Review article

Scrub typhus: An emerging scourge

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Abstract:

Scrub typhus is a public health problem causing severe morbidity and mortality. Clinical picture consist of high grade fever, severe headache, apathy, myalgia and generalised lymphadenopathy. A maculopapular rash may appear first on the trunk and then on extremities. Black eschar may be seen at the site of inoculation.Patients may develop complication like interstitial pneumonia, meningoencephalitis and myocarditis. Diagnostic approaches for scub typhus are based on several aspects. We can diagnose them clinically based on sign & symptoms or can be diagnosed serologically. Molecular methods can be used for their rapid identification as well as for epidemiological purposes. Most cases of fever were treated with drugs like chloromphenicol and tetracycline which effectively treat scrub typhus also. No vaccine is available for Scrub typhus but many vaccines using Sta47 and Sta56 antigens are under trial as a recombinant vaccine.

Key words: scrub typhus, Eschar, Rickettsial diseases

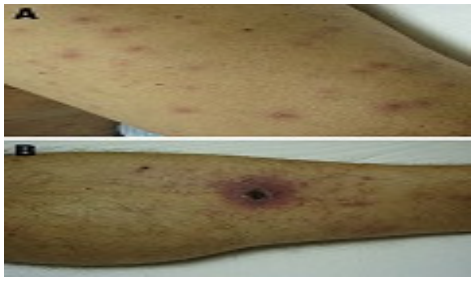
Introduction:

Scrub typhus is an acute, febrile, infectious illness that is caused by Orientia (formerly Rickettsia) tsutsugamushi. It is also known as tsutsugamushi disease. Mite borne typhus fever, tropical typhus and various other local names. Scrub typhus was first described from Japan in 1899. Humans are accidental hosts in this zoonotic disease. The term scrub is used because of the type of vegetation (terrain between woods and clearings) that harbours the vector. The infection is found in wide range of ecological conditions primary jungles, semi-desert, mountain desert, alpine meadows of Himalyas (1). scrub typhus is known to be prevalent in foot hills of Himalayas viz. Jammu & Kashmir, Sikkim, Manipur, Nagaland, Meghalaya, Himachal Pradesh etc. The disease has also been reported from Tamilnadu and Kerala. However, currently samples are being tested positive from Delhi, Haryana, Rajasthan, Maharashtra, Uttrakhand and Chhattisgarh. During 2012, outbreaks of scrub typhus were reported from many states in India. Over the years, the numbers of samples and areas which detected scrub typhus have also increased. The National Centre for Disease Control at its Zoonosis Division received 742 samples from suspected cases for scrub typhus from 11 states and 202 (27%) were found positive. In 2011, the number of samples received was 484 and in 2010, these were 204(2).

Clinical symptoms of scrub typhus: The chigger bite is painless and may become noticed as a transient localized itch. Bites are often found on the groin, axillae, genetalia or neck (3). An eschar is often seen in humans at the site of the chigger bite. The illness begins rather suddenly with shaking chills, fever, severe headache, infection of the mucous membrane lining the eyes (the conjunctiva), and swelling of the lymph nodes. A spotted rash on the trunk may be present. Eschars are rare in patients in countries of South-East Asia and indigenous persons of typhus-endemic areas commonly have less severe illness, often without rash or eschar (4).

Symptoms may include muscle and gastrointestinal pains. More virulent strains of O. tsutsugamushi can cause haemorrhaging and intravascular coagulation. Complications may include atypical pneumonia, overwhelming pneumonia with adult respiratory distress syndrome (ARDS)–like presentation, myocarditis, and disseminated intravascular coagulation (DIC). Patients with scrub typhus often exhibit leucopenia.

