ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Scrub Typhus- Epidemiology, Pathophysiology, Diagnosis, Treatment and Prophylaxis: A Review

Sambrinath Christopher*, Akash Suresh, Yuvaraj Dhinakaran, Chandran Massi, Ivo Romauld Sagayaraj, Gayathri P.K
Department of Biotechnology, Vel Tech High Tech Dr.Rangarajan Dr.Sakunthala Engineering College, Avadi, Chennai - 62.
***Corresponding author: E-Mail: sambrinath@gmail.com**

ABSTRACT

Scrub typhus or tsutsugamushi disease is an acute, febrile, fatal disease caused by an intracellular, obligate bacterium *Orientia tsutsugamushi*. It is transmitted to human by a chigger-borne vector, trombiculid mite that acts as a reservoir for the bacteria and transmits infection. It is commonly seen in Asia-Pacific region called tsutstugamushi triangle. However, recent reports suggest that scrub typhus is re-emerging in most of the endemic areas which influences many morbidity and mortality cases due to misdiagnosis and inappropriate treatment. This article reviews about virulencity, epidemiology, pathophysiology, diagnostic methods, treatments and their prophylactic methods to prevent scrub typhus, which may leads to development of vaccines against scrub typhus and also to promote appropriate management to this disease.

KEY WORDS: Scrub typhus, *Orientia tsutsugamushi*, pathophysiology, diagnosis, treatment.

1. INTRODUCTION

The emergence of an unknown origin fever or pyrexia becomes a challenging task while treating patients. Without identifying the root cause of infection, few cases were being treated as normal diseased cases which lead to misdiagnosis and mistreatment that increased into lethal cases. Scrub typhus is one among the PUO cases which is a rickettsial disease, is quite challenging for clinicians to identify/diagnose, due to its nonspecific clinical symptoms. This acute febrile disease is caused by the intracellular, obligate, gram negative bacterium *Orientia tsutsugamushi*. It is a life threatening fatal infectious disease that mainly transmits infection to humans by the larval bite of infected trombiculid mite (Chiggers), particularly *Leptotrombiculid deliense.* It is estimated that one billion people are at risk of it and about one million new cases occur annually throughout the world. The mortality rate in untreated patients ranges about 0-30%, depending upon the area, hence the disease named "scrub typhus". It produced considerable morbidity and mortality among troops in Southeast Asia.

In India, scrub typhus revolution took place during the World War II in Assam and Bengal and in Indo-Pakistan war in 1965 which caused many mortality and morbidity cases. It extends from Japan, Taiwan, China, South Korea, Nepal, Northern Pakistan, India and Srilanka. This endemic region is known as "tsutusugamushi triangle". However, there has been a severe decline of scrub typhus in the later decades, recent reports show that this disease is re-emerging in several parts of India which is an indication that there is a resurgence of scrub typhus infection. Clinical manifestations include prolonged fever, eschar formation in few cases, encephalitis, pneumonia, pericarditis, acute renal failure, hepatomegaly, splenomegaly, gastrointestinal disorders. Few cases were reported with meningo-encephalitis and regional lymphadenopathy. Early diagnosis is important in this case, since there is an excellent response to treatment in eliminating the disease, preventing various complications. Even though, diagnostic methods are available, they are found to have certain drawbacks in identification of the disease among many PUO cases. Scrub typhus is grossly under diagnosed in India because of its non-specific clinical presentation, low index of suspicion and scarcity of confirmatory diagnostic resources. Though many serological methods are available to diagnose scrub typhus infection, they are found to lack sensitivity and specificity. There are about six standard prototypes strains such as Karp, Gilliam, Kato, Shimokoshi, Kawasaki and Boryong. Among these strains Karp, Gilliam, Kato were formerly known as "original prototype strains". Many related strains have evolved with various antigenic variants in phenotypic as well as in genotypic characters.

