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Research Article

SEROPREVALENCE OF SCRUB TYPHUS AMONG FEBRILE PATIENTS: A PRELIMINARY STUDY

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ABSTRACT

Aim: Scrub typhus is a vector borne zoonotic diseases re-emerging in India. But there are no reports from Andhra Pradesh till date due to low index of suspicion and lack of diagnostic facilities. This emphasizes the need for a study on this re-emerging Zoonosis. Methodology: A total of 280 sera samples with pyrexia of unknown origin were screened by using Weil-Felix test, rapid immunochromatographic method and ELISA.
Result: Among the tested 280 febrile ill patients, 158 (56.42%) was found to be positive by Weil-Felix test, 163 (58.21%) was positive by ELISA and 160 (57.14%) was positive by immunochromatographic test.
Conclusion: By this study it can be inferred that awareness about vector borne zoonotic diseases is crucial. Clinical practice, epidemiological surveys and definite diagnostic tests have to be developed to study this neglected area, since this condition is very much prevalent in this part of the country.

Keywords: Febrile illness; Spotted fever group; Typhus fever group; Scrub typhus; Weil-Felix test; Immunochromatographic test; ELISA.

INTRODUCTION

Scrub typhus is the major causes of febrile illness throughout the Asia-Pacific region [1]. In India, the burden of rickettsiosis is under estimated as there is lack of both community based studies and availability of specific laboratory tests [2]. The presence of rickettsiosis has been documented from Jammu and Kashmir, Himachal Pradesh, Uttaranchal, Rajasthan, Assam, West Bengal, Maharashtra, Kerala and Tamil Nadu [3-6]. *Orientia tsutsugamushi*, a gram negative intracellular coccobacillus is the causative agent for Scrub typhus. Transmitted to humans and other vertebrates when bitten by larval trombiculid mites (known as Chiggers) harbouring the etiological agent. The mites normally feed upon single vertebrate hosts, usually rodents [7]. Febrile illness typically begins after the bite of an infected chigger and lasts for 7-10 days [8]. Major systems include fever, severe headache and myalgia. Other signs and symptoms include rash, lymphadenopathy, hepatosplenomegaly, cough, sore throat, abdominal pain and central nervous system involvement.

Weil-Felix test is the simplest and cheapest laboratory test for diagnosis of Rickettsiosis. It is based on the antigenic cross-reactions among Rickettsial antigens, mostly lipopolysaccharide and *Proteus vulgaris* strains OX19 and OX2 with typhus fever and spotted fever group respectively and *Proteus mirabilis* OXK with scrub typhus [9]. Immunochromatographic (ICT) test detects IgA, IgM and IgG. Indirect IgM ELISA uses recombinant antigens to detect antibodies.

The aim of this study was to identify the prevalence of scrub typhus by using serological methods and analyze their demographic profiles.

MATERIALS AND METHODS

Blood samples of the patients submitted to Microbiology Department, tertiary care hospital, Tirupati, Andhra Pradesh and reported negative for Typhoid, Malaria, Leptospirosis, Chikungunya and Dengue was used for this study. During the period April 2011 to December 2012 a total of 280 sera samples were tested by Weil-Felix test. Antigens were prepared in-house following standard protocol [10]. A doubling dilution of 1:20 to 1:320 was used and a titer ≥ 1:80 or fourfold rise in titer was considered positive. The samples were also analyzed by commercially available lateral-flow-format immunochromatographic test (SD Bioline tsutsugamushi assay, Inc., Korea.) and indirect IgM ELISA

The average baseline antibody titer against the *Rickettsiae* groups among the healthy people of various age groups which ranged from 18 to 50 years in the Chittoor region was also analyzed. The study protocol and objectives were duly explained and after obtaining an informed consent from the apparently healthy volunteers, non-repetitive blood samples were collected (n= 280).

RESULTS

In this study, we included 280 febrile ill patients, of whom 176 (62.85 %) were males and 104 (37.14 %) were females. The age of the patients with suspected scrub typhus ranged from 18-85 years with 85 % of patients falling in the age group of 25-65 years. Among the analyzed samples 61.78 % (173/280) were belonged to rural area and 38.21% (107/280) belonged to urban area. Most of the patients are farmers (55.00%) followed by Labours (26.0 %) and Dependents (18.92 %). A total of 72 patients presented with duration of fever with more than 5 days 118 patients presented with more than 10 days, where as 90 patients presented with fever of more than 2 weeks. They also showed clinical features like chills and rigors (62%), seizures (20%), vomiting (45%), headache and myalgia (29%), conjunctival suffusion (25%), lymphadenopathy (45%), hepato-splenomegaly (40%), abdominal pain (23%), altered sensorium (20%), rash (8%) and only 3 patients showed eschar. Respiratory failure was seen in 3 patients where as 2 patients were reported with sepsis and multiorgan failure. Those patients who are reported positive by serological methods are responded effectively to doxycycline treatment and some patients to chloramphenicol treatment.

