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EFFICACY OF PCR (POLYMERASE CHAIN REACTION) TO IDENTIFY RICKETTSIA INFECTION IN CLINICAL SAMPLES FROM INDIA

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ABSTRACT

Background: Infection with rickettsia is not well understood or valued in India. However, because there are no specific diagnostics available and these infections are typically detected with non-specific serological assays, the true impact of the disease is underestimated.

Objective: The current study used PCR (polymerase chain reaction) to identify Rickettsia infection in clinical samples from India.

Methods: The current study evaluated individuals of Indian descent whose blood samples were taken from patients who presented to the department with a referral or walk-in case of undifferentiated fever or PUO (pyrexia of undetermined cause). Before they were processed and put through a statistical analysis, the blood samples were kept in an EDTA test tube.

Results: According to the research's findings, 20.3% of the 28 participants in the samples evaluated tested positive for Rickettsia infection, and the study participants' gender distribution revealed that 57% of the participants had a higher incidence. Children were infected in 50% of the cases, and while several infections were observed, the severity was not very high. Severe clinical symptoms, including hospitalization, were observed in several patients.

Conclusion: The current study comes to the conclusion that there is a dearth of information on molecular diagnosis of Rickettsia infection in India and that early molecular diagnosis can assist to avoid and eliminate the major consequences.

Keywords: Molecular diagnosis, PCR, Rickettsia infection, pyrexia of unknown origin

INTRODUCTION

A group of organisms known as rickettsia are formed from eukaryotic vectors, such as lice, fleas, mites, and ticks, and they cause severe fever disease all over the world, including in India. The genus Rickettsia contains a variety of obligatory intracellular bacteria that cause rickettsial illnesses. These agents are classified into four groups: spotted fever, typhus, ancestral, and transitional.[1]

All four forms of rickettsial infection have a similar clinical presentation, despite regional differences in the causative species and epidemiology. The incidence and prevalence of rickettsia infections have been documented in India as well as other regions of the world. There have been reports of high rates of Rickettsia infection in West Bengal, Assam, Rajasthan, Himachal Pradesh, Uttaranchal, Jammu and Kashmir, Kerela, Karnataka, Tamil Nadu, and Maharashtra, among other Indian states.[2]

A Rickettsia infection can cause a number of symptoms, such as fever, headache, muscular soreness, coughing, and gastrointestinal issues. Rickettsia infections are also frequently accompanied with lymphadenopathies, splenomegaly, eschar, and rash in afflicted individuals. Due to their low threshold of suspicion and nonspecific signs and symptoms, lymphadenopathies are challenging to diagnose.[3]

Even when doctors are concerned, the therapy becomes questionable since serological tests used to diagnose lymphadenopathies are only reported as positive one week after the fever onset, whereas early diagnostic procedures like PCR (polymerase chain reaction) are not readily available.[4] Therefore, the current investigation used PCR (Polymerase Chain Reaction) to identify Rickettsia infection in clinical samples from Indian participants with PUO (pyrexia of unknown origin).

MATERIALS AND METHODS

The purpose of this prospective clinical investigation was to use PCR (polymerase chain reaction) to identify Rickettsia infection in clinical samples from Indian patients suffering with PUO (pyrexia of unexplained origin). The research participants were drawn from the Institute's Department of Microbiology's Outpatient Department. Before beginning the study, all participants and school officials gave their verbal and written informed permission.

Within the specified research period, 138 samples from participants with fevers of ≥99◦F and durations of ≥5 and ⩾15 days were sent to the Institute's Department of Microbiology for evaluation. Both urban and rural locations were represented among the research participants. Standard tests were performed on each participant to check for common infections, such as AFI, which is present in India as malaria, dengue, and/or enteric fever.

Blood samples were taken from each of the included specimens in a sterile test tube filled with ethylenediaminetetraacetic acid (EDTA). The samples were kept in a cold chain until they underwent further laboratory processing. 500 microliters of separated plasma were used to isolate the nucleic acid. The tropical fever kit was used to measure the amount of Rickettsia DNA in clinical samples, and nucleic acid extraction was carried out in accordance with the manufacturer's instructions.

To test the sensitivity, twenty-seven species of Rickettsia were used, as advised by the manufacturer. For every batch, internal control as well as positive and negative control were conducted. The primer sequences were matched to the reference genomes that were available. Following a 20-minute programming period at 50 degrees Celsius, the RT-PCR was first denaturated for 10 minutes at 94 degrees Celsius for one cycle, followed by 40 cycles of denaturation, annealing, and fluorescence measurement at 59 degrees Celsius for 15 seconds, and extension at 72 degrees Celsius for 15 seconds.

RESULTS

This prospective clinical investigation used PCR (polymerase chain reaction) to identify Rickettsia infection in clinical samples from Indian patients with PUO (pyrexia of unexplained origin). 138 samples were forwarded to the Institute's Department of Microbiology for evaluation. From the participants who experienced fever of ≥99◦F with fever duration of ≥5 and ⩾15 days within the specified research period.

Twenty-three percent (n=28) of the 138 members of the study that were evaluated tested positive for Rickettsia infection. Regarding the gender and age distribution of the study participants, 50% (n=14) of the participants were under the age of 18, followed by 28.57% (n=8) of the participants who were between the ages of 18 and 30, 14.29% (n=4) of the participants who were between the ages of 31 and 45, and 7.14% (n=1) of the participants who were between the ages of 46 and 60. In the current study, there were 42.86% (n=12) girls and 57.14% (n=16) men (Table 1).