The antigenic Antibiotics such as doxycycline, tetracycline, chloramphenicol, azithromycin, and rifampicin were found to be effective against the febrile illness. Introduction of chloramphenicol and tetracycline after the World War II seemed to save many lives, thus eliminated many morbidity and mortality cases. Live vaccines were not tolerated well due to the lack of availability of natural strains of *Orientia tsutsugamushi.* Even though vaccines were developed, they seemed to show low level of potential activity and seemed to be inactive in few cases as well. Researches were going on with development of DNA vaccines and recombinant proteins to develop immunity against scrub typhus.

Virulence Factors: The proteins encoded in the cell surface of antigen seemed to be considered as the virulence factors. Since, those proteins play a major role in infecting human.

56 kda antigen: It is the major integral membrane protein of *Orientia tsutsugamushi* that is distributed around the cell surface, involves in attachment or penetration of host cells. They were even capable of neutralizing antibody in

mice. Animal studies reveal that this antigen, is a major antigenic protein that influences the occurrence of scrub typhus.

47 kDa Antigen: It is also a major antigenic protein which is found in the outer membrane that contains both scrub typhus reactive group and strain specific.

110 kDa Antigen: The least protein in *Orientia tsutusugamushi* is 110 kDa that contains both group specific and strain specific epitopes. This antigen varies significantly in size from strain to strain.

22kDa Antigen: It is relatively a conserved protein which involves in inducing both humoral and cellular response. Cloning and sequencing of 22 kDa gene of Orientia species was performed and compared with various other strains to find out the similarity between the related strains.

Strain differentiation: The development in molecular biology leads to identification of differ rent types of *Orientia tsutsugamushi* strains. These virulence strains may vary dramatically among the Orientia species. Genetic analysis of Orientia strains were not only focused on 56 kDa gene, it involves in identification of 22 kDa and 47 kDa protein which has been resolved by 2D Gel Electrophoresis technique. Identification of these strains enables the comparison of drug susceptible and non-susceptible strains.

Methods involved in strain differentiation:

Cross-neutralization: In cross-neutralization, mice were immunized with either killed or live Orientia subcutaneously. The serum collected from immunized animal was injected into another mouse to check infectivity of organism. Infectivity of the organisms was reduced by the presence of specific neutralizing antibody. Strains variation may be observed by comparing neutralization between isolates. These were calculated on the ratio of survivors to non survivors which has been demonstrated by using a toxin neutralization assay.

Immunofluorescence and Immunoperoxidase: Antigenic characterization was done by using DFA (Direct Fluorescent Antibody) and IFA (Indirect Fluorescent Antibody). Extensive use of DFA plays an important role in identification of Orientia strains. DFA panel includes labelled anti-Karp, anti-Kato, anti-Gilliam, TA678, TA686, TA716, TA 763 and TH1817 sera. Cross linking of strains with specific antigen was involved in identification of strains. Similar to DFA method, IFA involves an additional incubation in the primarily unlabeled rabbit anti-Orientia serum is sandwiched between the organism and fluorescein Iso-thiocyanate labelled anti-rabbit Immunoglobulins.

Monoclonal antibody typing: Monoclonal antibodies which are derived from prototype strains are used to classify new strains by overcoming the methods like DFA, IFA and ELISA.

SDS-PAGE: This method yields about 30 major proteins which includes 110kDa, 70kDa, 60kDa, 56kDa, 47kDa and 25kDa. Since there is no outer membrane glycoproteins, proteins are obtained in a least manner. The antigenic protein 56kDa and 47kDa plays an important role in studying about scrub typhus by SDS-PAGE.

Epidemology: The mode of transmission in human occurs from trombiculid mite infected with *Orientia tsutsugamushi*, which acts as a mediator for the invasion of disease.

Reservoir: Trombiculid mite acts as primary source for the infection. Once the insect is infected with these bacteria, they maintain infection throughout their life stages and pass infection on to their eggs in a process called transtadial transmission.

