ISSN 0974-3618 (Print)
0974-360X (Online)
www.rjptonline.org

RESEARCH ARTICLE

Serological Evidence of Spotted Fever Group Rickettsial infection in North Karnataka Region

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ABSTRACT:
Background: Rickettsial diseases are important reoccurring infections that mostly go unnoticed or misdiagnosed due to low manifestation, non specific signs, symptoms and absence of widely available sensitive and specific tests. Failure of timely diagnosis leads to significant morbidity and mortality. Though the disease is rampant throughout the world, only few case reports and studies have been reported from only some states of India in the past 15-20 yrs. **Aim and Objectives:** To demonstrate the serological evidence for the existence of Spotted fever group Rickettsial infections in North Karnataka region and to develop a preliminary understanding of the distribution of these infections. **Materials and Methods:** During the period of 2015-16, a total of 231 patients sample presenting with consistent fever of more than 8 days, headache, myalgia, rashes and eschar were examined for Rickettsial infections by Weil Felix agglutination test. Further all the WF positive samples were screened for corresponding antibodies to SFG R. conorii by specific serological test IgM/ IgG ELISA. Results obtained with both the methods were tabulated. **Results:** Out of 231 cases, 105 samples showed agglutinins with Weil Felix antigens. 84 (36.36%) samples had titres ranging from 1:80 to 1:320 with OX2 antigen alone, 5 (2.16%) samples had titres of 1:80 and above for OX19 antigen and 16 (6.92%) samples had titres of 1:80 and above with both OX2 AND OX19 antigens. In R. conorii specific IgM ELISA 27 (25.7%) samples were positive with index >11 and 4 samples had equivocal index value. 74 samples were negative for R. conorii specific antibodies by ELISA. **Conclusion:** Findings of our study clearly demonstrated that among Rickettsial infections, SFG R. conorii infection seems to be common infection in and around Vijayapur. Inclusion of specific IgM ELISA method in routine diagnostic course is highly necessary for the proper treatment and patient management.

KEYWORDS: Weil Felix test, Spotted Fever Group Rickettsiosis, R. *conorii*, ELISA.

INTRODUCTION:
The Rickettsiae are small gram negative, aerobic cocco bacilli, that are obligate parasites of eukaryotic cells. The genus Rickettsia is included in the bacterial family Rickettsiaceae of the order Rickettsiales. This genus includes many species associated with human disease including those in Spotted fever group and Typhus group.

Rickettsial infections are escalating and are rampant right through the world. In India they are reported from states Maharastra, Tamil Nadu, Karnataka, Kerala, Jammu & Kashmir, Uttaranchal, Himachal Pradesh, Rajasthan, Assam and West Bengal[1,2,3,4,5].

Received on 04.06.2019 Modified on 31.07.2019
Accepted on 20.08.2019
Research J. Pharm. and Tech. 2019; 12(12): 6024-6026.
DOI: 10.5958/0974-360X.2019.01045.X

Rickettsial infections are one of the significant cause of fever of unknown origin, and these needs to be differentiated from other febrile illness like enteric fever, malaria, dengue etc.[6]. In view of low manifestation, non specific signs, symptoms and absence of widely available sensitive and specific tests, these infections are very difficult to identify and may pose a serious threat to public health if not diagnosed or misdiagnosed. Failure of timely diagnosis leads to significant morbidity and mortality[1,2,3].

Various serological tests are available for the diagnosis Rickettsial infections like Microimmunofluorescene, latex agglutination, ELISA, Weil felix test etc. IFA is the well recognized serological test and considered as gold standard technique, but very expensive and requires expertise[5]. As the specific serological tests are not easily available and expensive, Weil felix test is routinely used in most of the laboratories though the sensitivity and the specificity of the test is very low.

Since there was no complete study on Rickettsial infections in North Karnataka, this study was undertaken during the period 2015 - 2016 to know the serological evidence for the existence of Spotted fever group Rickettsial infections and to develop a preliminary understanding of the distribution of these infections.

MATERIALS AND METHODS:

The study group comprised of patients of all age-groups and both sexes attended and admitted to OP/IP departments of Medicine, Pediatrics and Dermatology of Shri B.M. Patil Medical College, Hospital and Research centre, Vijayapur and District hospital, Vijayapur (in and around Vijayapur) presenting with consistent fever of more than 8 days, headache and myalgia, rashes and eschar. The patients with cause of fever already known during the sample collection were excluded from the study. As there was no prevalence study on SFG Rickettsiae in north Karnataka region, we have considered the proportion of symptomatic cases tested for Rickettsial infections during the year 2010- 2011 for calculating the sample size. Calculation was done by using the formula $n= (Z)^2 \times p \times (1-p)/l^2$ where z= confidence level- 95%, p= proportion – 60%, I = marginal error – 4%. Ethical clearance certificate was obtained from ethical committee, BLDE University Vijayapur.

