Open Access Journal
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THOMSON REUTERS
International Journal of Medical Research and Pharmaceutical Sciences
Volume 5 (Issue 2): February 2018 ISSN: 2394-9414
DOI- 10.5281/zenodo.1173938 Impact Factor- 4.174

DIAGNOSIS OF EMERGING AND REEMERGING SCRUB TYPHUS

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Keywords: *Scrub Typhus; Orientiatsutsugamushi; Emerging; Reemerging; Diagnosis; Rapid Diagnostic Test.*

Abstract

Scrub typhus accounts for upto 23% of all acute febrile illness in endemicity in the Asia-Pacific region, and is also newly found in other continents. Despite of reemergence in Asia and emergence in other region, there is no effective human vaccine. And there are few convenient and rapid diagnostic methods, so scrub typhus has appeared as a significant threat to public health. Rapid diagnostic test (RDT) of scrub typhus can be recommended as point-of-care test (POCT) for on-site diagnosis in the area with resource-poor settings such as poor, remote and rural laboratories in which immunofluorescence assay (IFA), polymerase chain reaction (PCR) or enzyme linked immunosorbent assay (ELISA) is unavailable. Currently, the Scrub Typhus Detect IgM Rapid Test of InBios Co. and the SD BiolineTsutsugamushi Kit of SD Co. are widely used as RDT. On the other hand, ImmuneMed Co. developed reliable diagnostic recombinant proteins, which were used as a diagnostic antigen for development of the Scrub Typhus RAPID in RDT type that differentially and simultaneously detects IgM and IgG. A mixed antigen originated from 5 serotypes was used the RDT which showed high sensitivities and specificities in response to antibodies from patients infected with various serotypes. Thus, it can be used as a worldwide diagnostic tool for accurate and simple diagnosis of scrub typhus.

Introduction

Scrub typhus is also called as Tsutsugamushi disease, Japanese-river fever, Mite-borne typhus, Tropical typhus, Scrub fever and Trombidiasis[1,2]. This is an acute febrile exanthematous illness that is caused by *Orientiatsutsugamushi* that belongs to the scrub typhus group of Rickettsiaceae family, which are gram-negative, obligate, intracellular bacteria[3]. This disease has a high fatality rate[1,4,5].

Since scrub typhus was first described in Japan in 1899, and was found to be transmitted by mites, this disease was called tsutsugamushi (from ‘tsutsuga’ meaning ‘dangerous’ and ‘mushi’ meaning ‘insect or mite’)[3,6].

Distribution of Disease

Scrub typhus is present throughout the world, and in particular, is frequently found in so-called the ‘tsutsugamushi triangle’ that connects Japan, South Korea, China, Russia, Nepal, Pakistan, India, Malaysia, Northern Australia, Taiwan and the Philippine areas[1-3,7]. Scrub typhus accounts for upto 23% of all acute febrile illness in endemicity in the Asia-Pacific region[5,8]. It has gradually extended to other continents including Africa, Europe and South America, and became an emerging disease[7,9]. It is estimated that about 1 million of new cases infected by *O. tsutsugamushi* occur worldwide every year, and over 1 billion people are exposed to the risk of infection[7,10,11].

According to a report in 2013, author was mentioned that "scrub typhus was evaluated as the most prevalent, under-recognized, neglected and severe, but easily treatable disease”[12]. The 36% of patients who were admitted to the Christian Medical College of Vellore for tropic infection in South India was diagnosed with scrub typhus, which was higher than those of malaria and dengue fever[13].

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While scrub typhus was a common disease in Asia in the past, it has been easily cured by treatment of antibiotics, such as chloramphenicol and tetracycline. These antibiotics can be easily purchased in the pharmacy without prescription for febrile disease, so people thought that it was suddenly disappeared and previouslywas not a social problem. However, countries which is required the prescription for purchase of antibiotics for the treatment of febrile illness are reemerging for human febrile disease due to less frequently prescribed for chloramphenicol and tetracycline.

At present, there are rises of reemergence in Asia, while there is no effective human vaccine. And there are few convenient and rapid diagnostic methods, so that scrub typhus is a significant threat for public health [11].

