RESEARCH PAPER

Clinicoepidemiological and Genotyping Correlation of Pediatric Scrub Typhus from Chandigarh, India

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Objectives: We studied the clinical phenotypes and prevalent genotypes of *Orientia tsutsugamushi* in our area using indirect immunofluorescence assay (IFA). **Methods:** We prospectively screened all febrile children presenting to our hospital over three years. From among children who were scrub typhus positive by ELISA we selected a sample of convenience for IFA testing to determine the genotypes of *O. tsutsugamushi* using four strains namely Boryong, Gilliam, Karp and Kato. **Results:** Of all scrub positive patients (*n*=77), we tested 14 samples using IFA and all 14 samples were IFA positive. Karp genotype (*n*=7) was most prevalent followed by Kato (3), Boryong (1) and Gilliam (1) genotypes; 2 patients were positive for mixed genotype. There was high prevalence of organ dysfunction among IFA positive children. Three most common organ dysfunctions included hematological derangement in all, liver involvement in 10 (71%), and encephalopathy and shock in 4 each. **Conclusions:** Karp was the most prevalent genotype of *O. tsutsugamushi* in our area.

Keywords: *Antigenic variation, O. tsutsugamushi, Rickettsial infection, Outcome*.

Scrub typhus is an endemic infection in Tsutsugamushi triangle, putting one billion people at risk annually [1]. The clinical presentation in scrub typhus is variable ranging from acute undifferentiated febrile illness to shock and multiorgan dysfunction [2]. Scrub typhus caused by *Orientia tsutsugamushi* has more than 20 antigenic strains, classified into high virulence group (Karp, Kato and KN-3 genotypes), an intermediate virulence group (Gilliam genotype) and a low virulence group (Kuroki, Kawasaki and KN-2) [3-5]. The severity of infection and nature of complications in scrub typhus vary with the genotype [6,7]. It has been reported that strains closely aligned with Karp prototype are associated with manifestations like hepatitis, meningitis and multiorgan dysfunction in adults [5,8]. There is paucity of data in children, hence we studied the prevalent genotype of *O. tsutsugamushi* in our geographical area using an indirect immunofluorescence assay (IFA).

Methods

This cross-sectional study was carried out in the Pediatric emergency of a tertiary-care referral teaching hospital in Chandigarh, India from June, 2013 to December, 2017. Ethical approval was obtained from the Institute Ethics Committee. We screened all children aged 2 months to 14 years, who got admitted to our emergency with the diagnosis of scrub typhus (positive IgM ELISA cut-off optical density value ≥0.5) (InBios International Inc., USA) [9]. We excluded children with lethal malformations or known immunodeficiency. Out of the scrub positive cases, a convenient sample of children was tested for IFA. We obtained informed consent from one of the parents before enrolment in the study. The demographic profile, clinical presentation, laboratory manifestations and treatment history of all study children were noted.

IFA test for O. tsutsugamushi was performed to determine the prevalent genotypes in our area. IFA test kit (Fuller Laboratories, California, USA) semi-quantitatively determined IgM antibodies against four strains of *O. tsutsugamushi* namely, Boryong, Gilliam, Karp and Kato. The test samples were standardized for various dilutions ranging from 1:32 to 1:256. An IFA result in the acute phase was considered positive, if IgM antibody titres were ≥1:64 [10]. Additionally, all children were screened for malaria, typhoid, dengue, leptospira, and underwent other relevant tests, wherever indicated.

As we performed IFA on a convenient sample, we compared salient demographic and clinical characteristics of IFA-positive children with rest of scrub typhus positive children who were not sampled for IFA. Continuous variables were compared using independent *t* test or Mann-Whitney U tests as applicable. Proportions were compared using chi-square test. The analysis was performed using SPSS 20.0 (IBM, New York).

RESULTS

Out of 77 scrub typhus ELISA positive children during the study period, we performed IFA test in 14 children. All samples were IFA positive. The salient demographic and clinical characteristics of the IFA positive children were comparable to remaining Scrub typhus children who were not sampled for IFA (***Table* I**).

Salient demographic and clinical characteristics of the 14 IFA positive children is presented in ***Web Table* I**. We observed high incidence of organ dysfunction in the study children. Hematological derangements were universal [thrombocytopenia (*n*=11, 79%); anemia (*n*=10, 71%); and altered leucocyte count (*n*=5, 36%)]. Other common organ dysfunctions in our cohort include elevated serum transaminases (*n*=10, 71%), encephalopathy or seizures (*n*=4, 29%), shock (*n*=4, 29%), serositis (*n*=3, 21%), and pulmonary dysfunction (*n*=2, 14%). Physical examination did not reveal eschar in any child. Salient laboratory findings of the cohort included mean (SD) haemoglobin of 9.9 (1.7) g/dL, total leucocyte count of 10.3 (5.9) $\times 10^3$ cells /mm^3 and INR- 1.1 (0.1), the median (IQR) platelet count was 78.5$\times 10^3$ /mm^3 (45.0, 142.5); Asparate aminotransfenase - 174 IU/dL (116, 236), Alanine aminotransferase - 86 IU/dL (44,151), and total serum bilirubin of 1.0 mg/dL (0.2, 2.8).

