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A Study of Scrub Typhus, Its Various Clinical Presentation and Antigenic Types Detected by IFA

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A B S T R A C T

Scrub typhus is a bacterial zoonosis caused by *Orientia tsutsugamushi*. Timely diagnosis is necessary as the disease presents with varied signs, symptoms and may progresses to multiorgan dysfunction syndrome. Scrub typhus shows wide antigenic variation, one of the methods for its detection is IFA. There is scarcity of data regarding antigenic variations of *Orientia tsutsugamushi* which is of importance in our country. About 100 serum samples were collected from patients who had fever for > 7days (excluding other causes of fever). IFA detecting IgM antibodies to *Orientia tsutsugamushi* was done. Among 100 samples, 27 were positive by IFA. Common symptoms were abdominal pain (81.48%), vomiting (77.7%), myalgia (70.07%). O/E patients showed hepatomegaly (70.37%), lymphadenopathy (66. 6%), splenomegaly (51. 8%) and thrombocytopenia (77.77%) and Anaemia in (44.4 %) patients. Raised SGOT (81. 48%), SGPT (77.77%) with varied LFT was observed. Common Antigenic type was Gilliam (37. 03%), Karp (18.51%). Diagnosis of Scrub typhus is a challenge as it presents with various signs and symptoms. Antigenic variation of *Orientia tsusugamushi* will help in knowing a circulating strain which can be of use for a sensitive point of care test.

Introduction

Scrub typhus is a mite born acute febrile illness more commonly also known as tsutsugamushi disease. [1] Man is accidentally infected by an arthropod vector belonging to the trombiculidae family, more accurately called chigger borne Rickettsiosis. Scrub typhus is more commonly reported during the rainy season especially in mite infested areas which is made up of secondary scrub growth. [1, 2] People involving in the agricultural work are known to be more prone to develop Scrub typhus. It posses a major public health problem in South and Southeast Asia and the Asian Pacific rim. [3]

Recently it is been reported that there is a resurgence in Scrub typhus infection in India & other neighbouring countries. Scrub typhus infection is known for its unusual clinical presentation associated with significant mortality & morbidity. Most common presenting symptom is acute fever, along with

cough, breathlessness, nausea, vomiting, myalgia & eschar at the site of inoculation which may or may not be present since it as a variable presentation. [4]Clinical manifestations reported from India is variable when compared to other Asian countries which is said to be attributed to the infecting strains causing Scrub typhus, these strains have an higher range of antigenic variation. [6] *Orientia tsutsugamushi* is known for its wide antigenic diversity conventionally been classified by the reactivity with hyperimmune serum against prototype strains (kato, karp, Gilliam, Kawasaki, Kuroki or shimogoshi). [7] Knowing the epidemiology & diversity of *Orientia tsutsugamushi* is required for designing of diagnostic methods that can help in early diagnosis and also required for development of vaccine in endemic areas of Scrub typhus. [8]Various methods are available for detection of antigenic type, IFA is one of the method used for detection which is a standard and reliable method. In India very few studies have been carried out for identification of antigenic types in places like Vellore in South India, Shimla in Northern India and from Shillong in Northeast India. [9]

This study was taken up with the aim to know the prevalence, clinical presentation & antigenic types of Scrub typhus which help to differentiate it with other causes of acute febrile illness which helps in knowing the infecting strain in our place and knowledge of knowing the antigenic / infecting strain in the endemic area helps in early diagnosis of the disease.

Materials and Methods

This hospital based prospective study was carried out in the Department of Microbiology, J. S. S. Medical college & hospital over a period of 1 year. About 100 serum samples were collected from patients presenting with febrile illness of more than 7 days of all age groups with or without rash with detailed clinical history.

Other causes of fever like Malaria, Leptospira, Dengue, Enteric fever, urinary tract infection were laboratory confirmed & excluded from the study.

All 100 non consecutive serum samples were subjected to Indirect Immunofluorescence (*Orientia tsutsugamushi*) IFA IgM antibody kit (Fullerton California). Manufacturer's test protocol was followed for testing and result interpretation.

IgM immunofluorescence assay

Patient sera was diluted using IgM serum diluent to 1:16 dilution, further dilution was done using wash buffer to obtain a final dilution of 1:64 which was referred to as screening dilution.

Positive reaction was interpreted with the presence of bright staining (atleast 1+) of short pleomorphic rod forms in any of the 4 antigen areas (kato/karp/gilliam/boryong), size appearance, and density of each field is compared with positive and negative control. With no distinct and characteristic staining of the Orientia was considered negative. Patterns of reactivity different from the positive controls was considered non specific.

Results and Discussion

Out of 100 serum samples, 27 (27%, 27/100) gave IgMIFA positive for Orientiatsusugamushi. Most of the cases of Scrub typhus were seen in the month of June, July, November and December depicted in Table 1.

Most common presentation was fever along with either of the associated symptoms like abdominal pain, chills and rigor, myalgia,

abdominal pain, headache, cough, vomiting, rash Etc depicted in Table 2. More common clinical finding observed was hepatomegaly, lymphadenopathy & spienomegalyetc which has been depicted in Table 3.