Transmission: Humans acquire this disease through bite of trombiculid mite particularly by a species called *Leptrotrombidium deliense*. The adult mites have four stage of life cycle: Egg, Larva, Nymph and Adult. Transmission of disease occurs in the larval stage since they feed on vertebrate animals. In the adult stage it does not feed on vertebrates, hence no infection. This disease is not directly transmitted from person to person. The onset of this disease occurs during the rainy season and after the rainy season. People of all ages are affected by this disease.

Pathophysiology: The bacterium *Orientia tsutsugamushi* enter by making a hole in the skin, it multiplies at the site of inoculation where it becomes necrotic, evolving into an eschar and they get attached to the host cell with the help of 56 kDa surface protein, by a clathrin-dependent endosomal pathway it is further progressed to generalized lymphadenopathy. These bacteria mainly attack endothelial cells in organs such as brain, pancreas, lung, kidney and skin. Inflammatory mediators such as cytokines, prostaglandins, and leukotrienes are involved as response. Histopathology study reveals that the perivasculitis is the hallmark of scrub typhus. Vascular damage usually influences the thromboembolic and hemorrhagic condition. Few studies show the injury in cardiac mycoses, few others show elevation of liver enzymes and hepatic dysfunction. Multi-organ failure was also reported.

Immune response against scrub typhus: When a pathogen (*Orientia tsutsugamushi*) encounters our body, humoral and cellular immune response occurs as a response against infection.

Humoral immunity: Humoral immune response has been found to play a vital role in protection against scrub typhus. The antibody produced may inhibit infection by blocking surface components which is necessary for attachment or penetration into the cells. However, experiments with animals immunized with *O. tsutsugamushi* were found to have resistance. Moreover, patients whose antibodies produced seem to have short lived.

Cellular mediated immunity: Studies reveal that protective immunity to *Orientia tsutsugamushi* is associated with the development of strong cell-mediated immunity. Few other reports shows T- cells are involved in development of

immune response to scrub typhus. This shows that T-Cells are intricately involved in the development of immunity against scrub typhus. Patients with scrub typhus seems to develop increased concentration of cytokines (Interleukin 1β, tumour necrosis factor-α, Interleukin-10), chemokine (CXCL-9 AND CXCL-10), granzymes A and B.

Clinical Features: Scrub typhus is primarily characterized by high grade of fever, myalgia, and gastrointestinal disorders. Eschar formation in many cases reveals presence of scrub typhus. In few cases, eschar formation will be absent. Earlier identification and diagnosis may help the patient to get recovery, if untreated it leads to multi-organ dysfunction syndrome and even death. Various cranial nerve defects were also been noted in untreated patients. Pneumonia, myocarditis, splenomegaly, encephalopathy, ARDS (Acute Respiratory Distress Syndrome) have been reported in many cases. Laboratory studies reveal low WBC count, low platelet count and elevation of liver enzymes and in creatinine level.

Diagnosis:

Weil-felix test: This method detects antibody produced against *Orientia tsutsugamushi* in the body. Usually, antibodies produced will have cross-reactivity between *Proteus vulgaris* OX-K which results in agglutination reaction. Udayan (2014) performed a hospital based study where in out of 51 significant titres, 11 samples showed significant for OX-K antigen. By this method, results can be obtained within overnight and it is inexpensive method. However, it lacks specificity and sensitivity, which makes the test unfit.

Indirect Immuno Fluorescence Assay (IFA): IFA has been considered as a golden standard method for the detection of scrub typhus. It is more sensitive and results can be obtained within few hours. But the major limitations were very expensive and required skilled persons and it also required paired sera in acute and convalescent measures.

Immuno Chromatographic Test (ICT): A rapid diagnosis is achieved through binding of antibodies that were produced in the serum to those coated antigen in the strip. The occurrence or non-occurrence of band in the control and test line reveals positive or negative results. However though the sensitivity was high it lacked specificity.

Western blot: The r56 kD protein were used to detect the presence or absence of scrub typhus. In this method, IgG bound to r56 kD was detected by the addition of goat anti-human IgG conjugate. Results are interpreted as positive or negative with the presence or absence of 41-kD band.