Chigger mite



Eschar

Etiology:

O tutsugambushi belongs to the typhus group. In Greek the word typhos means '' stupor caused by a fever'' there is a high degree of antigenic heterogeneity among the different strains several serotypes coexist in an endemic area, one of them may predominate .The different strain vary in their virulence.Its distribution is uneven, since it depends upon the presence of the agent and the vector/resevoir complex, later consisting of trobiculid mites and the small mammals, especially rodents,on which they feed,when all these elements come together they form "typhus islands(1) It is an obligate intracellular gram-negative bacterium that has a large number of serotypes. Five major serotypes: Boryon, Gilliam, Karp, Kato and Kawazaki . Differentiation of serotypes is important for laboratory diagnosis. (5) This pathogen does not have a vacuolar membrane; thus, it grows freely in the cytoplasm of infected cells. Because they are intracellular parasites, they can live only within the cells of other animals. Even though it is recognized as one of the tropical rickettsioses diseases, O. tsutsugamushi has a different cell wall structure and genetic composition than that of the rickettsiae. Scrub typhus is transmitted to humans and rodents by some species of trombiculid mites ("chiggers", Leptotrombidium deliense and others). Most notably L akamushi, L arenicola, L fletcheri,L pallidum and L pavlovsky.the vector species differs depending on the particular ecosystem. e.g L akamushi is found in partially cultivated fields that flood in spring and early summer, whereas L deliense is associated more with jungle(1). The adult mites have a four-stage lifecycle: egg, larva, nymph and adult. The larva is the only stage (chigger) that can transmit the disease to humans and other vertebrates, since the other life stages (nymph and adult) do not feed on vertebrate animals. Both the nymph and the adult are free-living in the soil.

Global scenario

Geographic distribution of the disease occurs within Afghanistan and Pakistan to the west; Russia to the north; Korea and Japan to the northeast; Indonesia, Papua New Guinea, and northern Australia to the south; and some smaller islands in the western Pacific. It was first observed in Japan where it was found to be transmitted by mites. The disease was, therefore, called tsutsugamushi (from tsutsuga meaning dangerous and mushi meaning insect or mite). This is found only in areas with a suitable climate, plenty of moisture and scrub vegetation.

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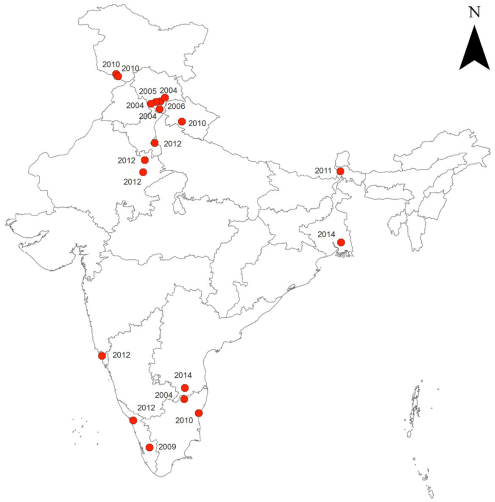
Recently, rickettsioses has been an emerging disease along the Thai Myanmar border. There are reports of emergence of scrub typhus in Maldive Islands and Micronesia. (6)

Indian scenario

In India, scrub typhus has been reported from Rajasthan, Jammu & Kashmir and Vellore. In addition, few cases have been tested positive for IgM antibodies for scrub typus in Sikkim , Darrjeeling, Nagaland & Manipur . In a study conducted from July through October 2004 in Himalayas, among several cases of acute febrile illness of unknown origin, O.tsutsugamushi was identified as causative agent by microimmunofluorescence and PCR (7) . In an entomolgic study in Himachal Pradesh, vector species Leptotombidium deliense and Gahrliepia (schoengastilla) spp. were recorded.(8)



Map showing Distribution of scrub typhus in india

Collection, storage & transportation of specimen

The collection, transportation and storage of specimens are extremely vital steps in laboratory diagnosis and hence, must be undertaken with utmost care

Specimen

Serum

Blood collected in tubes containing EDTA or Sodium citrate

Blood clot

Blood collection in tubes and vials

Aseptically collect 4-5 ml of venous blood.

Allow blood to clot at room temperature, centrifuge at 2000 rpm to separate serum.

