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PROPORTION OF SCRUB TYPHUS INFECTION AMONG PATIENTS ADMITTED WITH ACUTE FEBRILE ILLNESS IN A TERTIARY CARE SETUP.

Dr. Debasis Barman*	Assistant Professor, Department of Microbiology, Agartala Government Medical College, Kunjavan, AGARTALA -799006. *Corresponding Author
Anwesha Saha	Junior Research Fellow, Department of Microbiology, Agartala Government Medical College, Kunjavan, AGARTALA-799006.
Dr. Abhijit Sarkar	Tutor, Department of Microbiology, Agartala Government Medical College, Kunjavan, Agartala-799006.
Dr.Tapan Majumdar	Professor and HoD, Department of Microbiology, Agartala Government Medical College, Kunjavan, AGARTALA-799006.

ABSTRACT The serodiagnosis of Scrub Typhus fever is done by Indirect IFA, Indirect ELISA to to detect IgM class of antibodies against Orientia tsutsugamushi (etiological agent to cause Scrub Typhus infection) in the patients' serum. This study is to estimate the proportion of Scrub Typhus fever among patients admitted with acute febrile illness in a tertiary care setup in Tripura. Clinical data and serum samples have been collected from the 271 patients. The serodiagnosis of Scrub Typhus fever is done by indirect ELISA and IFA from the patients' serum samples. The Scrub Typhus infection is prevalent in Tripura. The proportion in the study group is calculated 14.76% (40/271) by combining Indirect ELISA and IFA. The Anti Boryong antibody was positive in 45% (18/40) IFA serology.

KEYWORDS : Orientia tsutsugamushi, serodiagnosis, IFA, Tripura

BACKGROUND

Scrub Typhus fever (ST) is a Rickettsial infection (RI) caused by *Orientia tsutsugamushi (OT), O.chutto*.[1, 2] Mites belong to family Trombiculidae is the vector of OT in India. Different studies estimated the seropositivity of ST is 20-50% of acute febrile illness (AFI) patients, depending upon endemicity of the infection. The endemicity of infection varies on seasonal vector activity, accidental human exposure to the vector which are also natural reservoir of OT. Variation in geo-climatic condition, deforestation, urbanization are compounding factor to the vector activity. In India, the incidence of Scrub Typhus is documented from the period of Second World War.[3,4,5,6,7] The infection is re-emerging in all the North Eastern states like Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura since 2010 onwards. Outbreaks has been reported from Manipur, Sikkim, Northern part West Bengal, Nagaland , Meghalaya.[3,8,9] It is difficult to separate out the ST patients from AFI; most of the cases are presenting as flu like illness, often associated with exanthematous rash; eschar may or may not be present. Presently increased awareness in the clinicians warrant the early laboratory diagnosis of ST which is specific, sensitive and reproducible.[10] Serological diagnosis by ELISA and IFA are the most popular method[11] following improved recombinant techniques for antigen preparation (for ELISA) and cell culture of OT strains (for IFA). IFA is considered as a "Gold Standard" to confirm the diagnosis of Scrub Typhus in absence of isolation by cell culture method in resource limited setup.[12] As there is no published data regarding the spectrum of ST among the AFI patients in Tripura, this study was proposed to estimate the proportion of ST infection among patients admitted with AFI in a Tertiary Care setup.

METHODS

After obtaining Institutional Ethical Clearance, the study undertook from September, 2018 to August, 2019. Number of patients included in the present study is 271 with following Inclusion and exclusion criteria,

1.inclusion Criteria Are,

a. Patient admitted in the hospital with diagnosis of AFI which is defined as a patient with fever of ≥38°C at the time of hospital admission that persisted for ≥5 days with no localizing source.[13]

b. Suspected case of ST is defined as fever persisting for ≥5 days with or without eschar (if eschar is present, fever of less than five days duration has also been considered as suspected case of ST). Other presenting features may be headache and exanthematous rash, lymphadenopathy, multi-organ involvement like liver, lung and kidney, central nervous system manifested as jaundice, interstitial pneumonitis, acute respiratory distress syndrome (ARDS), acute renal failure and acute encephalitis syndrome(AES). The differential diagnosis of dengue, malaria, pneumonia, leptospirosis and typhoid should be kept in mind.[4]

2. Exclusion Criterias Are,

a.Patients not giving consent by himself/ herself or by informant (in case of paediatric age group) in writing.
b. Incomplete filling of clinical data sheet
c. Inadequate collection of blood sample
d. Improper labelling/ storage of blood sample.

Collection of serum sample: After obtaining consent from the patient, Clinical data sheet is duly filled up and 2-3 ml of blood is collected from patient aseptically by venepuncture in a presterile vacutainer tube. Serum is separated out aseptically and preserved in 2(two) aliquots for serology; one for ELISA test and the second one for IFA test. Samples are stored in domestic refrigerator maximum for 2 days. If storage time is expected to be more than 2 days, samples are kept at -80°C.

