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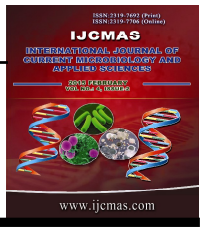

Original Research Article

FUO Cases Showing Prevalence of Scrub Typhus: A Comparative Study by ELISA and Rapid Test in a Tertiary Care Hospital in Andhra Pradesh, India

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Keywords

Fever of unknown origin, Orientia tsutsugamushi, Elisa-enzyme linked immune sorbent assay, Rapid test, Scrub typhus

A B S T R A C T

Fever of unknown origin (FUO) has multiple causes. Scrub typhus is less known cause of FUO in India. There was high mortality in undiagnosed cases of FUO which lead to the diagnosis of scrub typhus. Scrub typhus is a rickettsial infection which is caused by *Orientia tsutsugamushi* and transmitted by the bite of the chigger of a mite. Present study done to know the prevalence of scrub typhus as a causative factor in FUO cases by detection of IgM antibodies by ELISA and rapid test. This study was done over a period of six months in Narayana medical college and hospital. 223 serum samples of FUO cases were analysed for IgM antibodies to *Orientia tsutsugamushi* along with dengue, malaria, typhoid, tuberculosis and brucellosis. Scrub typhus IgM antibodies by ELISA were detected in 93 (41.7%) patients. Scrub typhus positivity was significantly higher among female in comparison to males. Maximum positivity of scrub typhus was found in females of 46-60 years age group. There was 98% correlation between ELISA and rapid method. The laboratory parameters were abnormal in most of the patients as evident by thrombocytopenia, deranged liver functions and renal functions. The present study emphasizes the importance of scrub typhus among cases of FUO especially after rainy season and during early cooler months.

Introduction

Fever of unknown origin (FUO) is said when the body temperature increases to 38.3°C (101°F) or more several times a day lasting longer than 3 weeks or failure to reach a diagnosis despite 1 week of inpatient evaluation [1]Although there are multiple causes of FUO but infections such as enteric fever, malaria, dengue, tuberculosis, brucellosis are among most common causes. [2] Scrub typhus (ST), a rickettsial disease caused by *Orientia tsutsugamushi*, is a very less known cause of FUO[3]. Rickettsial infections are re-emerging with increased reports from different parts of the world. Scrub typhus is an acute febrile illness and delay in diagnosis is associated with considerable morbidity and mortality. Although it is a neglected disease in India, but in recent years there are reports from Maharashtra, Tamil Nadu, Karnataka,

Kerala, Jammu and Kashmir, Uttaranchal, Himachal Pradesh, Rajasthan, Assam and West Bengal indicating the resurgence of the disease in this part of the world also. [4,5,6,7,8,9]

Scrub typhus or Bush typhus is a rickettsial infection which is caused by *Orientia tsutsugamushi* and transmitted by some species of trombiculid mites ("chiggers", particularly a *Leptotrombidium deliense*).[6] The name is derived from the prevalence of the mites in areas of heavy scrub vegetation. The disease is endemic in the geographical region known as "tsutsugamushi triangle" which extends from northern Japan and far-eastern Russia in the north, to the territories around the Solomon Sea into northern Australia in the south, and to Pakistan and Afghanistan in the west. The bite of this mite leaves a characteristic black eschar which is useful to the doctor for making the diagnosis. [5,10]

The observation of the eschar is often missed and other signs and symptoms of the disease are not characteristic leading to delayed diagnosis by the clinician. In view of low index of suspicion, non-specific signs and symptoms, and absence of widely available sensitive and specific diagnostic tests, these infections are difficult to diagnose. [11]

Failure of timely diagnosis leads to significant morbidity and mortality. With timely diagnosis treatment is easy, affordable and often successful with dramatic response to antimicrobials. As antimicrobials effective for rickettsial diseases are usually not included in empirical therapy of nonspecific febrile illnesses, treatment of rickettsial diseases is not provided unless they are suspected. Several tests are available with their own advantages and limitations. [12.] Among all the serological tests available Weil-Felix test is the cheapest and easily available, but this is notoriously unreliable. Indirect immunofluorescence test, the gold standard is beyond affordability especially in poor countries and needs expertise for interpretation as the choice of cut-off values for positive diagnosis is influenced by several factors such as antibody kinetics, geography, negative seroconversion and seasonality. [13,14]

IgM ELISA has been evaluated and found to be quite satisfactory in comparison to the gold standard, but samples need to be pooled for ELISA which can lead to delayed diagnosis thus influencing the overall outcome. Rapid tests which are economic, rapid and single tests can be carried out.