After obtaining the informed consent, 5 ml of blood was collected from the patients in plain tube and serum was separated. All the serum samples were tested for the detection of Rickettsial antibodies by Weil felix test, it exhibit agglutinins to Proteus vulgaris strain OX 19, OX 2 and OX K. (Tulip diagnostics, Goa). Samples with titres of 1:80 and above for OX2 and OX19 were considered as positive. For the serological confirmation of R. conorii infection, all the Weil felix positive samples were screened for Rickettsia conorii by IgM/IgG ELISA (Vircell, Granada, Spain), a specific serological test wherein antibodies present in the serum reacts with SFG R. conorii specific antigen Moroccan strain (ATCC VR-141) coated in the plate. Positive and negative controls were provided by SF ELISA kit manufacturers, Vircell Microbiologica, Spain. Test was performed strictly according to the manufacturer's instructions.

The interpretation of the result was made as follows: Antibody index <9 were considered negative, 9–11 were equivocal (needed repeat testing) and >11 were considered as positive for specific antibodies against R. *conorii*/ SFG Rickettsiosis.

RESULTS:

Weil Felix tube agglutination test:

All the serum samples were tested by Weil Felix tube agglutination test with different sample dilutions for the detection of anti –Rickettsial antibodies. Agglutination observed in the dilutions ranging from 1:80 to 1:320 with OX2 antigen, OX19 antigen and both O2 & OX19 antigens were considered as positives.

The results are reported in (Table 1).

Table 1: Year wise details of the patients tested for R. infections by Weil Felix test and ELISA

Sl. No	Year	n	Weil Felix test results			
			OX 2	OX 19	OX 2 & OX19	Negative
1	2015	109	38 (34.8%)	2 (1.83%)	9 (8.25%)	60 (55.04%)
2	2016	122	46 (37.7%)	3 (2.45%)	7 (5.73%)	66 (54.98%)

n = Total number of samples subjected in our study

R. conorii IgM ELISA:

All the Weil Felix positive samples were subjected to more specific immunological test Rickettsia conorii IgM ELISA for the confirmation of presence of anti – Rickettsial antibodies in the serum. OD values of all the samples were recorded and interpreted. The results in comparison with Weil Felix test are reported in (Table 2).

Table 2: Results of both WF test (antigens wise) and R. conorii IgM ELISA.

Sl. No	WF test - Antigens	WF Test Positives	IgM ELISA Positives
1	OX 2	84	24
2	OX 19	5	0
3	OX 2 & OX 19	16	3
Total		**105**	**27**

Gender wise analysis of the results obtained in the study is recorded in the (Table 3).

Table 3: Gender wise distribution of Weil Felix and IgM ELISA test results.

Tests	Male (128)	Female (103)
Weil Felix (n-231)	59(46.09%)	46 (44.66%)
IgM ELISA (n-105)	14 (23.72%)	13 (28.26%)

n- Samples subjected for test.

DISCUSSION:

Present work was carried out to serologically confirm the occurrence of SFG Rickettsial infection in and around Vijayapur, Karnataka by well established WF test and more specific serological test SFG R. *conorii* specific IgM ELISA test. We found significant number of cases positive by WF test. Out of 231 cases screened for R. infections 105 (45.45%) were positive for OX2 & OX19 suggestive of SFG Rickettsial infection. The results obtained in our study shows considerable disparity with similar studies reported by other investigators. In a study from Delhi by Veena Mittal *et.al* had reported 8 (27.5%)

SFG rickettsiae positive cases out of 29 cases positive by WF test[6]. Kumar *et.al* from Karnataka had reported seroprevalence of 23.3% in and around Davanagere[8].

For the serological confirmation, we screened all WF positive samples further by another serological test R. *conorii* IgM ELISA. IgM ELISA proved to be highly sensitive and specific in some of the studies carried out by other investigators during last 10 years. According to the manufacturers of the kit, the sensitivity and specificity of Vircell R. *conorii* IgM ELISA kits are 94 and 95%; and for IgG ELISA kits, these are 85, 100%, respectively[15]. Do *et al*, in their study observed that the sensitivity for R. *conorii* Vircell IgM + IgG ELISA was 90% and specificity was 100%[10]. In the present study out of 105 WF positive cases 27 (25.7%) cases found positive by R. *conorii* IgM ELISA. Though the present study clearly demonstrates the seroprevalence of R. *conorii*/ Indian tick typhus infection in this region, the figures obtained in our study notably vary from some of the studies reported by other investigators in India. Kalal *et al*, from Karnataka reported that 37.1% hospitalized children were seropositive for SFG rickettsia based on the IgG ELISA result in acute samples and some paired sera[9]. Somashekar *et al* had reported seroprevalence of SF in children of Tamil Nadu was found to be 7.78% during 2003–2004[3]. Tripathi *et al* from Uttar Pradesh reported seroprevalence of spotted fever in 42.15% in children and 17.10% adults. Their observation was based on IgM ELISA and IgM IFA positivity on acute samples only[11]. In a study by Stephen *et al* demonstrated a high seroprevalence of SFG rickettsiosis (44.38%) in Puducherry region of south India. A moderately higher rate of ST and SFG coinfection/cross-reactivity (34.50%) was also observed in the SF IgG positive patients[7]. In the present study more seropositivity was observed during cooler months between August to December, same was reported by most of the investigators in their study[2,12,13,14].

ACKNOWLEDGEMENT:

I am very much thankful to all the patients who participated in my study. I acknowledge my sincere thanks to all the Non Teaching staff of Dept. Microbiology, BLDEUs Shri B.M Patil Medical College, Vijayapur for their support during the sample collection and performing the tests.

CONFLICT OF INTEREST:

None.

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