When it came to the clinical characteristics of the study participants, the most common symptom observed was recurrent fever, which occurred in 100% of the subjects (n = 28). This was followed by myalgia in 35.71% of the subjects (n = 10), high NLR ratio, ketonuria, hepatorenal failure, thrombocytopenia, and leucocytosis in 7.14% of the subjects (n = 2), and cough and rash were not observed in any of the study participants. Table 2 shows that 21.43% (n=6) of the study participants were admitted to the hospital.

Additionally, it was observed that the number of cases was greater during the monsoon and post-monsoon periods, and that the prevalence of rickettsial infection was considerably higher in people living in rural regions throughout the year ($p<0.01$). A significantly greater proportion of male participants (42.8%; n = 16/28) had a rickettsial infection ($p<0.01$).

Furthermore, 50% of the patients (n=14/28) who were less than 18 years old had substantially greater positive instances; there were 8 female and 6 male participants in the research. According to the study's findings, no subjects older than 60 had any instances. The research participants' clinical history revealed that two toddlers had a very high CRP level of 73 mg/L and that four male individuals under the age of ten had simultaneous diagnosis of Salmonella species. Leukocytopenia was seen in a 2-year-old female child, however a CRP test was not performed. Two patients experienced hepatorenal failure as a result of thrombocytopenia and other comorbidities.

DISCUSSION

The current study evaluated 138 samples from people who had fevers of ≥99◦F and fever durations of ≥5 and ⩾15 days that were sent to the Institute's Department of Microbiology throughout the designated study period. Twenty-three percent

(n=28) of the 138 members of the study that were evaluated tested positive for Rickettsia infection. Regarding the gender and age distribution of the study participants, 50% (n=14) of the participants were under the age of 18, followed by 28.57% (n=8) of the participants who were between the ages of 18 and 30, 14.29% (n=4) of the participants who were between the ages of 31 and 45, and 7.14% (n=1) of the participants who were between the ages of 46 and 60. In the current study, there were 42.86% (n=12) females and 57.14% (n=16) men.

These results were similar to those of earlier studies by Stewart AGA5 in 2020 and Cohen R et al.6 in 2018, where the authors evaluated participants who had Rickettsial infection and similar demographics to the current research. Clinical characteristics of the study participants showed that the most common symptom was recurrent fever, which occurred in 100% of the subjects (n = 28). Myalgia was observed in 35.71% of the subjects (n = 10), high NLR ratio, ketonuria, hepatorenal failure, thrombocytopenia, and leucocytosis were observed in 7.14% of the subjects (n = 2) each, and cough and rash were not observed in any of the study participants. 21.43% (n=6) of the trial participants were admitted to the hospital.

The present study's findings were in line with those of Lin IF et al. (2020) and Kumar S et al. (2019), who stated that the clinical features of the study participants with rickettsial infection were similar to those of the authors' current study.

According to the study's findings, all year round, participants living in rural regions had a considerably higher rate of rickettsial infection ($p<0.01$), with a larger number of cases occurring during and after the monsoon season. 42.8% (n=16/28) of the male individuals had a rickettsial infection, which is a significantly larger percentage than the female patients ($p<0.01$). Also, 50% of the patients (n=14/28) who were less than 18 years old had substantially greater positive instances; there were 8 female and 6 male participants in the research.

The results of this investigation were consistent with that of Premaratna R9 in 2022 and Khrouf F et al.10, who found that male individuals and those living in rural regions had considerably higher rates of rickettsial infection. Additionally, it was observed that no participant older than 60 years had any instances. The research participants' clinical histories revealed that two youngsters had a very high CRP level of 73 mg/L and that four male individuals under the age of ten had simultaneous diagnosis of Salmonella species. Leukocytopenia was seen in a 2-year-old girl, however a CRP test was not performed on her. Two participants experienced hepatorenal failure as a result of thrombocytopenia and other comorbidities.

These findings aligned with those of Biswal M et al. (2020) and Mansoor T et al. (2021), who found no evidence of Rickettsial infection in participants older than 60 years, and who also found Salmonella species linked to the illness.

CONCLUSION

The present study indicates, taking into account its limitations, that there is a lack of data about molecular diagnosis of Rickettsia infection in India and that early molecular diagnosis can assist avoid and eliminate the significant sequelae. Nevertheless, further longitudinal research is required to draw a firm conclusion.

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S. No	Characteristics	Number (n)	Percentage (%)
1.	**Age range (years)**		
a)	<18	14	50
b)	18-30	8	28.57
c)	31-45	4	14.29
d)	46-60	1	7.14
2.	**Gender**		
a)	Males	16	57.14
b)	Females	12	42.86

Table 1: Demographic data of study subjects with Rickettsia infection

S. No	Clinical characteristics	Number (n)	Percentage (%)
1.	**Symptoms**		
a)	High NLR ratio	2	7.14
b)	Ketonuria	2	7.14
c)	Hepatorenal failure	2	7.14
d)	Thrombocytopenia	2	7.14
e)	Leucocytosis	2	7.14
f)	Cough	0	0
g)	Myalgia	10	35.71
h)	Rash	0	0
i)	Recurrent fever	28	100
2.	**Hospital admission**	6	21.43

Table 2: Clinical characteristics of the study subjects with Rickettsia infection