Collect the serum in sterile dry vial.

Fix the cap with adhesive tape, wax or other sealing material to prevent leakage during transport.

Use adhesive tape marked with pencil, indelible ink, or a typewritten self adhesive label to identify the container. The name of the patient, identification number and date of collection must be indicated on the label.

Precautions while collecting specimen:

Collect sufficient quantity of specimen

Avoid contamination by using sterile equipment and aseptic precautions.

Despatch the specimen immediately to laboratory at 2-8ºC (ice box) as soon as possible.

Don't freeze whole blood as haemolysis may interfere with serology test results.

In case the delay is inevitable, keep the specimen at + 4ºC in a refrigerator.

Label all specimens accurately and send all pertinent information to laboratory which will help in better interpretation of the laboratory findings.

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Isolation of the organism

As rickettsiae are highly infectious and have caused several serious and fatal infections among laboratory workers, it comes under Risk Group 3 organisms. Isolation should be done in laboratories equipped with appropriate safety provisions preferably Biosafety level-3 laboratory following strict biosafety precautions.

Rickettsia may be isolated in male guinea pigs or mice; yolk sac of chick embryos; vero cell line or MRC 5 cell lines from patients in early phase of the disease.

Isolation of Rickettsiae: only research laboratories that had biosafety level 3 are able to isolate rickettsiae from clinical specimens but,. Since different rickettsial diseases may have indistinguishable clinical manifestations, the isolation of new isolates followed by their molecular characterization is critical for the discovery of new rickettsial diseases. The isolation of rickettsiae may be attempted with several samples: buffys coat of heparinized blood, defibrinated whole blood, triturated clot, plasma, necropsy tissue, skin biopsy, and arthropod samples.

(A) **Embryonated chicken egg yolk sacs**: have been widely used in the past, but they are now being replaced by cell culture systems. The mouse is the species of choice for the isolation of R. akari, Rickettsia australis, and especially O. tsutsugamushi. Blood clot ground in skimmed milk or any suitable medium is inoculated intraperitoneally. The animals have to be observed for 3-4 weeks. Smears from peritoneum, tunica and spleen of animal, stained by Giemsa or Gimenez methods demonstrates rickettsiae**.**

(B) Cell cultures: Cell culture, is now the most widely used method for isolating rickettsiae from clinical samples. Verocells, MRC 5 cells are being used frequently but L929 mouse fibroblast cell monolayer in tube culture is best suited for the isolation of R. rickettsii and O. tsutsugamushi from blood (9**).**

(C) Shell Vial Assay: More recently, the shell vial assay, detection of the microorganism being possible in 48 to 72 h in most cases. Inoculation should be made onto two types of cells. Vero or L929 cells have been shown to allow better and faster isolation (10).

Serological diagnosis

Diagnosis of the etiology of rickettsial diseases can be accomplished most easily and rapidly by demonstrating a significant increase in antibodies in the serum of the patient during the course of infection and convalescence. Several serological tests are currently available for the diagnosis of rickettsial diseases like Weil-Felix Test (WFT), Indirect Immunoflourescence (IIF), Enzyme linked Immunosorbent assay (ELISA) etc. Although many techniques have been used successfully for rickettsial serodiagnosis, relatively few are used regularly by most laboratories. BSL-3 Lab is not required for performing serology, when the test is to be used for seroepidemiologic studies, it should be highly specific to prevent false-positive results due to cross-reacting antibodies. In primary infection with O. tsutsugamushi, a significant antibody titer is observed at the end of the first week, concomitant with e detection of IgM antibodies, whereas IgG antibodies appear at the end of the second week. In the case of reinfection with O. tsutsugamushi, IgG antibodies are detectable by day 6, with IgM antibody titers being variable(11) . Following tests can be used for diagnosis **: -**

1. Weil-Felix Test:The cheapest and most easily available serological test . The Weil-Felix test is based on the detection of antibodies to alkali based carbohydrate antigen which are shared by some rickettsiae and certain strains of Proteus species,

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P.vulgaris OX19, and OX2 and P.mirabilis OXK. The OX-K strain of Proteus mirabilis was demonstrated to agglutinate with sera from scrub typhus patients. .By the Weil-Felix test, agglutinating antibodies are detectable after 5 to 10 days following the onset of symptoms, with the antibodies detected being mainly of the immunoglobulin M (IgM) type (12).