Common haematological findings seen was thrombocytopenia depicted in Table 4 which was noted in about 21patients (77. 77%), followed by increased ESR level seen in about 15 patients (55. 55%) and Anaemia seen in 12 patients (44. 4 %) leucocytosis seen in 11 patients (40. 74%).

Common lab finding of liver function test observed in our study was raised SGOT level seen in 22 patients (81. 48%), followed by raised SGPT level seen in 21 patients (77. 77%), raised alkaline phosphatase level seen in 18 patients (66. 66 %), low serum albumin level seen in 12 patients (44. 44 %) depicted in Table 5.

Scrub typhus is one of the important differential diagnosis in patients who present with undifferentiated febrile illness, often the presentation is nonspecific. Early diagnosis helps to prevent development of complication and reduce mortality and morbidity. Knowing the antigenic type helps to identify the common strain present in a particular endemic area. So, our study has included to know the local prevalence of different antigenic types with various clinical presentation in Scrub typhus patient.

Out of 27 positive Scrub typhus cases, it was observed that more number of positive cases were seen during the month of June, July, November and December. Chiggers live in wide range in vegetation type from scrubs (terrain between woods and clearings) in forests and gardens & these infected mites occurs more frequently during and after rainy season. Also probably the reason of spurt of cases where activities of people bring them into contact with vector chigger maybe recently of more gardening & man loving to live closer to nature. NarendraRungta *et al.,* showed in his study that more no. of cases was reported between July and December which correlates with our findings. [10]

Fever was the most common presentation (100%) observed in our study, which was similar to the study done by Vishal dasari *et al.,* and Narendrarungta *et al.,* fever was the most common presentation, this was in concordance with our study[10, 11]In the present study, abdominal pain was the second most common clinical presentation of about (81. 48%), which was higher in percentage when compared to the study conducted by Shirishinamdar *et al.,* where abdominal pain was seen in about 56% but similar observation was made in the studies that, abdominal pain was the more common clinical symptom associated with fever. [12]Vomiting was seen in about (77. 7%) in present study which was higher in contrast to a study done by George M Vargese *et al.,* where nausea/ vomiting were seen in about (54%) [13.] Study done by Upasanasingh *et al.,* (n=114) among positive cases of Scrub typhus, myalgia and headache were seen in all patients (100%) however in our study only 74% of patient presented with myalgia. [14]

Cough was one of the presenting symptom seen in about (37. 03 %) which was in concordance with the study conducted by George M. Vargese *et al.,* where cough was seen in about (38%). [13] Rash (eschar) was noted in 7. 40 % in the present study, in comparison with the study conducted by Senaka Rajapakse *et al.,* prevalence of eschar varied from 7% -80%. This difference in observation or presence of eschar may be because of difficulty in detecting the eschar in dark skinned individuals or eschar inducing capacity of different strains of *Orientia tsutsugamushi*[15].

Table.1 Showing seasonal distribution of positive cases of Scrub typhus

Month	No. of patients	%
January	1	3. 70%
February	1	3. 7%
March	2	7. 40%
April	2	7. 40%
May	2	7. 40%
June	4	14. 81%%
July	7	25. 92%
August	1	3. 70%
September	0	0%
October	0	0%
November	4	14. 81%
December	3	11. 11%

Table.2 Showing fever with associated symptoms in Scrub typhus

Symptoms	Number	Percentage
Fever	27	100%
Chills and rigor	18	66. 6%
Myalgia	20	74. 07%
Abdominal pain	22	81. 48%
Headache	20	70. 07%
Cough	10	37. 03%
Vomiting	21	77. 7%
Rash	2	7. 40%

Table.3 Showing Clinical findings in patients of Scrub typhus

Signs	Number of cases (n=27)	Percentage
Hepatomegaly	19	70. 37%
Splenomegaly	14	51. 80%
Lymphadenopathy	18	66. 60 %
Conjuctival congestion	7	25. 90%
Tachypnea	4	14. 80%
Eschar	2	7. 40%

Table.4 Variation of Haematological parameters seen in Scrub typhus cases

Findings	No. of cases (n=27)	Percentage
Hb	12	44. 4%
Leucocytosis	11	40. 74%
Thrombocypenia	21	77. 77%
Increased ESR	15	55. 55%

Table.5 Showing Altered Liver function test in patients of Scrub typhus

Findings	Number of patients (n=27)	Percentage
Serum albumin (<3. 5 gm%)	12	44. 44%
Total bilirubin (>1. 5mg%)	11	40. 70%
SGPT (>40 IU/ L)	21	77. 77%
SGOT (>40 IU/L)	22	81. 48%
Increased Alkaline phosphatae (ALP)	18	66, 66%

Table.6 Showing antigenic types of Scrub typhus as detected by IFA

Antigenic type	No. of samples (n=27)	%
Gilliam	10	37. 03%
Karp	5	18. 51%
Kato	3	11. 11%
Boryong	1	3. 70%
Gilliam + karp	1	3. 70%
Kato+karp	1	3. 70%
Kato+boryong	2	7. 40%
Kato+Gilliam	2	7. 40%
kato+Gilliam+boryong+karp	2	7. 40%

Since eschar are very small in size, the physician may miss or delay the recognition and also because of similarity of eschar with the scab formed during trauma. [16]Chills and rigor was noted in (66. 6%) patients, similar observation was also made by Mahajaan *et al.*, study where chills & rigor were present in (72%). [17]Various symptoms not conclusive for Scrub typhus were noticed in our study. This can create a lot of confusion while diagnosing a particular disease. High suspicion is always required for diagnosis Scrub typhus.