Etiology

Mites act as both vector and reservoir through trans-ovarial transmission of *O. tsutsugamushi*[2]. Most mites are belonging to genus *Leptotrombidium*, and *L. akamushi*, *L. scutellare*and *L. pallidumia*are reported in Japan.*L. pallidumia*(52.6%), *L. scutellare* (27.1%), *L. palpale* (8.2%), *L. orientale* (5.6%) and *Neotrombiculatamiyai* (1.7%) have been reported in South Korea[1,14]. Mites infected by *O. tsutsugamushi*maintain infection throughout the life cycle, and infection is passed to eggs from adult females[2]. During the life cycle, mites in the larva stage suck blood of warm blooded animals, while nymphs or adult mites do not[3]. When larval-stage trombiculid mites bite the human skin and suck blood,*O. tsutsugamushi*is systemically transmitted through hematogenous and lymphogenous routes[1,2]. On the other hand, larva sucks blood of vertebrates including rodents infected with *O. tsutsugamushi* and then is infected. *O. tsutsugamushi* is maintained throughout the life cycle and is transovarian transmission to female offspring. Humans are accidental hosts that are infected mainly during outdoor activities such as working, camping, hiking, and rafting, but there is no report on person-to-person transmission[3].

Pathogenesis and Treatment

Pathogenicity of *O. tsutsugamushi* is caused by systemic vasculitis after attacking into vascular endothelial cells. After invading to the blood, *O. tsutsugamushi* grows in vascular endothelial cells, lead to damage to the blood vessels, which causes microvascular thrombosis and inflammation around the blood vessels. Thus, its histopathological characteristics caused by the infection are damage to the blood vessels in multiple organs, which leads to dermal necrosis, rash, meningitis, hearing loss, myocarditis, and heart failure. In addition, hypofibrinogenemia by intravascular coagulation was also reported [3, 15-17]. Early treatment with antibiotics such as chloramphenicol, tetracycline, doxycycline, azithromycin, and rifampicin can be completely cure scrub typhus without complication[2,4].

Clinical Symptoms

After about 10 days (1-3 weeks) from being bitten by larval-stage trombiculid mite, chills, fever and headache occur, and rash spread from the human body to the extremities within about 1 week from the onset of disease. At the bitten site, a blister is formed, which leads to 0.5-0.8 cm of ulcer formation, and then covered by black crust. This is called eschar [3,4,15] which can be developed in any area of the body, but often appear in the areas with soft, thin, moist and wrinkled skins such as axilla, groin and genitals. Although most patients recover without any complications, severity of illness varies from sub-clinical illness to severe illness including multi-organ systems. Clinical features of scrub typhus are manifested generally after one week from infection[3,16]. Some patients complain respiratory symptoms, and pulmonary infiltration was observed in about 50% of the cases.In severe cases, it invades into the central nervous system, resulting in unconsciousness or death [15]. Therefore, it can be fatal illness if it is without early diagnosis and treatment [3].

Serotype of Scrub Typhus

*O. tsutsugamushi*shows different symptoms and epidemiologic characteristics depending on serotype.Recently a novel *Orientia* species (*O. chuto*) was isolated from a patient infected in Dubai [18]. Tsutsugamushi bacteria is serologically associated to other serotypes, but has no or rarely related to protective immunity with other serotypes.

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While O. tsutsugamushi is classified into one species, there are more than 20 serotypes with different antigenicity over the world.For example, serotype is classified into Gilliam, Karp and Kato depending on antigenicity, and these 3 serotypes are prototypes [15].

Antigenicity of Scrub Typhus

Antigenic serotypes of *O. tsutsugamushi*are classified by serological cross-testing, which follows antigenicity of immunodominant 56 kDa major surface proteins containing serotype-specific epitopes[8,19]. Though analysis by SDS-PAGE and immunoblotting after purification of *O. tsutsugamushi* proteins, major antigens can be found in 70, 60, 54-56 and 46-47 kDa, of which 46-47 kDa, 54-56 kDa and 70 kDa proteins have antigenicity, and 70 kDa and 46-47 kDa proteins are strain specific antigens[15,20]. The 56 kDa protein is localized at the outer membrane of *O. tsutsugamushi*, accounting for 10-15% of total bacterial cellular proteins[19]. The 56 kDa protein is highly reactive in sera of scrub typhus patients due to its strong antigenicity and abundances, so that it can be the first antigen candidate to be used for diagnosis of scrub typhus[8].

Diagnosis

In general, scrub typhus has mild clinical symptoms, and its clinical diagnosis has been easy to medical expert by symptoms and signs. However, it was reported that there were many cases with atypical symptoms and signs. Hence, when there were no specific symptoms of scrub typhus such as systemic lymphoid hyperplasia or eschar formation, it is difficult to discriminate scrub typhus from other acute febrile illnesses including fever of unknown origin such as enteric fever, typhoid, dengue hemorrhagic fever, other rickettsioses, tularemia, anthrax, leptospirosis, malaria, other hemorrhagic fevers, and infectious mononucleosis [3, 15, 21].