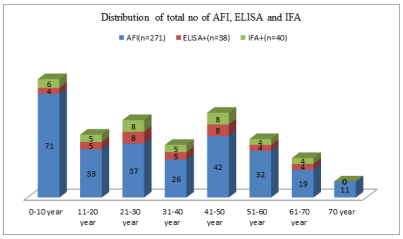
Sero-diagnosis of ST is done by antibody (IgM) detection against OT by indirect enzyme linked immunosorbant assay (ELISA) using the commercially available ELISA kit (InBios International.Inc; www.inbios.com). The test has been done as per manufacturer's protocol. The test is considered positive for ST when the Optical Density (OD) value is more than > 0.5(cut off value).

Serological detection of antibody (IgM) against OT by indirect Fluorescence assay (IFA) done with the second aliquot serum sample. The stored serum thawed at room temeperature before the IFA test. Detection of IgM class antibody against different strains of OT is done by IFA kit (*Orientia tsutsugamushi* IFA IgM Antibody Kit, Fuller Laboratories, California, USA; www.fullerlaboratories.com). Within the each slide well of the IFA slides provided in the kit utilize 4 strains (Boryong, Gilliam, Karp and Kato) propagated in L292 (mouse fibroblast cell line)[11] cells *in vitro* and presented in a linear array of infected cells. Patient sera are diluted at least 1:64[14] in IgM Serum Diluent and incubated in the individual slide wells to allow reaction of serum antibody with the strains of *Orientia* as per manufacturer's protocol. The resulting reactions are visualized using Fluorescence microscope with filter system for FITC (maximum excitation wavelength 490 nm, mean emission wavelength 530 nm) and 400X magnification. A positive reaction is seen as small sharply defined fluorescent rod forms dispersed within each antigen spot, both intracellular (cytoplasmic) and extracellular. A negative reaction is seen as counterstained (red) cells alone or fluorescence different from

that seen in the Positive Control wells.

RESULTS

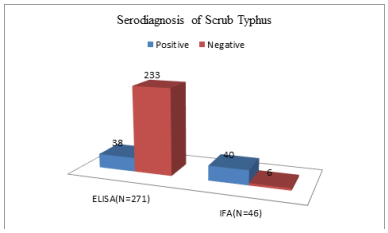
During the study period, total number of 271 tested for ST, out of which 40 cases constituting 14.76% of AFI is seropositive for ST.



Bar Diagram 1. Age group wise distribution of total no of AFI, ELISA Positive and IFA Positive case.

The demographical profile of the positive cases consists of 40 patients of AFI comprised of 19 (47.5%) males and 21 (52.5%) females. Mean age is calculated as 34.9 years. The oldest and youngest patients are of age 65 years and 4 years. Sero positivity is seen high (8 out of 37) in the age group is 21-30 years (21.6%) and male (n=4) and female (n=4) are equally affected (Bar diagram. 1).

All the 271 serum samples have been tested to detect antibodies (IgM) against OT by indirect ELISA. 38 samples have been found to be seropositive for ST infection (14.02%; 38/271) in the AFI patients. Indirect IFA has been done in 46 cases which include 38 ELISA positive and 8 ELISA negative but clinically highly suspicious of ST infection. All the ELISA positive serum samples are also positive by IFA (Bar diagram.2). Considering IFA serology as the "Gold standard" for confirmation of diagnosis of ST, 2 samples have been found to be positive (False negative by ELISA) for anti *O.tsutsugamushi* (IgM) by IFA. Another 6 serum samples negative by ELISA has also been found to be negative (True negative by ELISA) by IFA.



Bar Diagram 2. Analysis Of ELISA And IFA Serological Test Results.

While analysing 40 IFA positive serum samples (Chart. 1), Boryong strain (n=18; 45%), Kato strain (n=10; 25%) and Gilliam strains (n=2; 5%) has been detected. In the remaining 10 (n=10; 25%) serum, antibodies to ≥2 different strains of OT detected. In the later group, antibodies to Karp strain of OT were also observed in 3 serum samples.

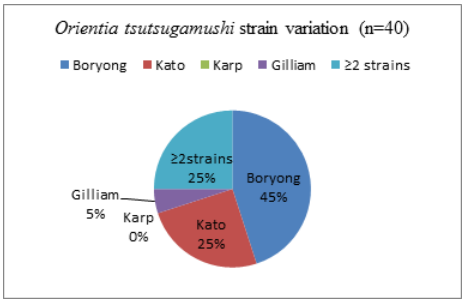


Chart 1. Distribution Of *Orientia Tsutsugamushi* Strains Detected In Serodiagnosis By IFA.

The common symptoms noted were Fever (40/40; 100%), Headache (16/40; 40%), Myalgia (18/40; 45%), Gastrointestinal complains (10/40; 25%), Arthralgia (7/40; 17.5%), Rash (6/40; 15%), Eschar (5/40; 12.5%). Complications of ST like Hepatitis (2/40; 5%), Pulmonary Involvement (24/40; 60.5%) have been observed in the ST group.