This study was carried out to know the seroprevalence of scrub typhus in FUO cases and to compare a rapid test with IgM ELISA for the diagnosis of scrub typhus.

Materials and Methods

This is a cross-sectional study carried out on serum samples from FUO cases received over a period of 10months extending from March to December 2014. The samples were processed for the detection of IgM antibodies for the diagnosis of scrub typhus by ELISA and Rapid test. Samples were also tested for dengue fever, typhoid fever and leptospirosis, tuberculosis and malaria.

Detection IgM antibodies by ELISA- was done using In Bios International TM IgM ELISA. Detection of IgM antibodies by Rapid method was done using SD Bioline Tsutsugamushi, one-step scrub typhus antibody test. Clinical features of the patients were retrieved from hospital medical records. Statistical analysis was done using SPSS 11.5 version. *P*-value was calculated using Chi-square test.

Results and Discussion

A total of 223 patients of FUO reported to the Narayana hospital tested for ST IgM antibodies were detected in 93 (41.7%) cases. Among these 93 diagnosed cases 54 (58.06%) were females and 39 (41.94%) were males. Positivity for ST was higher among female who were suffering from FUO in comparison to males. The age of the patients ranged between 20 and 80 years. In females and males positivity for ST IgM antibody was highest in 40-60 years.

There was 98% correlation between ELISA and rapid method. Of the 93 samples, only 2 samples positive by ELISA were negative by rapid method.

Scrub typhus is a rickettsial disease caused by *O. tsutsugamushi* which is a Gram negative, intracellular bacterium. It is transmitted by the bite of mite belonging to the genus Leptotrombidium (*L.delienis*)india.[15] The prevalence of scrub typhus varies from 0-8% to 60% in different countries. [13,16]In a study from Thailand, the positivity for scrub typhus was 59.5% with highest prevalence in 40-49 (77.7%) year age group with no difference between the two sexes[17]. Gurung *et al.*, tested 204 patients with fever of unknown origin of which 63 were confirmed positive of which 42 were males and 21 were females. [18] The study shows more positivity of ST in females particularly above 30 years of age. Vivekanandan M *et al*. also reported female preponderance in their study.[19], In our study also there is female prepoderence and high prevalence seen in 40-60 years age group. Fever with chills and rigors was the most common presentation in our study which is similar to a hospital-based study in Taiwan and a study by Dass *et al.*, from the state of Meghalaya, India. [20]

The present study reports 9.7% mortality in concurrence with previous studies.[21,22,23,24] The most common cause of death in our study was acute respiratory failure as most of cases presented with fever, cough and dyspnoea showing bilateral interstitial pneumonia in high-resolution computerized tomogram. Fever, cough and vomitings have been reported as chief complaints in other studies also. [20,25]Kammili *et al.*, from Secunderabad, India tested for the prevalence of scrub typhus among patients who were provisionally diagnosed as dengue fever. Among the 100 patients studied, 19 were found to be positive for antibodies for scrub typhus by rapid immune-chromatography and Weil-Felix test. Seropositivity was equal among males and females with preponderance in old age group. Chief complaints included arthralgia (2patients), haemorrhagic manifestations (3patients) and rash (3patients). [26] Boorugu *et al.*, reported a case from Andhra Pradesh.The patient presented with fever which was associated with myalgia, loose stools and dry cough. Patient was finally diagnosed with scrub typhus associated with thrombocytopenia, hepatitis, hypoalbuminemia and bilateral pleural effusion. [27] A study from Chennai reported seizures, signs of consolidation, thrombocytopenia, elevated serum alkaline phosphates and renal failure in 25%, 40%, 37.5%, 52% and 33% patients, respectively. 6.25% of patients died of multi organ failure[28]

In the laboratory parameters, the most important abnormality noticed was thrombocytopenia (63%). Other laboratory findings include elevation of liver enzymes, serum urea and serum creatinine. Similar abnormalities have been observed by Vivekanandan M *et al.* in their study[19].