188 (67.14 %) were tested positive for rickettsiosis by Weil-Felix test and 92 (32.85 %) were tested negative. The prevalence of antibodies to scrub typhus was highest 158 (56.42%) followed by typhus fever group 54 (19.28%) and spotted fever group 46 (16.42%) respectively. **(Figure: 1)**

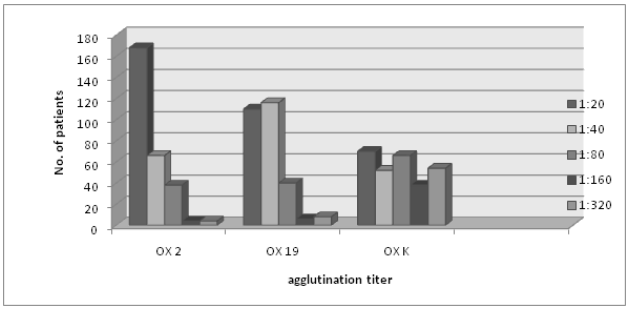

Figure1: Graphical representation of antibody (agglutination titers)

54 (19.28%) patients were seropositive to more than one rickettsiosis and 16 (5.71%) were seropositive to more than two rickettsiosis by Weil-Felix test. By ELISA test 163 (58.21%) patients was positive and by immunochromatographic test 160 (57.14%) patients was positive. **(Table 2)**

Table 2: Seroprevalence of Rickettsiosis among febrile ill patients

Response	Number of positive patiens (%)
Positive patients	188 (78.33)
SFG only by WFT	08 (2.85)
TG only by WFT	06 (2.14)
ST only WFT	120 (42.85)
SFG+ TG only by WFT	16 (5.71)
SFG+ST only by WFT	06 (2.14)
TG+ST only by WFT	16 (5.71)
SFG +TG+ST by WFT	16 (5.71)
ST only by ELISA	163 (58.21%)
ST only by ICT	160 (57.14%)

TG – Typhus fever group; SFG – Spotted fever group; ST – Scrub typhus. ICT- Immunochromatographic test; ELISA- Enzyme Linked Immunosorbent Assay.

Of the 280 tested healthy volunteers, none had showed a titer of 1:320 for OX 2, OX 19, OX K and 16 (5.71%) had a WFT titer above 1:80 (OX2- 8; OX19- 4, OXK- 4). **(Table 3)**

Table 3: Antibody titre of serum samples from healthy volunteers measured by Weil-Felix test

Type of Antigen	Weil Felix test Agglutination Titers (%)				
	1:20	1:40	1:80	1:160	1:320
OX 2	168 (60.00)	104 (37.14)	04 (1.42)	04 (1.42)	0 (0.00)
OX 19	150 (53.57)	126 (45.00)	02 (0.71)	02 (0.71)	0 (0.00)
OX K	136 (48.57)	140 (50.00)	04 (1.42)	0 (0.00)	0 (0.00)

DISCUSSION

Rickettsial infections are re-emerging and lead to significant morbidity and mortality if failed to diagnose at appropriate time [11]. It constitute a very significant, but often unrecognized portion of the acute febrile disease contributing to financial burden on many populations, especially in developing countries [12]. These zoonotic infections should be considered in differential diagnosis of any acute febrile illness. Rickettsiosis should be differentiated from other infections like meningococcemia, brucellosis, malaria, viral illness and typhoid fever as there is an overlap of clinical features [13]. A high index of suspicion is required to diagnose rickettsiosis especially in endemic areas. Due to lack of simple diagnostic tools, the diagnosis of scrub typhus cannot be easily made in many laboratories. For diagnosis of scrub typhus, gold standard diagnostic tests like indirect immunofluorescence antibody (IFA) and indirect Immunoperoxidase (IIP) requires highly trained persons to perform and production of antigens may vary among different laboratories, leading to inconsistency in interpretation of results. is expensive and not available in India [14].

In several regions around the world, Weil-Felix test has been used in documenting the presence of rickettsial infections for the first time [15]. Usually Weil-felix results may be negative during the early stages of the disease because agglutinating antibodies are detectable only during the second week of illness [16]. Isaac *et al*. have demonstrated that the sensitivity of Weil-Felix test is 30% at a break point titer of 1:80, but the specificity and positive predictive value were 100% [17]. In another study by Prakash *et al.* Weil-Felix test showed specificity of over 98% and a sensitivity of about 43% [18]. Instead of misdiagnosing rickettsial infections Weil-Felix test can be used to detect more positive cases [19]. By performing Weil-Felix simply we can get some information regarding the nature of infection, which can be cross confirmed by other techniques, if available. There is good correlation between the results of Weil-Felix test and detection of IgM antibodies by IFA/ELISA [20].

In this study we used single acute phase sera from patients with febrile illness attending the hospital for determining antibodies against SFG, TG and ST. A study conducted by Mittal *et al.* on fever of unknown origin patients sera showed that 42.6% were positive for OXK, 39.3% were positive for OX2 and 8.1% were positive for OX19 [21]. In another study conducted by Kamarasu *et al.* showed 9.2 % of patients sera were positive for scrub typhus and 4.6% for other rickettsiosis [22]. There results show that scrub typhus seems to be more common when compared to other rickettsiosis. Kulkarni *et al.* from Western part of India reported higher incidence of spotted fever group [23]. Our study showed more positives for scrub typhus followed by typhus fever and spotted fever group. In the present study more number of scrub typhus cases was observed from October to February. In southern India outbreak of scrub typhus occur during cooler months as reported by Mathai *et al.* [24].

In our study more scrub typhus cases were reported by ELISA and ICT tests than Weil-Felix test. Gurung *et al* also reported more scrub typhus cases were positive by ELISA and ICT tests than Weil-Felix test. [25] Although Indirect IgM ELSA may give false positive results due to rheumatoid factor [26] and false negative results due to rise of IgG levels occur at the time of secondary infection [27]. Primary infection produces a rapid rise in IgM antibodies within 8 days, whereas secondary or re-infection is characterized by a sharp rise in IgG levels, with a variable IgM response [27].

In conclusion Weil-felix test was found to be promising as a screening test for diagnosis of scrub typhus in correlation with clinical feature in a hospital setting where gold standard tests are not available like India. ELISA based system is helpful for detection of IgM antibodies where labs are developed. This study also helps the authorities to undertake therapeutic as well as preventive measures to prevent the morbidity and mortality. As our region is pilgrim place so many travelers will be coming from other states, so further studies has to be done to understand epidemiological aspects and strain variability of this re-emerging infection.

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