2. Complement Fixation (CF) Test: It is a serological test to detect specific antibody or specific antigen in a patient's serum. Each patient's serum is systematically tested against five O.tsutsugamushi serotypes. An IgM titer >1:32 and/or a four-fold increase of titers between two sera confirm a recent infection. However, due to cross-reactions among serotypes, it is difficult to identify accurately a specific serotype.

3.Indirect Hemagglutination Test: The indirect hemagglutination test detects antibodies to an antigenic erythrocyte-sensitizing substance (ESS) used to coat human or sheep erythrocytes that are either fresh or fixed in glutaraldehyde. The ESS is rickettsial group specific with cross-reactivity among Rocky mountain spotted fever (RMSF) and rickettsialpox .This test detects both IgG and IgM antibodies, but agglutination is more efficient with IgM antibodies.

4.Latex Agglutination Test: In the latex agglutination test, ESS is used to coat latex beads. The reactivity is not exactly the same as that of the indirect hemagglutination test, because the ESS on latex beads probably contains more antigenic fractions than the ESS adsorbed onto erythrocytes. This test is rapid (15 min) and does not require elaborate instrumentation.This test allows the demonstration of antibodies within 1 week after the onset of illness. Significant antibody titers disappear after 2 months.

5.**Enzyme-linked Immunosorbent Assay (ELISA):** ELISA was first introduced for detection of antibodies against Rickettsia typhi and Rickettsia prowazekii. The use of this technique is highly sensitive and reproducible, allowing the differentiation of IgG and IgM antibodies.

6.**Immunofluorescence Antibody (IFA):**IFA is the gold standard and is used as a reference technique in most laboratories. . Detection of rickettsiae by using immunofluorescence allows the confirmation of infection in patients prior to their seroconversion. Samples can be tested fresh or after formalin fixation and paraffin embedment. Biopsy specimens of the skin with a rash around the lesion, preferably petechial lesions are the most common samples used. In animals or patients with fatal cases of infection, bacteria are detectable at autopsy in the tissues of numerous organs.(13)

7.**Indirect Immunoperoxidase (**IIP): IIP is a modification of the standard IFA method that can be used with a light microscope, The procedure is the same as IFA, but fluorescein is replaced by peroxidase. The advantage of the immunoperoxidase assay is that the results can be read with an ordinary light microscope. In addition, it provides a permanent slide record.(14)

8.Microimmunofluorescence.The micro-IFA has the advantage that it can simultaneously detect antibodies to a number of rickettsial antigens (up to nine antigens) with the same drop of serum in a single well containing multiple rickettsial antigen dots. IFA allows the detection of IgG and IgM antibodies or both. This technique is, furthermore, affected by RF, thus requiring the use of a RF absorbent before IgM determination(15.)

9.Western Immunoblot: Western immunoblot assay with sodium dodecyl sulphate gelelectrophoresed andelectroblotted a ntigens is a powerful serodiagnostic tool for seroepidemiology

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and confirmation of serologic diagnoses obtained by conventional tests. It is especially useful in differentiating true-positive from false-positive results created by cross-reacting

10.Line blot Assay:The line blot assay allows the testing of more than 45 antigens simultaneously. It is a useful test for large-scale screening of sera when quantitative titers are not needed might be considered for patients with nonspecific or atypical clinical presentation.(16).