We observed maximum prevalence of Karp genotype (7) (***Web Fig.*1**), followed by Kato (3), Boryong (1) and Gilliam (1). Two patients were positive for mixed genotype *i.e.* one had both Karp and Kato and another was positive for Karp and Boryong. IgM IFA titres in most of the cases were 1:128 or more (***Web Table* I**). Five of the 7 children (71%) with Karp genotype had multisystem involvement. All 14 children received doxycycline in recommended doses for 7 days [9,11] and showed defervescence within 3 days of doxycycline. All patients survived, and no adverse reaction was noted following doxycycline.

Table I Characteristics of Children with Scrub Typhus (*N*=77)

Characteristics	*Sampled for IFA (n=14)*	*Not-sampled (n=63)*
Age, mo*	92.7 (39.9)	85.8 (42.2)
Weight, kg*	23.3 (10.3)	21.0 (8.9)
Male	8 (57)	35 (57)
Fever duration (d)[#]	7 (7, 10)	6 (5, 8)
Encephalopathy	5 (36)	13 (21)
Hepatosplenomegaly	8 (57)	26 (77)
Shock	4 (10)	12 (19)
Death/LAMA	1 (13)	4 (6.3)

*Values in no.(%) except, *Mean (SD), [#]Median (IQR); All P >0.05, LAMA: Left against medical advice, IFA: Immunofluorescence assay.*

DISCUSSION

In our study group of 14 cases, Karp was the commonest genotype with a high prevalence of multiorgan dysfunction. Eschar was notably absent in all children.

Previous studies published from India and Himalayan regions of India reported Karp as the most prevalent genotype [1,8,12]. Findings of other endemic regions of tsutsugamushi triangle *i.e*. Thailand and Vietnam were similar [6,7]. In contrast, Kato strains are more frequently reported from Southern India [8]. This antigenic diversity is due to varied intragenomic rearrangements and recombinations, exact process of which is not clear. The virulence of Karp, Kato and Gilliam genotypes have been reported previously in mice and adults [4-6]. Acute encephalitis syndrome (AES) presentation was seen in 4 children, which has also been reported in young children with scrub typhus from Gorakhpur and north-eastern India [1,13]. An absence of eschar in predominantly Karp prototype has also been corroborated in other adult studies, especially from the Himalayan region [1,7,12,13]. Hematological and liver dysfunction was also conspicuous in children with scrub typhus from North-eastern and Southern India [13,14]. In the absence of eschar, diagnostics like serology attain importance for therapeutic and epidemiological purposes [9,12]. The sensitivity of scrub typhus IgM ELISA is 99.9% and specificity 99.1%, respectively and IgM IFA sensitivity 96.8% and specificity 99.7%, respectively across different regions in India [9,10]. Response to therapy occurred within 72 hours in all children following doxycycline, thereby reaffirming efficacy [9].

The study has few limitations. It is a single centre study with limited samples of scrub typhus children being tested by IFA. Two children in our cohort had mixed infections with dengue virus, which possibly suggests co-infection. The diagnosis of scrub typhus was supported by both IgM ELISA and IFA positivity. In endemic areas, co-infections are not uncommon [13]. However, a possibility of false-positive results of serologic tests cannot be completely ruled out. We did not perform molecular diagnosis by PCR. PCR has added advantage of phylogenetic tracing; however, results are best within the first week for blood samples because of presence of rickettsemia [1,8,9,11]. However, in the absence of availability of PCR and after the first week of illness, IFA remains an attractive option. Also PCR yield is higher on eschar samples [15]. As not all strains are likely to produce eschar, this could skew results [1,8].

WHAT THIS STUDY ADDS?

- Karp was the most prevalent genotype isolated by Immunofluorescence assay among scrub typhus cases, which correlated with severe systemic manifestations in children.

IFA is the reference serological gold standard for diagnosis but cost and expertise limit its use. In clinical care, ELISA usually suffices [9,11].

Out data; although from a small number of children, provides important information for diagnostic and epidemiological purposes.

Contributors: NS: performed the serological tests, edited the manuscript and approved the final manuscript; VM: conceived the idea, enrolled the patients, managed the cases, analysed the data, wrote the 1st draft; JC: supervised laboratory analysis, critically evaluated the manuscript; VG: supervised the clinical management of the children, critically evaluated the manuscript. All authors approved the final manuscript.