Diagnostic methods of scrub typhus include isolation of pathogen and identification of antibody by indirect immunofluorescent assay (IFA). Recent popular methods include enzyme-linked immunosorbent assay (ELISA) that can diagnose many patients easily, rapidly and simultaneously, serological tests such as point-of-care test (POCT) that can be performed on-site easily, rapidly and individually, and molecular diagnostic test targeting to DNA of *O. tsutsugamushi* [9]. Since easy and early diagnosis is essential for implementing rapid treatment and preventing various complications, serological tests are preferred. Thus, it is necessary to develop simple, convenient and accurate diagnostic methods [8].

1. Weil-Felix

Weil-Felix (WF) test which is affordable and can be performed in laboratories uses in the antigenic cross reaction between Rickettsia and Proteus, whereas it is non-specific, and has a low sensitivity, so the results should be interpreted only in clinical context [17, 22]. Therefore, WF is considered as a supplementary test, and is not accurate standard to determine infection status of scrub typhus. Nevertheless, WF is still used in many regions such as India. It is anticipated that IFA and PCR will be more used for diagnosis in many regions including India. Before regular usage of these methods, however, all local strains should be identified and standardized for further use.

2. Immunofluorescence Assay (IFA)

IFA is the gold standard diagnostic method for an accurate serological diagnosis of scrub typhus [3, 22, 23]. IFA has high sensitivity and specificity for detection of antibody for *O. tsutsugamushi* [8]. However, *O. tsutsugamushi* is an obligate intracellular pathogen, so it is some difficult to culture, and category B/Bio-safety level 3 containment facilities are required for the standard of the Centers for Disease Control and Prevention (CDC) [24]. In addition, an expensive fluorescent microscope is required to perform IFA, and the procedure is difficult and relatively takes a long time, so an expert is needed to perform this test. Moreover, interpretation of the results is subjective, so each reader may have different conclusions [8, 22]. Therefore, IFA is not conducted in many laboratories in epidemic or endemic regions with poor economic state. In addition, if IFA was used to diagnose scrub typhus as golden standard method, it is essential to use bacterial antigens for all prevalent serotypes of the relevant area, which makes it

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difficult to screen accurately all patients who are suspected to have scrub typhus. If there are unknown serotypes in the area, accurate diagnosis of scrub typhus by IFA is difficult in that area.

For example, IFA is not broadly used in India yet. Despite of a recent report that sensitivity and specificity of IFA were 100% and 93.5%, respectively, it is difficult to include all numerous *O. tsutsugamushi* serotypes of the relevant area from different geographical locations of India in IFA slide. According to a report, Gilliam, Karp, Kato, Ikeda and Neimeng-65 genotype strains were identified in India, among which Karp and Kato were the most prevalent serotypes. In addition, information about *O. tsutsugamushi* serotypes in different areas of India is still incomplete [9]. Therefore, even IFA result is not positive in endemic region such as India, it should not exclude infection by serotypes that were unregistered in IFA slide.

3. Recombinant Protein

During the last 15 years, recombinant proteins of *O. tsutsugamushi* using *E. coli* were expressed for development of scrub typhus diagnosis in many laboratories. An immunodominant 56 kDa outer membrane protein was used, which was expressed singularly or in combination with proteins from various *O. tsutsugamushi* serotypes (Gilliam, Karp, and Kato). The expressed recombinant 56 kDa antigens were used for passive hemagglutination assay, IFA and ELISA.

4. ELISA

Due to the characteristics of IFA requiring for high interpretational skills, ELISA is recommended by many researchers. IgM ELISA developed by using the 56 kDa recombinant antigen is simple and easy to apply compared to IFA, its specificity and sensitivity are considered comparable to those of IFA. Many researchers reported that IgM ELISA (InBios, USA) had a satisfactory performance as an alternate reference test replacing IFA [22, 25]. However, even if IgM ELISA used the recombinant 56 kDa outer membrane antigens of *O. tsutsugamushi* from well-characterized strains and was developed by evaluation under the clinical setting with an appropriate statistical model, IgM ELISA was found negative in some patients who were diagnosed with scrub typhus [26]. Accuracy of diagnostic tests varies depending on prevalence, clinical variability, and availability as well as timing of convalescent-phase sample [27]. Thus, it should be further studied on evaluation of optimal cutoff titer for the accuracy of IgM ELISA.

It should be recommended the use of antigens from geographically specific serotypes and determination of diagnostic cutoff, and application of appropriate statistical models, for IgM ELISA is used as an alternative serological reference test for relatively accurate diagnosis of acute scrub typhus [25].