On admission to the hospital, laboratory parameters show anemia in 5.3% and leukocytosis in 39.5% cases. Elevated liver enzymes (SGOT, SGPT) were found in 39.5% and 15.8% cases. Elevated serum urea and serum creatinine were observed in 52.6% and 42.1% respectively.

DISCUSSION

In our study, seropositivity of ST infection has been found to be 14.76% (40/271) of all the AFI patients. Depending up the endemicity the incidence can be as high as 30% with case fatality rate in India varies between 12-13%.[3,7,10,16,17,28] Scrub typhus can occur in areas where scrub vegetation consisting of low lying trees and bushes is encountered, and also in habitats as diverse as banks of rivers, rice fields, poorly maintained kitchen gardens, grassy lawns which can all be inhabited by chiggers.[4] Outdoor activity, especially occupational exposure, is a critical risk factor.[4,8]

The maximum number of cases has been detected throughout the months of May to December. The disease is seasonal in many parts of India, which correlates with the appearance and activity of mites.[8,9,16,17]

Seasonal variations has been observed with scrub typhus; as in our study, incidence increases towards the end of the rainy season and the beginning of winter months (July–November) in Southeast Asia.[16] Seropositive cases are found in all age groups (upto 70 years of age) found in males and females. In our study, proportions of seropositive cases are high in the young adult (21-30 years of age) and the adults of 41-50years age group (8/40;20%).

Most scrub typhus cases are mild and characterized by acute onset fever, myalgia, generalized lymphadenopathy, maculopapular rash, and splenomegaly.[4] Eschar is variably seen in 1–97% of cases,[17] typically on torso, axilla, or groin, and may be diagnostic.[4] Vasculitis is the basic pathologic changes occur following ST infection.[18] Pulmonary involvement occurs in up to 60.5% of patients followed by Hepatitis in 5% of the seropositive patients. Acute abdomen has been observed in patients from hyperendemic zones of China.[9] Hepatitis and Pulmonary involvement[17] are common complications in India.[16] Overall mortality rates for scrub typhus range from 1.4% to 6% with early initiation of treatment but can be as high as 30%-70% without antibiotic treatment.[17] Genetic diversity [3,11,17,19,20] has association with clinical manifestations as well as response to antimicrobial. Scrub typhus with a severe clinical picture is due to prominent inflammatory response in the accidental host (human), which causes multiorgan dysfunction, meningoencephalitis, persistent neurologic deficits.

No patient died in our study group of patients. Strain variations of OT has its impact on the bacterial virulence, clinical presentation and response to antibiotics as observed.[17]

In our study, we found Boryong strain infection is predominant of the 4 strains (Gilliam, Kato, Karp and Boryong) by IFA. Boryong like strains also prevalent in other Indian states.[21] The Boryong strain of OT is predominant in Korea,[22] Molecular studies describe prevalence of Karp, Karp like, Kato like,Gilliam, Neimeng-65 strains in North Eastern region of India.[21,22,23,24,25] The genetic diversity of OT strains observed in India might cause variation in the efficacy (sensitivity and specificity) of commercial kits in India.[26]

Scrub typhus remains severely underrecognized, mainly because of diagnostic difficulties and lack of awareness.[10] Sensitivity and specificity of the ELISA depends upon the determination of cut off value can vary due antigenic diversity of the O.tsutsugamushi prevalent in that geographical region.[11,19,20] In a study undertook in India, the sample OD value of ≥0.87 has improved the sensitivity and specificity of the indirect ELISA test to 100% and 94.12%, respectively.[14]

The study also shows that, sensitivity and specificity of indirect IFA are100% and 93.5%, respectively at 1:64 serum dilution.[14] However, IFA has several limitations, like high cost of the kit, expensive fluorescence microscope apart from trained man power. As the IFA kit is expensive, we are unable to bear the cost to perform IFA study of all 271 serum samples. As per protocol we only confirmed the ELISA positive samples (n=38) by IFA. In addition to 38 samples, we have selected ELISA negative serum samples of 8 more patients who are clinically highly suspicious of ST for IFA study (n=46). This modification detects 2 more cases of ST totalling 40 (n=40) confirmed cases of ST. Phylogenetic characterization of OT shows much higher genetic diversity than we thought earlier. It certainly makes difficulty in selecting IFA kit relevant for a geographical area (i.e., antigenic

strains used and antibody isotypes measured) and titers selected as positive may vary for diagnostic and epidemiologic studies.[16] At lower dilutions of patient serum cross-reaction with other related bacteria such as Anaplasma phagocytophilum, Ehrlichia chaffeensis, and even unrelated Coxiella should be kept in mind.[16]

CONCLUSION

More funding in the research of ST is necessary to look for of new vector[28] and to vector control[8] using advanced molecular technologies like Real Time Polymerase Chain Reaction (RT-PCR), Multiplex Nested PCR and Whole genome sequencing and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS).[29,30] The ST surveillance is also necessary to combat emerging drug resistance in OT[20] and thus to prevent ST outbreak.[9]

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