ELISA: Enzyme-Linked Immuno Sorbent Assay was prominently used for the detection of antigen. When a disease encounters the cells, IgM/IgG antibodies are produced. Strains of *Orientia tsutsugamushi* such as Karp, Kato and Gilliam were used and final concentration of antibodies was measured by microplate reader. The major limitation for this method is that it is difficult to culture *Orientia tsutsugamushi* as it grows in cell lines and it required biosafety level-3 facilities.

PCR: Polymerase Chain Reaction provides more sensitive test when compared with the other diagnostic test. Nested and non-nested have been used for targeting various gene targets. Mostly 56 kDa antigen gene was targeted in order to identify the disease. Jiradejmanosroi (2003) performed a nested PCR and compared with Weil-Felix and Immunofluorescence assay which showed 19 positive results in PCR whereas 13 and 3 in Weil-Felix and immunofluorescence assay out of 80 patients. The primers described by Furuya (1993) were used by Prakash (2011), to target 56kDa gene using nested PCR.

16S rRNA gene for detection of Scrub Typhus: Detection of *Orientia tsutsugamushi* infection was achieved by targeting 16s rRNA gene through polymerase chain reaction. Since 16s rRNA gene played a vital role in differentiating the various bacterial species as well as in the same species, it is used to identify various related strains. The 16s rRNA gene was amplified using PCR with the specific Orientia primers. Primers were designed to be specific for gene sequence for *Orientia tsutsugamushi* strain Kato, Karp, Gilliam. Sonthayanon (2006) obtained as an amplicon size of 220 bp in molecular testing for *Orientia tsutsugamushi*. The verification of primer specificity was performed by using genomic DNA from *Orientia tsutsugamushi* strain Kato and other Rickettsial species. 16s rRNA gene shows sensitivity of 86.8% and with a specificity of 100%. These 16s rRNA genes are feasible candidates for analyses because they are unaffected by the host immune response.

Treatment: Scrub typhus can be easily treated whereas diagnosis part was quite difficult. Doxycyclines, are the foremost antibiotics that were administrated for treating this disease. Tetracyclines were also given to the patients suffering from scrub typhus. Clinical trials showed that azithromycin and rifampicin showed superiority to doxycycline in such cases. Eventhough, they are superior, standard therapy for scrub typhus is doxycycline and chloramphenicol. There is a fatal risk associated with pregnant women in treating scrub typhus, since doxycycline is classified as class D drug according to U.S Food and Drug Administration which may lead to risk factors associated with foetus. Intake of chloramphenicol (class C Drug) will also lead to risk to foetus at the time of delivery. In the case of pregnant women, ciprofloxacin can be selected with caution for the treatment.

Vaccines: Many studies were conducted to develop a vaccine for scrub typhus infection which lead to aggressive failure and to stimulate protective immunity against *Orientia tsutsugamushi*. In the past, formalin killed Orientia were administered which provided a short-term protection but were also also not that successful to protect in the long run. Further studies conducted had proceeded with usage of live Orientia strains with low virulence that fetched solid

protection in humans. Many vaccines revealed failure to protect against Orientiastrains, they are effective towards few strains. Current approaches involved understanding natural immune response against Orientia that does not produce sterilizing, long lasting and cross-protective immunity.

Prophylaxis: Proper care should be taken for scrub typhus patient. Administration of doxycycline in the initial level may prevent the disease leading to various complications. When there is any case of fever of unknown origin, one should be investigated for proper diagnosis and treatment. Vector control should be aided in such a way that vegetation area should be cleared. Applications of mite repellents like Diethyltoluamide, Benzyl benzoate are employed to clear the mite populations.

Prevention and control: As this disease occurs accidentally with the mite bite (chiggers), proper dressing should be done while travelling in the scrub vegetation area. Intake of doxycycline (200mg) will afford resistance to this disease to people who are at the high risk exposure to the area where scrub typhus is prominent.

REFERENCES

Akira Tamura, Norio Ohashi, Hiroshi Urakami, Sadao Miyamura, Classification of Rickettsia tsutsugamushi in a New Genus, Orientia gen. nov., as *Orientia tsutsugamushi* comb. Nov., Int J of syst Bacteriol, 45, 1995, 589-591.