5. RDT

Scrub typhus rapid diagnostic test (RDT) is recommended as a point-of-care test (POCT) in poor regions where IFA, PCR, or ELISA facilities are unavailable or in regions where patients are rare.

The Scrub Typhus Detect IgM Rapid Test (InBios) was reported the reliable sensitivity and specificity, but was able to detect IgM antibody alone. IgM detection can allow identification of acute scrub typhus, which facilitates early treatment and prevention of complication [28]. Since RDT is a cost effective POCT, RDT would be helpful for the areas that are poor or far from clinical diagnostic laboratories or for rural clinics. Of RDT tools of scrub typhus, SD BiolineTsutsugamushi kit is currently the most popular product used as RDT in the world [29].

6. ImmuneMedScrub Typhus RAPID

Recently, Kim et al. [30]has developed a reliable diagnostic recombinant protein,cr56 protein which was produced by PCR amplification of chimeric genes encoding important epitopes of 56 kDa antigenic proteins from *O. tsutsugamushi* Gilliam, Karp and Kato serotype. This protein has an improved sensitivity compared with single recombinant protein from single gene of a serotype [30]. In addition, each gene encoding antigenic protein of 21 kDa (r21) from Boryong and 56 kDa (kr56) from Kangwon was separately cloned and expressed. All of these recombinant antigens were mixed as an antigenic mixture [15]. Using this mixed antigen as diagnostic antigen of

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RDT, the ImmuneMed Scrub Typhus RAPID (South Korea) was developed having remarkably improved sensitivity [31, 32].

According to the report, even cr56 alone shown high sensitivity and specificity. Addition of kr56, one of the 56 kDa protein, and r21, distinct antigenicity, drastically improved sensitivity and specificity [8]. In a form that IgM and IgG were detected in two separate windows, scrub typhus was early diagnosed through IgM detection, and the current infection and progress situation could be predicted through IgG detection. Thus, it was considered that the ImmuneMed Scrub Typhus RAPID has accurate sensitivity and specificity in diagnosis.

Although parts of antigenic motifs in 5 serotypes were used to produce the mixture of antigen for RDT, the sequences of these recombinant proteins showed a high homology with serotypes in different geographical regions [8, 33]. Thus, it seems to be possible to diagnose scrub typhus not only in South Korea but also in the rest of the world.

When clinical performance of the ImmuneMed Scrub Typhus RAPID RDT was evaluate based on IFA (South Korea: sensitivity – 97.3 %, specificity – 99.5 %; Sri Lanka: sensitivity - 92.1 %, specificity - 96.1 %; South India: sensitivity – IgM 86 %, IgG 92 %) [1], some of the RDT negative in sensitivity may be real negative because of the difficulty in interpretation of IFA results. Also some of the RDT positive in specificity may be real positive because patients infected with rare or new serotype could be failed to be diagnosed with scrub typhus due to the limitation of in-house IFA. However, it was unable to exclude the situation that the RDT result was interpreted as real positive. In other words, even though IFA was not positive in endemic regions such as Sri Lanka and India, it is possible to be positive in the RDT by infection of region specific serotype that was not used in IFA.

Conclusion

There are about 20 serotypes of *O. tsutsugamushi*in the world and protective immunity to a same serotype sustains only a few years.These reasons are limitations in development of preventive vaccine. However, once early diagnosed, it can be easily treated with doxycycline or tetracycline. Therefore, it is highly important to have an affordable, rapid and accurate method for early diagnosis.

According to a report on scrub typhus in 2013, "scrub typhus was evaluated as the most prevalent, under-recognized, neglected and severe, but easily treatable disease"[12]. The 36% of patients who were admitted to the Christian Medical College of Vellore for tropic infection in South India was scrub typhus, which was higher than those of malaria and dengue fever[13]. Thus, morbidity and mortality should be minimized through early diagnosis and appropriate treatment for scrub typhus.

The Immune Med Scrub Typhus RAPID has been designed to respond to antibodies for most serotype by using 5 serotype-originated antigen mixture including prototypes. This RDT was demonstrated to be an accurate and simple diagnostic tool for scrub typhus in the world due to its high sensitivity and specificity in South Korea, Sri Lanka and India.

Author Contributions

Sungman Park wrote the manuscript. Young-Jin Kim collected and classified literatures. Yoon-Won Kim supervised the review article.

Conflicts of Interest

The authors declare no conflict of interest

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DOI- 10.5281/zenodo.1173938 Impact Factor- 4.174

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