The diagnosis of scrub typhus poses a problem due to low index of suspicion, non-specific signs and symptoms, absence of the specific presentation of an eschar and the lack of diagnostic facilities in India.

Tests are available like Weil-Felix, indirect immunofluoresce, PCR, culture and ELISA all of which have their own limitations. In poor countries some of these tests like PCR, indirect immunofluorescence which provide accurate and specific diagnosis are either not available or are too expensive. Weil-Felix is a commonly used inexpensive serological test which lacks both sensitivity and specificity. [29] There is need for a rapid, technically simple and economic test. ELISA is an easy and comparatively economic test but the results of this test may not be available on the same day as samples need to be pooled for testing thus causing delayed diagnosis and treatment, which at times may be fatal. A rapid method which can provide the diagnosis on the same day.

Therefore, we compared the rapid method with IgM ELISA for the diagnosis of scrub typhus. Chinprasatsak *et al.*,[30] evaluated a rapid dot blot assay for rapid diagnosis and reported the sensitivity and specificity of 87% and 94%, respectively. They concluded that the dot blot immunoassay dipstick was accurate, rapid, easy to use and relatively inexpensive. No false positives were observed. Jang *et al.,* evaluated IgM ELISA for the diagnosis of scrub typhus and reported sensitivity of 96.3% for IgG IFA-positive samples and of 100% for IgM IFA-positive samples. The specificity of the IgM capture ELISA was 99%, for IgM-positive samples. [31]

