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Research Article

PREVALENCE AND CLINICO LABORATORY STUDY OF SCRUB TYPHUS IN PAEDIATRIC PATIENTS ATTENDING A TERTIARY CARE HOSPITAL

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Abstract- Scrub typhus is a febrile disease caused by Orientia tsutsugamushi (Previously called Rickettsia) and is carried to humans by an arthropod vector of the Trombiculidae family (*Leptotrombidium deliense* and *L. akamushi*). Scrub typhus is a Rickettsial infection re-emerging with increased reports from different places in India and other South East Asian countries with considerable mortality and morbidity. Our aim of the present study is to report the magnitude of scrub typhus antibodies and to assess the clinical features and outcomes of paediatric scrub typhus in children attending a tertiary care hospital, Katuri Medical College, Guntur, from August 2014 to February 2017 with differential diagnosis and scrub typhus was diagnosed by positive IgM antibodies against *O. Tsutsugamushi*. A total of 87 children diagnosed as scrub typhus on the basis of eschar and specific tests were included in the study. They presented with subacute fever 100%, with eschar 29.68% hepatomegaly 36.90%, splenomegaly 45.31%, lymphadenopathy 56.25%, cough 25%, vomiting 48.43%, rigor 100% and Pain abdomen 23%. The counts of blood leucocyte were usually normal but 18.75% of patients exhibited thrombocytopenia. Elevated SGOT /SGPT and bilirubin were seen. There was 96.85% correlation between ELISA and immunochromatographic method. Scrub typhus cases were misdiagnosed due limited awareness of disease and no specific clinical presentation, and lack of proper diagnostic facilities. The clinical features of Scrub typhus mimics with other common infectious conditions, that should be ruled out diagnostically. Both ELISA and Immunochromatography test (ICT) found to be effective in diagnosis. Empirical treatment should be started with Doxycycline and Azithromycin, in suspected cases of Scrub typhus, as the patients responds well to with these antibiotics.

Keywords- *Scrub typhus, Eschar, Orientia tsutsugamushi, Enzyme Linked Immunosorbent Assay, Doxycycline, Azithromycin*

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Introduction

Scrub typhus, was described in Japan in 1899, it is an acute infectious disease, caused by *Orientia tsutsugamushi* formerly called Rickettsia. Rickettsia infection, also called as rickettsiosis, is an acute febril zoonotic disease caused by an obligate intracellular, small Gram-negative bacteria of the genera Rickettsia, Neorickettsia, *Ehrlichia Orientia* and Anaplasma[1]. In India the causative agent of scrub typhus is *O. Tsutsugamushi*, which is antigenically different from other members of Rickettsiae, about 8 serotypes have been recognized [2]. Typhus fever is transmitted to humans by larval mites or "chiggers, Leptotrombidium *deliense* and *Leptotrombidium akamushi*. This is found only in areas with a suitable climate, plenty of moisture and scrub vegetation [3] and the larval stage feeds only on blood meal [2]. Scrub typhus is an endemic disease in the geographical region which is known as "*tsutsugamushi triangle*" that 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 in the west Pakistan and Afghanistan countries [4]. Scrub typhus is an endemic disease in many parts of India, in 1960s and 1970s. Drastic usage of insecticides in the later years, reduced the burden of this disease from our country. However, in the recent years, there had been resurgence and re-emergence of the Scrub typhus in India, several cases of scrub typhus were reported from India and especially number of positive cases were increasingly reported from southern region [5]. In tropical areas scrub typhus transmission occurs throughout the year, in case of temperate zones seasonal transmission is seen.

