processing

The blood samples were collected by in a sterile EDTA (ethylenediaminetetraacetic acid) tube. The cold chain was maintained for transportation of the samples till it reached the lab. For isolation of nucleic acid, 500 microlitre of separated plasma was used. Total DNA was extracted by using total Nucleic Acid extraction kit [Cat. No. 3B207, 3B Black Bio Biotech India Ltd.] as per the manufacturer's instructions. The aliquots of plasma were stored at -20 degree Celsius for further use. Tropical Fever kit [Cat.No. 3B1423/3B1424, 3B Black Bio Biotech India Ltd.] was used for the detection of Rickettsia DNA in clinical samples. Twenty-seven Rickettsia sp were included for inclusivity analysis by the manufacturer. Both positive and negative control along with internal control were run in each batch. The primer sequences were aligned with all available reference genomes in NCBI database using BLAST package (Version 2.9.0). The Real time PCR was programmed at Quant Studio 5, Applied Biosystem, with 50°C for 20 min, followed by initial denaturation of 94°C for 10 min for 1 cycle and 40 cycles for denaturation for 94°C for 15 s., annealing and fluorescence measurement at 59°C for 15 s and extension at 72°C for 15s.

3. Result

A total of 14 patients (14/69; 20.3%) were positive for Rickettsia infection. Age and gender wise distribution of Rickettsia positive patients are presented in Table 1. The patients positive for Rickettsial infection, were also evaluated noting their clinical history (Table 1).

In our study, the positive patients were from rural areas of Madhya Pradesh, India. Rickettsial infections were detected throughout the year though significantly [$p < 0.01$] higher number of the cases were detected during monsoon and post monsoon period from June to November [06/14; 42.8%] (Figure 1). Gender wise distribution revealed a male preponderance [08/14; 57%], though it was not significantly high ($p < 0.01$) (Figure 2). A significant number of positive cases were detected below 18 years of age [07/14; 50%];

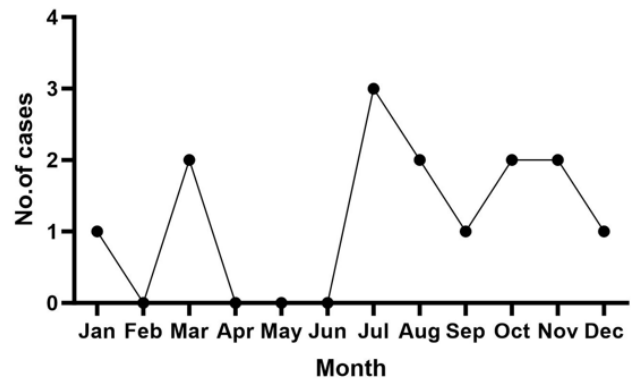


Figure 1: Monthly distribution of Rickettsia infection. Cases were higher during the monsoon period (July-August)

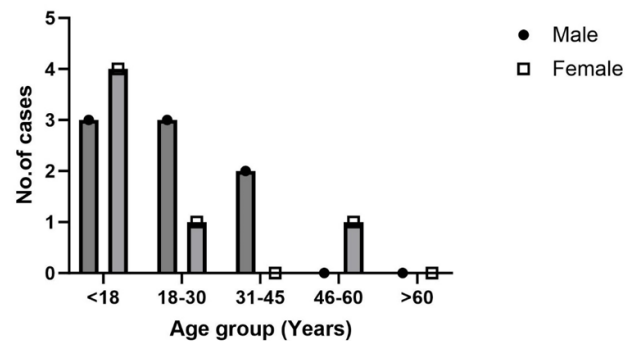


Figure 2: Age and gender wise distribution of Rickettsia positive cases. Children (<18 years) are mainly affected by the infection

of them three were males and four were females. Cases were not detected in older age groups [> 60 years of age]. Clinical investigations referred by the Clinicians were also investigated in the Pathology Division (Table 1). Clinical history revealed the simultaneous detection of Salmonella sp. in two male children, aged below 10 years; C reactive proteins were much higher in one of them [73 mg/L]. A two-year old female child reported leukocytopenia, though CRP was not done in this patient. Two patients were referred from Chattarpur, Madhya Pradesh, reported severe comorbid conditions with Thrombocytopenia and complications leading to hepatorenal failure.