Hepatomegaly was seen in 70.37%, lymphadenopathy was seen in 66.6% in our study. Hepatomegaly as a clinical finding was in concordance with the study conducted by ShirishImandar *et al.,* where hepatomegaly was seen in (74%). Lymphadenopathy as a clinical finding was comparable with the study conducted by Sanjeevkumardigra *et al.,* lymphadenopathy was seen in (61. 9%) [12, 18]In the current study, splenomegaly was seen in 44. 4% which was in concordance with the study conducted by Man je park *et al.,* where splenomegaly was observed in (46. 8%). [19]Tachypnea was observed in about 14. 8% which was in concordance to the study conducted by Silpapojakul *et al.,* where he stated scrub typhus was characterised by fever, tachypnea and hepatomegaly. [20]In the present study, conjunctival congestion was seen in (14. 8%) however a study done by Annilmahajan *et al.,* where conjunctival congestion was observed in (30%) of the cases. [21] Abdominal pain with hepatomegaly was a very common symptom among scrub typhus patients in our study population.

Most common lab finding observed in our study was thrombocytopenia (77. 77%) and leucocytosis (40. 74%) which was comparable with the study done by George

M. Varghese *et al.,* where thrombocytopenia was (79%), leucocytosis was (46%). These laboratory findings were also similar to the study done by Raman Sharma *et al.,* where thrombocytopenia was seen in (85. 6%), leucocytosis was seen in (44. 8%). [13, 22] In our study, thrombocytopenia was also a common symptom which can confuse diagnosis with other viral infections like Dengue.

Anaemia as a clinical finding was seen in 12(44. 4%) patients which was similarly seen in the study conducted by Nitingupta *et al.,* in new Delhi where anaemia was seen in 45. 6% patients. [26] Most common observation in our study was altered LFT i. e. raised AST which was seen in 22(81. 48%) patients which was comparable with the study done by Ming Luen Hu *et al.,* where raised AST was seen in 89. 3% patients. Raised ALT was observed in 21(77. 77%) which was slightly less when compared to Ming Luen Hu *et al.,* study where raised ALT was (91. 7%). [23]

Increased bilirubin level observed in our study was (40. 70%) similar findings was seen in study conducted by Ming Hu *et al.,* where increased bilirubin level was (38. 5%). [23] Raised ALP level observed in our study was (66. 66%) which was less in comparison to the study conducted by Ming Hu *et al.,* where raised ALP level was seen in 84. 2% patients.

Raised transaminase levels as a common lab findings seen in our study was in concordance with the study conducted by Raman Sharma *et al.,* where raised transaminase level (AST & ALT) was seen in 98.4% patients. [22] Low albumin level as a result of altered LFT was seen in (44. 4%) which was less in comparison to the study conducted by Ming Luen Hu *et al.,* where low albumin level was seen in (83.3%). [22, 23] Liver function test abnormality is one of the important finding in patients with scrub typhus as evidenced in this study.

Four antigenic types (Gilliam, Kato, Karp, Boryong) were detected in our strains, most common type obtained was Gilliam (37. 03%). which was comparable with the study done by Liu Yun-xi *et al.,* in China where predominant strain was Gilliam (21 isolates out of 23). The high predominance of this antigen may be because of the antigenic heterogenecity using serological method like IFA, because of the existence of homology of Gilliam to Kawasaki type. [24] The antigenic types reported in other studies like study done by George M vargeseetal, Kato-like strains predominated (61. 5%), especially in the South and Northeast, followed by Karp-like strains (27. 7%) & Gilliam and Ikeda strains (2. 3% each) strains were observed in the Northeast. This predominance was seen in wide geographical area like 95 cases from Vellore in South India, 72 cases from Shimla in Northern India, and 96 cases from Shillong in Northeast India. [9] In our study among the 4 antigenic types, the most common type obtained was Gilliam (37. 03%), second common antigen type obtained in our study was karp (18. 51%) and kato 11. 11%. When compared to the study performed by Kelly *et al.,* using IFA, karp isolates obtained were (60%-64%) which was in discordance with our study. Reason may be due to size of study population included in Kelly *et al.,* study where population included were from 5 geographically distinct regions in Thailand and factors like geographical distribution and epidemiological prevalence which has to be considered [25].

In our study predominant infecting antigenic strain was Gilliam followed by karp. Four mixed antigenic types were seen in few patients which may be explained due to mixed infection. This study was done on a small population and PCR was not done. Knowing antigenic types in a region will definitely help to understand the epidemiology of Scrub typhus and improvise the diagnosis. Varied

clinical presentation of this disease is a hurdle to diagnosis.

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