Rainfall influences the occurrence of *Leptotrombidium deliense*, in the wetter months a greater number of chiggers were attached to the rodents, which may be the reason for a greater number of cases during the rainy season and number of outbreaks was reported during the cooler months in southern parts of India [6]. These larvae usually feed on the small rodents in particular the wild rats which belongs to subgenus Rattus. Humans will get infected accidentally, when they trespass an area of infected mites especially in rainy season. The chiggers after grasping a passing host, they preferably feed the thin, tender and wrinkled areas of skin. After attaching, they will inject a liquid, which dissolves the tissue around the feeding site. This liquefied tissue is then sucked up assustenance for the chigger. As large numbers of the *R.tsutsugamushi* organisms present in the salivary glands of the chigger, they are injected into its host when it feeds After an incubation period of 6-21 days, the rickettsia proliferates at the site of the chigger bite to form in <50% of cases a necrotic eschar with an erythematous rim [7]. The observation of the eschar is often missed and other signs and symptoms are not characteristic for the disease thus posing the problem of delay in diagnosis by the clinician [4]. Clinical manifestations of scrub typhus vary widely from a mild and self-limited febrile illness to a more severe course which may be fatal [8]. the onset of disease is characterized by fever, headache, myalgia, cough, and gastrointestinal symptoms. Patients often present with pyrexia of unknown origin (PUO) [9].

Scrub typhus is usually under-diagnosed in India, due lack of specific clinical presentation and least awareness. so, high index of suspicion should be required in febrile children, pertaining to endemic regions. The classical presentation of fever with eschar and accompanying with clinical symptoms such as headache, myalgias and organ dysfunctions especially, acute renal failure ARF, hepatitis in liver involvement, acute respiratory distress syndrome (ARDS) with lungs, meningitis with central nervous system involvement and circulatory collapse with haemorrhagic manifestations. Scrub typhus is one of the differential diagnoses (in addition to enteric fever, leptospirosis, malaria or dengue fever) in patients with haemorrhagic fever, especially if associated with jaundice and/or renal failure [10-12].

Scrub typhus in high risk group

In Pregnancy: Vertical transmission occurs transplacental and perinatal blood-borne infection occurs during labour, has been reported in earlier studies [13,14].

In children: The febrile illness may be mild or severe. Most common clinical presentation is fever with regional or generalized lymphadenopathy. Appearance of painless eschar, maculopapular rash, splenomegaly, hepatomegaly and gastrointestinal symptoms like diarrhea, vomiting and abdominal pain) may be seen. Case fatality rate in case of untreated may be as high as up to 30%, although deaths in children are infrequent [15].

The immunofluorescence test assay (IFA) is considered to be a 'gold standard' in diagnosing rickettsiosis, but fluorescent microscope equipment expensive and not available in every laboratory setting. Moreover, the test requires an expert for its use ideally needs cell culture facilities for sustaining rickettsia antigens and often can requires a week to get the results. The most widely used serological test for rickettsia screening is the Weil-Felix test, the organism possess a polysaccharide antigen which have antigenic relation with proteus OX-K, which is utilized in Weil-Felix test for confirmation of scrub typhus, although its reliability is suspected due to the poor sensitivity and specificity. Recent outbreaks are reported by detecting the antigen-specific IgM or IgG antibodies by ELISA [16].

Materials and Methods

This Prospective cross-sectional, diagnostic study was conducted at paediatric medical unit of our tertiary care hospital GEMS Srikakulam in, India. Institutional ethical and research committee, consent form, was taken from children's parents. All children with undiagnosed acute febrile illness below the age of 12 years, admitted in the department of paediatrics were enrolled in this study. All the suspected samples were processed tested for IgM antibodies of scrub typhus by ELISA and by Immunochromatography assay. All the clinical samples were also tested for typhoid, dengue, tuberculosis, leptospirosis, and malaria. Detection of IgM antibodies by ELISA was done using In Bios International TM IgM ELISA. Detection of IgM antibodies by rapid method was performed using SD Bioline *Tsutsugamushi*, one-step scrub typhus antibody test method.

Results

Highest numbers of cases were seen in the month of October. This study evaluates varying clinical presentations in different cases, pattern of morbidity, and associated complications with scrub typhus. The various observations were analyzed. Among the 87 pediatric cases tested, 64 cases were diagnosed positive for Scrub typhus, the ages of the cases ranged from 1-12 yrs [Table-1]. 23 cases were excluded, as the patients had other comorbidities like tuberculosis, malaria, dengue and enteric fever, which are other febrile illness endemic in this area, whether the Scrub typhus may be positive due to cross reactivity or there were coinfections. All the 64 cases were positive by ELISA IgM method whereas 62 cases (96.87%) were positive by Immunochromatography method, there was 96.85% correlation between ELISA and rapid method. Demographic and clinical profile of scrub typhus patients is enlisted in the [Table-1] and [Table-2]. Different laboratory investigations in patients with positive Scrub typhus were listed in [Table-3]. Fever was the most common manifestation (100%). Skin rash was seen only in 9.6% of patients [Table-2] and [Table-3]. Eschar was seen in seven patients and skin rash was seen in five patients over chest and abdomen.

