

A Bacterial Profile of Scrub typhus Infections in North Karnataka and their Isolation by Serological and Molecular Method

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Scrub typhus infections are caused by *Orientia tsutsugamushi* and similar to febrile infections which makes more difficult in diagnosis. Present study revealed the incidence of scrub typhus infections. A total 521 samples which are Weil-Felix positive were selected in the present study and analysed by PCR over a period of one year in Khaja Banda Nawaz Institute of Medical Sciences, Gulbarga. The incidence rate was 29.36 % (153/521) of cases were positive and 39.06 % (84/215) of cases were males and 22.54 % (69/306) of cases were females. More than 30 years of age group population had highest incidence rate. Species specific gene 56 kDa was selected for the confirmation of scrub typhus. Multiple clinical features was observed along with rash and eschar. Early diagnosis help to reduce the morbidity and mortality.

Keywords: Scrub typhus, eschar, Weil-Felix test, RT-PCR.

India being a tropical nation, fevers are caused by various etiological micro organisms. At the point when a patient presents with history of fever with rash/thrombocytopenia, scrub typhus is one of the differential diagnosis alongside dengue, chikungunya, measles, rubella, meningococcal disease, jungle fever, leptospirosis and other viral exanthemas.¹ These diseases have a worldwide distribution and are accounted for from all parts of India.² Scrub typhus broadly varies in seriousness from self limited gentle illness to serious infections. Scrub typhus infections are re-emerging diseases which are vector borne zoonotic diseases in India present time.

Scrub typhus caused by gram negative coccobacilli bacteria *Orientia tsutsugamushi*. The

incubation time is six to 18 days, and the most widely recognized clinical features are onset of headache, fever (enduring up to 19 days) with chills. Presence of eschar in groin and armpits oftenly. Infections like deafness, conjunctival suffusion and tinnitus. Meningoencephalitis, pneumonitis, myocarditis, jaundice, multiple organ failure, intense renal disorder and other clinical manifestations also caused by *O. tsutsugamushi*.

Clinicians face many problems like no proper symptoms are present in the patients in first week of illness and does not grow in cell free cultures. But the test like Weil-Felix(WF) test gives a preliminary confirmation for the diagnosis. The best diagnostic test for the early diagnosis is PCR and useful where serological results were unclear. There are some limitations are present in India for the PCR because scrub typhus infections are common in rural areas where the large and

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high-cost setups are not available, people can not affordable for this PCR test.³

MATERIALS AND METHODS

A prospective study was done at Khaja Banda Nawaz Institute of Medical Sciences, Gulbarga from June 2016 to May-2017, Suspected clinical feature samples like signs and symptoms of fever, chills, rash, eschar, edema, PUO, lymphadenopathy, hepatosplenomegaly and history of tick or flea exposure were included. Patients diagnosed with malaria, enteric fever, other febrile diseases like dengue were excluded in the present study. A total number of 736 samples were tested by Weil-Felix test for preliminary confirmation of

the infection. The titer value of 1:80 with OX K antigen was taken as scrub typhus positive cases. A number of 521 samples were found positive and PCR (RT-PCR) test was performed for further confirmation of scrub typhus.

DNA extraction and Sequencing

Whole blood was used to extract the DNA as per manufactures manual instructions of the kit (Qiagen, USA) and 56-kDa gene was sequenced, the primers were selected with the help of software BLAST (<http://blast.ncbi.nlm.nih.gov>) in Gen Bank. The standard sequence of the gene was 5'-AATTGCTAGTGCAATGTCTG-3' in forward and 5'-GGCATTATAGTAGGCTGAG-3' was in reverse of the oligonucleotide primers.^{4,5} Thermal cycler (GeNei™, Merk specialties Pvt. Ltd) was

Table 1. Incidence of scrub typhus among male and female

Sex	Positive		Negative		Total	
	No	%	No	%	No	%
Male	84	39.06 %	131	61 %	215	41.26 %
Female	69	22.54 %	237	77.45 %	306	58.73 %
Total	153	29.36 %	368	71 %	521	100

n = 521; Chi-square test $\chi^2 = 15.829$; p<0.001

Table 2. Age and sex wise distribution of scrub typhus

Age group	Male Number	Female Number	Total Number
<30 years	25 (58.13%)	18 (42%)	43
> 30 years	59 (54%)	51 (46.36)	110
Total	84 (55%)	69(45.09%)	153

Chi-square test $\chi^2 = 0.253$, p= 0.615

used for the amplifications. At 94°C for 5 min first amplification was performed followed by denaturation at the temperature of 94°C for 30 min, next annealing was at 56°C for 1 min and at 72°C extension was done for 5 min. Again extension was performed at same temperature 72°C finally for 5 min. The end products were observed under UV light after the electrophoresis in an agarose gel (1.5 % agarose gel+ ethidium bromide).