Figure.1 Age wise distribution of scrub typhus

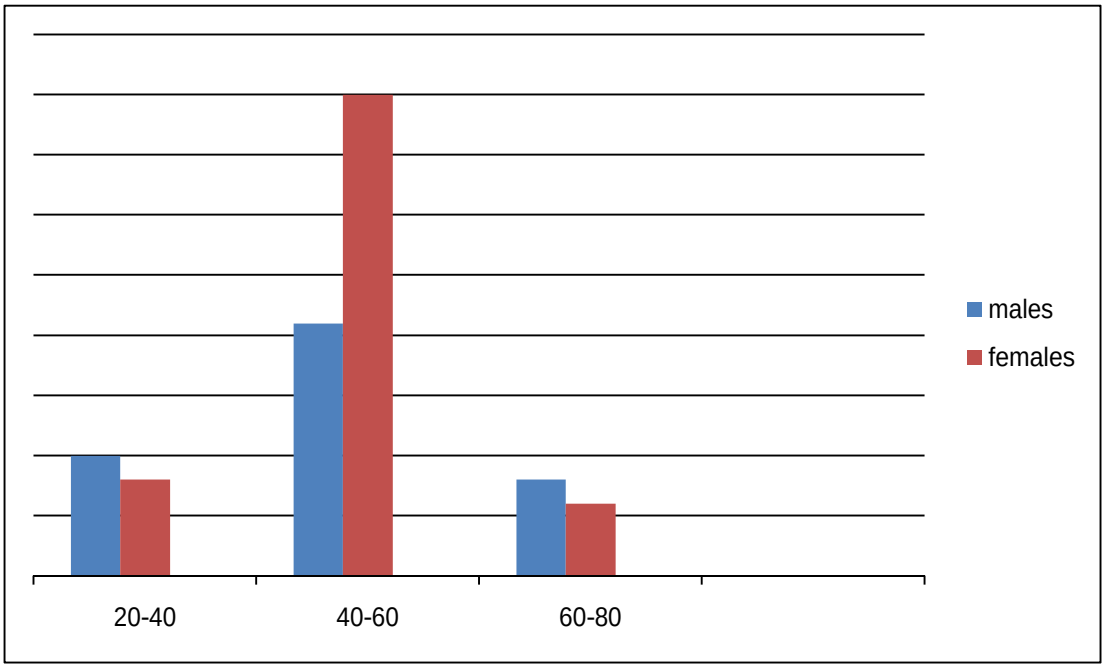


Figure.2 Sex wise distribution of scrub typhus

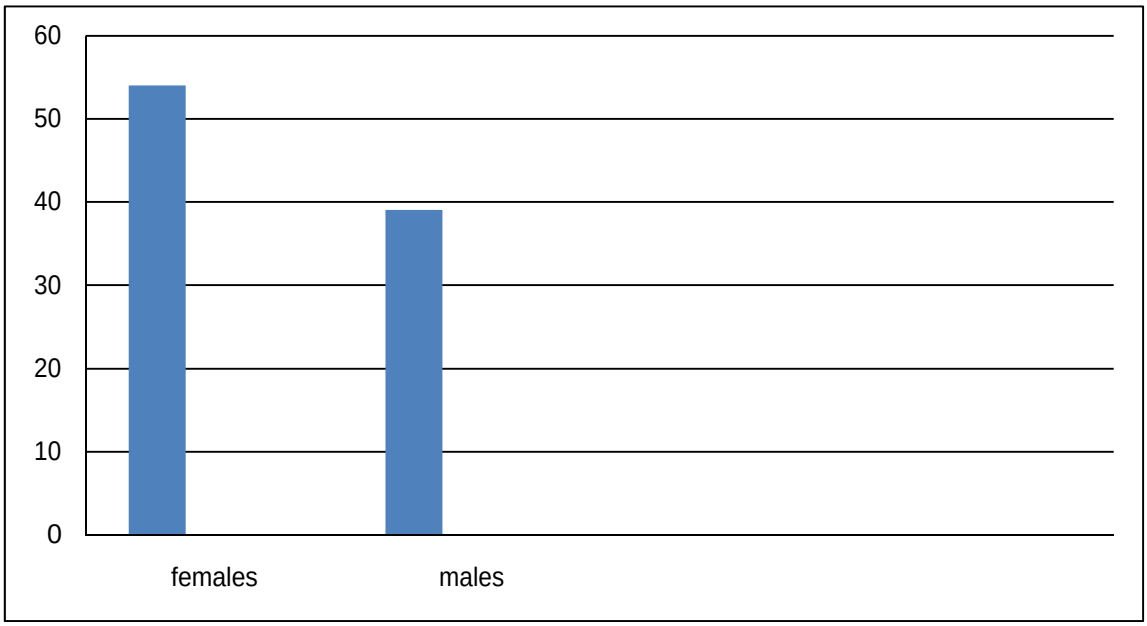


Figure.3 Clinical presentation of seropositive patients

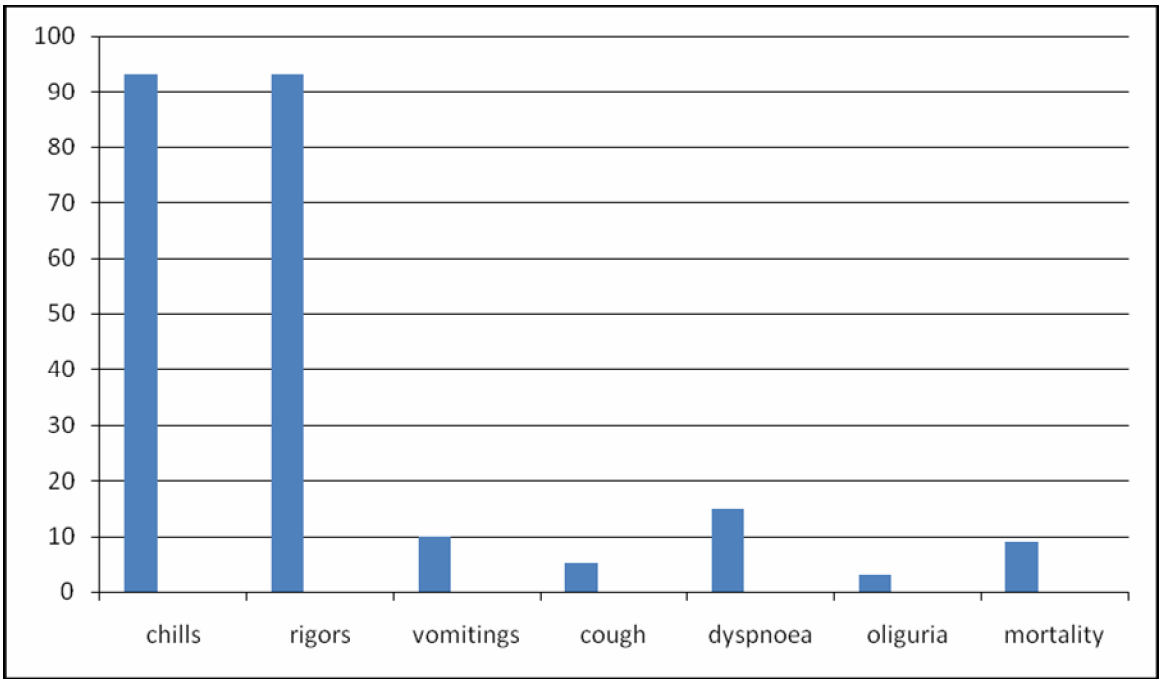


Figure.4 laboratory parameters of scrub typhus patients

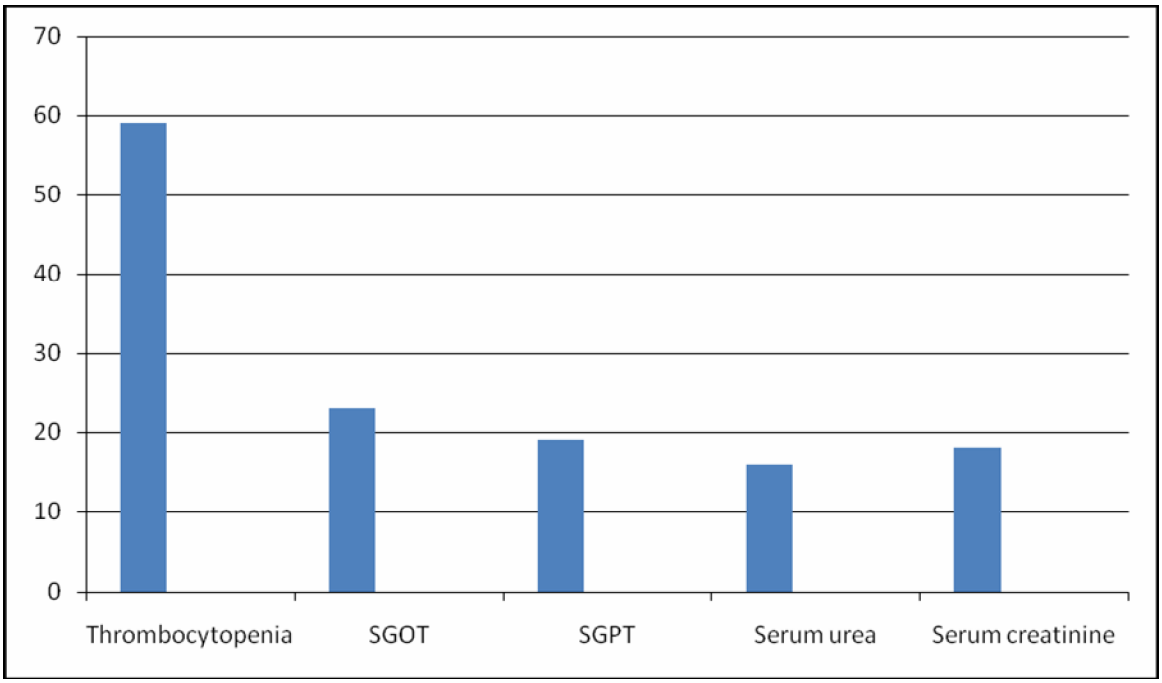
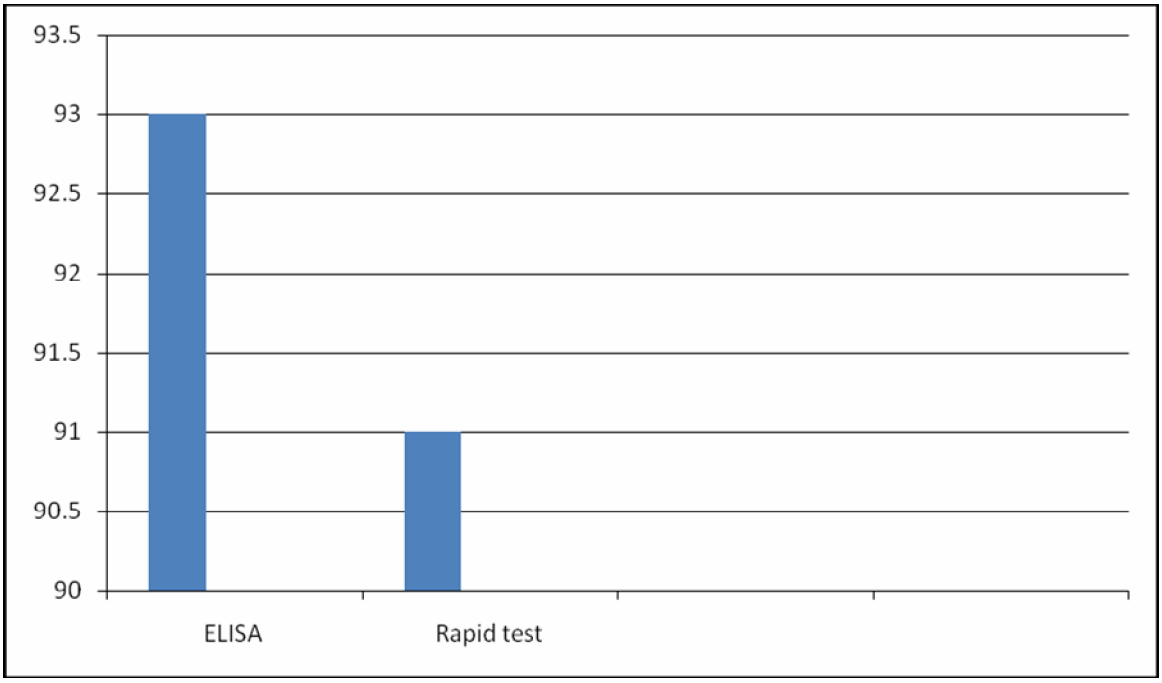


Figure.5 Comparison of efficacy ELISA and rapid test



Gurung *et al.*, used ELISA and rapid method for the diagnosis of scrub typhus. In their study one sample positive by rapid method was negative by ELISA. [18]In our study using IgM ELISA and rapid test, there was 97% correlation between the two tests. The difference was statistically significant indicting the superiority of ELISA as compared to rapid test. Therefore, it can be concluded that in centers with high workload, ELISA should be used but with less number of samples rapid method may be used for early diagnosis of scrub typhus.

Scrub typhus infections have been reported from neighbouring states like Tamil Nadu, Kerala, Karnataka and Orissa. The climatic conditions in Andhra Pradesh are more or less similar to these states therefore, it is highly likely that the various infections prevalent may be similar. Our study provides an evidence for the seropositivity of scrub typhus in this state.

Scrub typhus is prevalent but an undiagnosed disease in India. It should be considered in the differential diagnosis of patients suffering from acute febrile illness . This is particularly important after the rainy season and early cooler months, i.e. between August and October months. Rapid and specific diagnostic methods using ELISA can be carried out timely for early diagnosis of scrub typhus in patients with FUO.

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