Alvarez Elcoro S, Enzler MJ, The macrolides: Erythromycin, clarithromycin, and azithromycin, Mayo Clin Proc., 74 (6), 1999, 613-634.

Balasubramaniyan Bharathiraja, and Jayaraman Jayamuthunagai, Production and Kinetics of Cellulase Enzyme from saw Dust Hydrolysate using Trichoderma reesei 992 6a, Advanced Biotech, 2008, 32-35.

Bithu R, Kanodia V, Maheswari RK, Possibility of scrub typhus in fever of unknown origin (FUO) cases: An experience from Rajasthan, Indian J Med Microbiol., 32 (4), 2014, 387-390.

Blacksell SD, Bryant NJ, Paris DH, Doust JA, Sakoda Y, Day NP, Scrub typhus serologic testing with the indirect immunofluorescence method as a diagnostic gold standard, A lack of consensus leads to a lot of confusion, Clin Infect Dis., 44 (3), 2007, 391-401.

Blacksell SD, Jenjaroen K, Phetsouvanh R, Wuthiekanun V, Day NP, Newton PN, Accuracy of Access Bio Immunoglobulin M and Total Antibody Rapid Immuno chromatographic Assays for the Diagnosis of Acute Scrub Typhus Infection, Clin Vaccine Immunol., 17 (2), 2010, 263-266.

Bleeker-Rovers CP, Van der Meer JW, Oyen WJ, Fever of Unknown Origin, Semin Nucl Med., 39 (2), 2009, 81-87.

Cecilia G Moron, Sevolod V, Popov L, Hui-Min Feng, Douglas Wear, David H Walker, Identification of the target cells of *Orientia tsutsugamushi* in Human cases of scrub typhus, The United States and Canadian Academy of Pathology, 14 (8), 2001, 752-759.

Chattopadhyay S, Allen L Richards, Scrub typhus vaccines: past history and recent developments, Human Vaccines, 3 (3), 2007, 47-54.

Chogle AR, Diagnosis and treatment of scrub typhus-The Indian Scenario, J Assoc Physicians India, 58, 2010, 11-12.

Daniel H Paris, Thomas R Shelite, Nicholas P Day, David H Walker, Review Article: Unresolved Problems Related to Scrub Typhus: A Seriously Neglected Life-Threatening Disease, Am J Trop Med Hyg, 89 (2), 2013, 301-307.

Daryl J Kelly, Paul A Fuerst, Wei-Mei Ching, Allen L Richards, Scrub typhus: The Geographic Distribution of Phenotypic and Genotypic Variants of *Orientia tsutsugamushi*, Clin Infect Disease Society of America, 48S3, 2009, 203-230.

Enatsu T, Urakami H, Tamura A, Phylogenetic analysis of *Orientia tsutsugamushi* strains based on the sequence homologies of 56kDa type-specific antigen genes, FEMS Microbiol let., 180 (2), 1999, 163-69.

Ge Y, Rikihisa Y, Subversion of host cell signaling by *Orientia tsutsugamushi*, Microbes Infect., 13 (7), 2011, 638-48.

George Cowman, Rickettsial diseases: the typhus group of fevers- a review, Postgrad Med J., 76 (895), 2000, 269-272.

George M Varghese, Jeshina Janardhanan, Paul Trowbridge, John V Peter, John A Prakash, Sowmya Sathyendra, Kurien Thomas, Thambu S David, Scrub typhus in south india: clinical and laboratory manifestation genetic variability and outcome, Int J of Infect Dis., 17 (11), 2013, e981-987.

Isaac R, Mahajan S, Gupta ML, Kanga A, Sharma V, Investigation of an outbreak of scrub typhus in the Himalayan region of India, J Infect Dis., 58 (4), 2005, 208-10.

Jacobson RH, Validation of serological assays for diagnosis of infectious diseases, Rev Sci Tech., 17 (2), 1998, 469-526.