11.**Molecular Biology-Based Identification:** The first proposed molecular biology-based identification method was based on PCR-restriction fragment length polymorphism (RFLP) analysis of the gene encoding the OmpA protein. Molecular detection using polymerase chain reaction (PCR) is possible from skin rash biopsies, lymph node biopsies or ethylenediaminetetraacetic acid (EDTA) blood.O. tsutsugamushi can be demonstrated by standard and by nested PCR. Realtime PCR assays are as sensitive as standard PCR but are more rapid and can give quantitativeresults.PCR-based detection in published reports has been based on amplification of the gene encoding the 56kDa antigen for O.tsutsugamushi.(17)

Treatment

Prompt institution of effective antibiotic therapy against rickettsiae is the single most effective measure for preventing morbidity and mortality due to rickettsial diseases. Anti rickettsial therapy improves the outcome of all rickettsioses. If the illness is severe, the cardiac, pulmonary, renal, and central nervous systems should be assessed and additional measures instituted to prevent complications.

Tetracyclines and chloramphenicol remain the only proven therapy for the rickettsial diseases. Doxycycline in a dose of 100 mg twice daily for 7-15 days or Chloramphenicol 500 mg four times a day PO for 7-15 days (for children 150 mg/kg/day for 5 days) is recommended. , tetracycline should not be used for children under 8 years of age and for pregnant women.

Prevention and control

The mite vectors of scrub typhus are especially amenable to control because they are often
found in distinct areas (Typhus Island).

These foci can be eliminated by treating the ground and vegetation with residual insecticides, reducing rodent populations, and destroying limited amounts of local vegetation.

Persons who cannot avoid infested terrain should wear protective clothing, impregnate their ing and bedding with a mitecide (e.g. benzyl benzoate) and apply a mite repellent, diethyltoluamide, to exposed skin. Chemoprophylaxis should also be considered.

In a controlled trial, the weekly administration of 200 mg doxycycline decreased the incidence of clinical illnesses but not of inapparent infection.

An effective vaccine for humans has not been developed till now, mainly due to serotypic heterogeneity of the organism.(18)

Vaccines:

Scrub typhus vaccine were tried earlier during World War II in Britain using cotton rat.. Formalin killed volner vaccine-prepared from rat lung-spleen extracts and Inactivated Karp vaccine trials were also unsucessful .Then a polyvalent Gamma irradiated vaccine which elicited protection against heterologous serological types of scrub typhus was developed(19) . Recent advances in molecular biology and immunology have lead to detailed antigenic structure of Orienta tsutsugamushi which includes proteins with molecular masses of 70, 58, 56, 47,110 and 22KD. 47kd and 56kd protein are the major surface antigens and are called as [Sta 47, Sta 56] scrub typhus antigens. These proteins have now become the focus of modern research for the development of scrub typhus vaccine. The 47kd protein [Sta47] is found in outer membrane of Orienta tsutsugamushi and contains both group

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reactive and strain specific epitopes .Sta-56 protein has an immense capability to induce CMI against Orienta tsutsugamush.In addition, Sta-56 also plays a vital role in the adhesion and internalization of Orienta tsutsugamushi into host cells(20, 21,22,23,) , the gene for Sta-56 was cloned into the DNA vaccine vector pvR1012 as a vaccine candidate (pkarp 56(24). , the Sta-47 and Sta-56 proteins were fused together by ligating their genes and the fusion product was expressed in E.Coli cells.In this way immune system was effectively stimulated to generate a high level of humoral and cellular immune responses against the disease . Future vaccines are focusing more on immunodominant protein combinations which can provide long term, effective and heterologous protection against scrub typhus. newer adjuvant-Vaccine combinations (titermax+Kpr56,liposome+pKarp110, FIA+pKarp47) are also tried. Sta-56-47 fusion product is also looked upon as a suitable candidate for recombinant vaccine against the scrub typhus. (25)

Conclusion:

A high index of clinical suspicion, prompt diagnosis and early institution of appropriate antimicrobials can decrease the morbidity and mortality. Scrub typhus is easily treatable disease if we focus on case identification, public education, rodent control and habitat modification to control the impact of Scrub typhus on public health. Vaccines are under trial sooner we will be able to control this scourge of mankind.

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