Discussion

Scrub typhus is a re-emerging febrile illness, seen spread widely in the areas of eastern Asia and the western Pacific regions, which clinically presents as an acute febrile disease with no specific clinical signs and symptoms. The disease transmission doesn't occurs from one individual to other, infected larval stage only involves in transmission of the disease, infected chigger bites humans, during feeding on humans, chiggers inoculates *O.tsutsugamushi* pathogens. The organism multiplies at inoculated region with and forms a papule, leads to ulceration and necrosis results and forms an eschar. Regional lymphadenopathy occurs, within a few days it advances to generalized lymphadenopathy [10, 17].

Orientia tsutsugamushi, targets humans endothelial cells throughout the body, also the macrophages and cardiac myocytes. Rickettsia infection occurs, causing focal or disseminated vasculitis and perivasculitis with significant vascular leakage leading to end-organ damage. Injury to liver, spleen, lung, heart, and central nervous system, thus explaining the various unexpected clinical manifestations [17].

Table-1 *Age distribution of Scrub typhus patients*

SN	Age wise distribution	No. Of Patients	Percentage
1	0-2	3	4.68%
2	2-4	10	15.62%
3	4-6	8	12.50%
4	6-8	12	18.75%
5	8-10	17	26.56%
6	10-12	14	21.87%

Table-2 *Clinical profile of patients with scrub typhus*

SN	Clinical Conditions	No. of cases	Percentage
1	Fever	64	100%
2	Rigor	64	100%
3	Vomiting	31	48.43%
4	Eschar	19	29.68%
5	Skin Rash	17	26.56%
6	Pain Abdomen	24	23%
7	Diarrhoea	20	31.25%
8	Seizures	4	6.25%
9	Cough	16	25%
10	Lymphaedenopathy	36	56.25%
11	Icterus	6	9.30%
12	Pallor	14	21.87%
13	Hepatomegaly	31	36.90%
14	Spleenomegaly	29	45.31%
15	Jaundice	4	6.25%
16	Mortality	-	-

Table-3 *Laboratory investigations*

SN	Investigations	Number	Percentage
1	Leukocytosis	14	21.87%
2	↓Platelets	12	18.75%
3	↑Bilirubin >1.2mg/dl	13	20.31%
4	↑Creatinine	5	7.81%
5	↑ SGOT	21	32.81%
6	↑SGPT	19	29.68%
7	↑Alkaline Phosphate	14	21.87%
8	↓ Albumin	8	12.50%
9	↓Decreased Sodium	21	32.81%

It induces the generation of various kinds of cytokines like interferon γ, macrophage-CSF, tumor necrosis factor-α (TNF-α), and Granulocyte – Colony - Stimulating factor (GSF) The cytotoxic T-lymphocytes and the NK T-cells plays a vital role in attacking the infected cells of host. The organism suppresses the host defence mechanism by down regulating the GP-96 on the macrophages and the endothelial cells, which is essential for antigen presenting, dendritic cells functioning, production of antibody, and cross - priming of the immune system. The immune response for *Orientia tsutsugamushi* is both antibody mediated and cell mediated. Antibody mediated immunity takes part in of strain-specific antibodies which will reduce the organism's ability in entering the cells by altering nonspecific attractions between target cells and infectious agents [18].