Table 3. Pattern of *Orientia tsutsugamushi* isolated from various clinical features among male and female (n=153)

Clinical features	Male (n=84)		Female (n=69)		Total (n=153)		Testing proportions
	No	%	No	%	No	%	
History of insect bite	05	6 %	06	9 %	11	4.34 %	Z=0.653, p=0.257
Fever with chills	23	27.38 %	21	30.43 %	44	29.00 %	Z=0.415, p=0.339
Eschar	05	6 %	06	9%	13	11.59 %	Z=0.653, p=0.257
Rash	27	32.14 %	20	29 %	47	39.13%	Z=0.421, p=0.337
Multiple clinical manifestations	23	27.38 %	17	20.28 %	40	15.94 %	Z=0.384, p=0.350

Ethical consideration

The present study was approved by the Institutional Ethics Committee (IEC) of Khaja Banda Nawaz Institute of Medical Sciences, Gulbarga-Karnataka, India (IEC No KES/KBNIMS/IEC/2016-17/24; meeting held on 21-08-2016). An informed consent form (questionnaire in English/local language) was provided; the procedure was informed to concerned staff and Head of Departments. Confidentiality was maintained where it was found necessary. Bio-safety measures were taken for sample processing as per the hospital standard operating procedures.

RESULTS

A total number of 521 samples were performed to PCR test, in that 29.36 % (153/521) of cases were positive and 39.06 % (84/215) of cases were males and 22.54 % (69/306) of cases were females as shown in Table no.1. Highest rate of incidence (72 %) was observed in above 30 years of age group in both genders. Table no.2 shown the age & sex wise distribution of scrub typhus. As per symptom wise incidence rate was also included in present study, highest bacteria was isolated from rash (32.14%) and lowest from eschar and insect bite history(6%). After the these symptoms from other symptoms incidence rate was observed as shown in the Table no.3.

DISCUSSION

Different studies from India were reported the occurrence and mortality of scrub typhus by using serological and molecular tests.⁶⁻⁹ However, so many gold standard methods available but still many laboratories are using widely Weil-Felix test for the diagnosis of *O. tsutsugamushi*. The sensitivity of this WF test is low and specificity is high. Weil-Felix test had specificity 94 % and sensitivity of 59 %¹⁰. Other tests like ELISA had good sensitivity and specificity for the diagnosis within the first onset of the disease.⁷⁻¹⁰

PCR played a major important diagnostic tool for the accurate identification of scrub typhus where clinical symptoms less patients also. Specific treatment allows the patient to recovery which reduces the mortality. The main disadvantages of PCR test are high-cost, skilled technicians to

perform the test and need of a very good laboratory set up to prevent contamination. The specific species gene 56-kDa was used for the confirmation of diagnosis of *Orientia tsutsugamushi* infections.¹¹ High prevalence rate was seen in male than females as comparison of genders in present study ($p < 0.001$). Because of recreational habits of males and exposure to tick-borne infections always.¹² Females were protected from the infection by the females hormones probably.¹³ Varghese *et al.*, has reported the incidence of gender rate was 96 cases in males 116 cases in females, the mean age group was 45.6 ± 14.8 and observed eschar in 55%(86) of cases.¹⁴ In present study highest incidence was seen in above 30 years of age group with $p = 0.615$.

Yun-Xi *et al.*, has observed the presence of eschars and positive to 56-kDa gene in all 7 cases from acute-phase patients¹⁵. Rajapakse *et al.*, have found the maximum incidence of 7% to 80% in children with eschars.¹⁶ In present study about only 6 % of cases were have eschar. In India detection of eschar is very tuff because of the dark skin incidence of 4% to 46% reported and few social customs while physical examination by Mathai *et al* and Priya *et al.* studies.^{17,18} In clinical features highest incidence rate was observed in rash (32.14%) next was in fever with chills (27.38%). Less incidence rate was observed in insect bite history. This might be patients were not aware of insect bite due to bite was painless. The other clinical manifestations were also observed in 27.38% of cases in our study. Complications like involvement of renal, cardiovascular, respiratory and most in hepatic system with 7.8 % of fatality rate¹⁴. Observed rash in 22 % of cases and next was myalgia with 52% as a most frequent clinical feature¹⁷. Study by Saisongkorh *et al.*, reported 56-kDa gene positive in 7 cases along with symptoms like vomiting, lymphadenopathy, thrombocytopenia and hepatomegaly.¹⁹

CONCLUSION

Scrub typhus infection is much prevalent but underdiagnosed disease in this area. Variations in the clinical presentation could complicate diagnosis. Serological and molecular tests are useful to confirm the disease. Weil-Felix test can serve as initial but not sole method to recognize and diagnose scrub typhus infections. 56 kDa

protein gene is a group specific and useful for the diagnosis of scrub typhus. Early diagnosis helps in reducing the morbidity and mortality of scrub typhus and these infections are co-endemic along with bacterial and viral diseases.

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