4. Discussion

Human rickettsioses have been giving recognition in the Asia-Pacific region including Taiwan, Vietnam, Bhutan, and Malaysia for clinical management.^{3,8} Till now broad studies have not been conducted in India to determine the incidence per million people and the annual case fatality rate for these diseases. Recent data from the Central part

Table 1: Demographic and clinical presentation of Rickettsia positive cases

Characteristics	No. of cases	Percentage (%)
Gender		
Male	8	57.14
Female	6	42.86
Age (in years)		
<18	7	50.00
18-30	4	28.57
31-45	2	14.29
46-60	1	7.14
>60		
Current symptoms		
Fever (recurrent)	14	100.00
Rash	0	0.00
Myalgia	5	35.71
Cough	0	0.00
Leucocytosis	1	7.14
Thrombocytopenia	1	7.14
Hepatorenal failure	1	7.14
Ketonuria	1	7.14
High NLR ratio	1	7.14
Admission to hospital	3	21.43

of India is also not available. Most of these infections might have gone undiagnosed because of the low index of clinical suspicion due to the non-specific symptoms and lack of a suitable diagnostic test.⁹ Non-specific serological investigations such as the Weil–Felix test have been used to differentiate among different rickettsial diseases. However, seroprevalence studies in both North India and South India have shown evidence of *R. conorii* infection also.¹⁰ Clinical cases of spotted fever are well described. Most of these studies discussed the low sensitivity of the test is based on cases of scrub typhus rather than cases of spotted fever or typhus fever.¹¹

Real-time quantitative PCR [qPCR] is a rapid and sensitive test.^{10,12} PCR based diagnosis, unlike serology, provides the detection of Rickettsial sp. presence in patients' specimens in the first week of illness.⁹ It also has an advantage in endemic areas with high background levels of antibodies in the population. In our study a total of 14 patients with acute undifferentiated febrile illness were detected for Rickettsial infection; of which 50% were children. Rickettsia among children has been reported in many parts of India though in majority cases they usually recover from severe complications.^{13,14} Among three hospitalized patients, one 2 years old female child reported leukocytosis. Two children reported high CRP levels [>50]. Studies by Lin et al suggested CRP [C reactive protein] values might be useful in clinical investigations and in predicting the response to doxycycline treatment for patients with rickettsioses.¹⁵ In India, the IAP Guidelines suggested that the practicing paediatricians need to be familiar with clinical scenarios, epidemiological features, differential

diagnoses and suggestive laboratory features, which include Thrombocytopenia, raised ESR and CRP, hyponatremia, hypoalbuminemia and elevated hepatic transaminases to make accurate diagnosis of these infections.⁹ None of our patients reported rash in our study which was also noted by other investigators.¹⁰ Detection of *Salmonella* sp. through blood culture were found in two children during diagnosis. In India, concomitant infection of *Salmonella* and Rickettsia have been reported in some recent studies.¹⁶ Cohen et al. reported one such dual infected case and suggested that the dual infection must be considered in post-travel patients and in immigrants from developing countries. Seow et al. from Singapore also reported Typhoid and Scrub Typhus Coinfection in one India Returned Traveler.¹⁷

There are several limitations in our study. Though the clinical history was available, the patients were lost to follow up. So the prognosis of the positive patients was not assessed. The correlation between PCR and serological tests that demonstrate antibodies to rickettsial antigens themselves (indirect fluorescence antibody test or latex agglutination) was also not available.

5. Conclusion

The public health impact of Rickettsial infections is enormous in India. To conclude, rickettsial infections occur in India but are probably underdiagnosed due to a lack of awareness. Moreover, they are consistently misdiagnosed as leptospirosis, dengue, or *Salmonella typhi* infection due to overlapping clinical symptoms. Clinicians and microbiologists should be aware of the epidemiology and

diagnostic tests for these infections. More PCR-based investigations along with other serological tests as well as seasonal variations with tick activity and their association with the incidence of cases and distribution in rural, semi-urban, and urban areas—need to be explored further.

6. Source of Funding

Nil.

7. Conflict of Interest


The authors declare that he has no conflict of interest.

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Cite this article: Taraphdar D, Hawaldar R, Sodani S. A prospective study on Rickettsia infections in Central India. *Indian J Microbiol Res* 2024;11(1):34-37.