Scrub typhus is usually under-diagnosed in India, because of limited awareness, diverse and nonspecific clinical presentation, and low index of suspicion among clinicians and lack of diagnostic facilities. This study was designed to evaluate the level of antibodies specific for scrub typhus among paediatric patients attending a tertiary hospital. Immunofluorescence test, is the gold standard test in the serologic diagnosis of scrub typhus, IgM, IgG and IgA antibodies against *O. tsutsugamushi* from the serum of suspected febrile paediatric patients were diagnosed using SD Bioline, Standard Diagnostics, Korea. The endemic strain differs geographically, hence the clinical course of the illness and prognosis varies. Kalal, *et al*., (2016) [16] reported a raise in scrub typhus cases during the cooler months, this phenomenon was also observed in our study where maximum number of cases was observed in the months following the rainstorm leading to winter. Highest numbers of cases were seen during the month of October resembling autumn-winter type scrub typhus. Similar seasonal distribution has been reported [17-19]. Appearance of eschar, was seen among (13.4%) of cases in our present study, similar to the findings seen with Kedareshwar, *et al*., (2012) [11] (13.3%), Oberoi and Varghese (2014) [12] (14%), Kumar, *et al*., (2012) [1] 11% and Rajoor, *et al*., (2013) [19] (10%) whereas, some other studies from India reported the appearance of eschar in 50% of cases [20] 17.5% in Sharma, *et al*., (2015) [21] study, 43.5% in Narendra Rungta, (2014) [22] 47.36% in Jain and Jain, (2012) [23] of an eschar in 50-70% of cases [23], Radha Kumar and Purusothaman Srinivasan (2017) [24], Shah, *et al*., (2010) [25]. Often individuals are not aware of existence of eschar as it won't produce any signs of discomfort. Some reports from south India, didn't found an eschar in any of their cases.

Fever with chills and rigors were the common presentation seen in our study similar to a study from Tirupati by Ramyasree, *et al*., (2015) [4]. Regional or generalized lymphadenopathy has always been a characteristic feature of rickettsia infection. Present study shows generalized lymphadenopathy and hepatomegaly was the most common complication seen in 34% of patients, other studies by Deb, *et al*., (2016) [26] (13%), Ajith Babu, *et al*., (2016) [4] 22%, Manish Kumar, *et al*., (2012) [1] (37%), Jain and Jain, (2012) [23] (42.1%). from different areas reported varying degrees of complication.

Antibiotics of choice for Scrub typhus treatment is Doxycycline, Macrolides, Chloramphenicol and Tetracycline. All the cases were treated with Doxycycline or Azithromycin. In case of children above eight years, regimen Doxycycline 4 mg per kg weight per day orally or IV divided every 12 hours, maximum 200mg per day) (10 mg per kg per day) for one week. Alternative antibiotics are Tetracycline (25-50mg per kg weight per day orally divided every 6 hours, maximum 2 gms per day) or Chloramphenicol (50-100 mgs per kg body weight per day divided every 6 hours IV, maximum 4 gms per 24 hrs). Therapy should be continued for a minimum of five days and till the patient becomes afebrile for more than three days to avoid relapse. Chloramphenicol is used in late trimester to prevent fetal transmission [27, 28], Doxycycline, a category D drug, is not recommended for pregnant women. Bhat, *et al*., (2016) [29] 90%, and a study Radha Kumar from Chennai reported 94.1%, of cases became afebrile with doxycycline treatment within 48hrs of initiation. Our patients responded well to therapy with antibiotics, 96.1% of patients became afebrile with in 48hrs of antibiotic therapy. In our study there was no mortality seen.

Conclusion

The present study assesses the clinical and laboratory findings of severe scrub typhus in children. Scrub typhus should be suspected as one of the differential diagnosis of high-grade fever, with presentation of lymphadenopathy, hepatosplenomegaly and other nonspecific symptoms like nausea, vomiting, pain abdomen, especially in a condition where no other definite diagnostic could be ruled out. History of bite with mites should be elicited and whole body is searched for Escher. Both ELISA and lateral flow Immunochromatography test found to be effective in diagnosis. High level of clinical suspicion with thorough empirical therapy covering the regimens, susceptible for Scrub typhus is required for better outcome and complete recovery. Specific treatment with doxycycline should not be delayed waiting for confirmation of diagnosis to avoid mortality. Inappropriate diagnosis and delay in treatment may proceeds to severe complications like gastro intestinal bleeding, shock, meningoencephalitis, pneumonitis and acute renal failure with notable morbidity and mortality in children. In the recent years number of studies has reported the resurgence of Scrub typhus from different parts of India, but there is paucity in community-based data, more research is required on this entity with respect to epidemiology, pathogenesis, clinical findings in context of the Indian subcontinent.

Application of research: Study assesses the clinical and laboratory findings of severe scrub typhus in children's

Research Category: Medical microbiology

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