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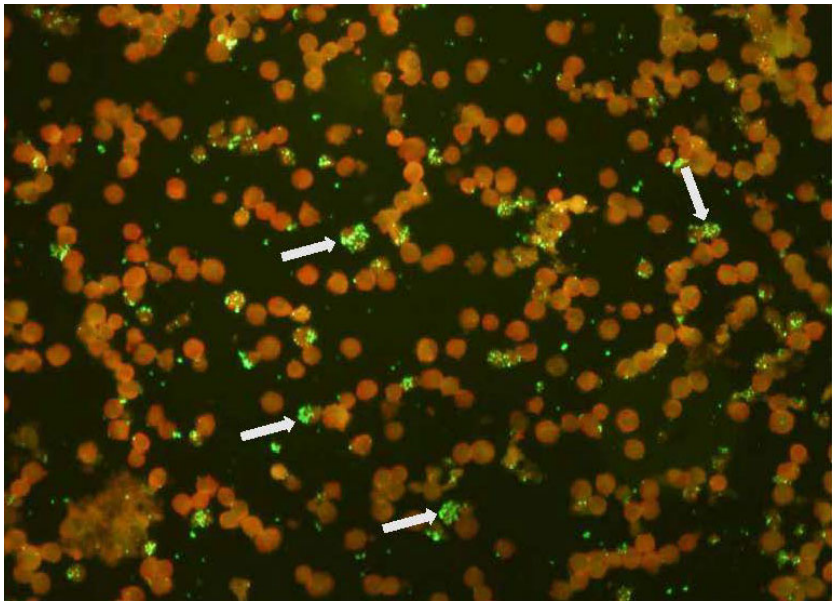
Web Table I PHENOTYPE-GENOTYPE CORRELATION IN CHILDREN POSITIVE FOR IMMUNOFLUORESCENCE ASSAY FOR SCRUB TYPHUS IN CHANDIGARH, INDIA

S No	*Genotype*	*IFA titre*	*Phenotype*	*Clinical presentation*	*Lab investigations*
1	Karp	1:256	F/10 y	Acute undifferentiated febrile illness; hepatitis; Fever ×15 d; abdominal pain & vomiting; serositis, hepatomegaly	Leucocytosis
2	Karp	1:256	F/10 y	Acute undifferentiated febrile illness; Fever ×6 d	Thrombocytopenia; leucopenia
3	Karp	1:256	M/8 y	Acute encephalitis; shock; hepatitis; Fever × 7 d; seizures, altered sensorium; hepatomegaly	Anemia, thrombocytopenia
4	Karp	1:256	M/6 y	Acute encephalitis; shock; hepatitis; pneumonia; Fever × 9 d; seizures, altered sensorium, respiratory distress, crepitations	Anemia, hrombocyto-penia, leucocytosis
5	Karp	1:128	M/2 y	Acute undifferentiated febrile illness; Fever × 5d	Anemia, thrombocytopenia
6	Karp	1:128	M/4 y	Acute encephalitis; hepatitis; Fever x 4 d; seizures, altered sensorium, hepatosplenomegaly	Anemia, thrombocytopenia
7	Karp	1:256	M/6 y	Acute undifferentiated febrile illness; hepatitis; shock; Fever × 5d	Anemia, thrombocytopenia
8	Kato	1:256	F/11 y	Acute undifferentiated febrile illness; shock; hepatitis; Fever × 7d	Anemia, thrombocyto-penia; leucocytosis
9	Kato	1:256	F/11 y	Acute undifferentiated febrile illness; Fever x 14d	Anemia
10	Kato	1:128	F/7 y	Acute undifferentiated febrile illness; hepatitis; Fever × 10d; ascites, serositis	Anemia
11	Gilliam	1:128	M/7 y	Acute undifferentiated febrile illness; hepatitis; Fever × 10 d; hepatomegaly	Thrombocytopenia
12	Boryong	1:256	M/12 y	Acute encephalitis; shock; hepatitis; Fever x 4 d; seizures, altered sensorium, hepatosplenomegaly	Anemia, thrombocytopenia
13	Karp + Kato	1:128	M/3 y	Acute undifferentiated febrile illness; hepatitis; Fever × 10 d; hepatosplenomegaly; ascites, serositis	Anemia, thrombocytopenia
14	Karp + Boryong	1:128	F/13 y	Acute undifferentiated febrile illness; Fever × 7d	Thrombocytopenia, leucopenia



Web Fig. 1 *Immunofluorescence assay of scrub typhus IgM ELISA positive child showing Karp genotype seen as immunofluorescent bodies (white arrows); orange rods are the controls*.