Jerells TR, Palmer BA, Osterman JV, Gamma-irradiated scrub typhus immunogens: Development of cell-mediated immunity after vaccination of inbred mice, Infect Immun., 39 (1), 1983, 262-69.

Kayoko Hayakawa, Masayuki Oki, Yusuke Moriya, Atsushi Mizuma, Yoichi Ohnuki, HidetakaYanagi, Ryuki Fukuda, Hideki Ozawa, Shunya Takizawa, Atsushi Takagi, A case of scrub typhus with acalculous cholescystitis, aseptic meningitis and mononeuritis multiplex, J Med Microbiol., 61(pt 2), 2012, 291-294.

Kim YS, Yun HJ, Shim SY, Koo SH, Kim SY, Kim S, A comparative trial of a single dose of azithromycin versus doxycycline for the treatment of mild scrub typhus, Clin Infect Dis., 39 (9), 2004, 1329-1335.

Luyan Zhang, Zhongtang Zhao, Zhenwang Bi, Zengqiangkou, Meng Zhang, Li Yang, Li Zheng, Risk factors associated with severe scrub typhus in Shandong northern China, Int J Infect Dis., 29, 2014, 203-207.

Mathai E, Rolain JM, Verghese GM, Outbreak of scrub typhus in Southern India during cooler months, Ann N Y Acad Sci., 990, 2003, 359-64.

Mathai E, Rolain JM, Verghese L, Mathai M, Jasper P, Verghese G, Case reports: Scrub typhus during pregnancy in India, Trans R Soc Trop Med Hyg., 97 (5), 2003, 570-572.

Ni YS, Chan TC, Chao CC, Richards AL, Dasch GA, Ching WM, Protection against scrub typhus by a plasmid vaccine encoding the 56kD outer membrane protein antigen gene, Am J Trop Med Hyg., 73 (5), 2005, 936-41.

NY, Cho NH, Kim YS, Choi MS, Kim IS, An autotransporter protein from *Orientia tsutsugamushi* mediates adherence to nonphagocytic host cells, Infect Immun., 79 (4), 2011, 1718-1727.

Prakash J, Kavitha ML, Mathai E, Nested polymerase chain reaction on blood clots for gene encoding 56 kDa antigen and serology for the diagnosis of scrub typhus, Indian J medical microbial., 29 (1), 2011, 47-49.

Ramyasree A, Kalawat U, Rani ND, Chuhaudry A, Seroprevalence of scrub typhus at a tertiary care hospital in Andhra Pradesh, Indian J Med Microbiol., 33 (1), 2015, 68-72.

Saifudheen K, Sajeeth Kumar KG, James Jose, Veena V, Abdul Gafoor VA, First case of scrub typhus with meningoencephalitis from Kerala: An emerging infectious threat, Ann Indian Academy Neurology, 15 (2), 2012, 141-144.

Sandeep Dogra, Recent Advances in Understanding Pathophysiology of scrub typhus, JK Science, 12 (2), 2010, 70-71.

Senaka Rajapakse, Chaturaka Rodrigo, Deepika Fernando, Scrub typhus: Pathophysiology, Clinical Manifestations and prognosis, Asian Pac J Trop Med., 1 (2), 2011, 261-264.

Sinha P, Gupta, Dawra R, Rijhawan P, Recent outbreak of scrub typhus in North Western part of India, Indian J Med Microbiol., 32 (3), 2014, 247-50.

Smadel JE, Ley HL, Diercks FH, Cameron JAP, Persistence of *Rickettsia tsutsugamushi* in tissues of patients recovered from scrub typhus, Am J Hyg., 56 (3), 1952, 294-302.

Traub R, Wisseman Jr. CL, The ecology of chigger-borne rickettsiosis (scrub typhus), J Med Entomol., 11 (3), 1947, 237-303.

Uttam Udayan, Meena Dias, Santhosh Machado, A hospital based study of rickettisial disease evidenced by Weil felix test in a tertiary care hospital, J Health Research, 1 (3), 2014, 150-153.