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RESEARCH ARTICLE

Incidence of Scrub Typhus in a Tertiary Care Hospital in Uttarakhand.

Dr Sulekha Nautiyal, Dr Shalabh Jauhari, Dr. Noopur Goel, Dr. B.S. Mahawal

Shri Guru Ram Rai Institute of Medical & Health Sciences

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*Corresponding Author

Dr Sulekha Nautiyal.

Abstract

Context: Scrub typhus is an important cause of acute undifferentiated febrile illness. Due to non specific clinical presentation cases are often missed, although endemic in our country. IgM Ab detection by ELISA and Rapid Immunochromatographic test (ICT) are commonly employed for its diagnosis. **Aim:** To study the incidence of Scrub typhus in population attending our hospital in Uttarakhand. **Settings and Design:** This prospective study was carried out from September 2013 to November 2013 in cases of PUO with high suspicion of scrub typhus. **Material and Methods:** A total of 294 samples were subjected to a spectrum of tests (ICT for antibodies against *Orientia tsutsugamushi*, IgM, IgG Ab & NS1Ag for Dengue, IgM to *Salmonella Typhi*) based on clinical suspicion of febrile illness. Positive samples for *Orientia tsutsugamushi* were retested by ELISA for IgM antibodies against *O. tsutsugamushi*. **Results:** Out of 294 cases of PUO, 61 /294 (20.7%) were found to be positive for IgM Ab to *Orientia tsutsugamushi*, 27 /294 (9.18%) IgM, IgG Ab & NS1Ag for Dengue, and one out of 294 (0.003%) for IgM Ab to *Salmonella Typhi*. One case was found to be reactive for both IgM Ab for *Orientia tsutsugamushi* and Dengue. Data was analyzed in correlation with clinical features. **Conclusions:** This study implies emergence of scrub typhus in our region. It is thus suggested that high index of suspicion for scrub typhus should be maintained for cases presenting with acute febrile illness to avoid false negative reporting owing to absence of eschar formation which is pathognomic feature of disease and rarely seen in Indian sub-continent.

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Introduction:-

Scrub typhus, a widely endemic disease in Asian Pacific regions, is caused by *Orientia tsutsugamushi*, a Gram negative obligate intracellular bacterium in the family *Rickettsiaceae*. Scrub typhus is usually transmitted by chigger of trombiculid mites in Asia, northern Australia and the western Pacific region.¹ It was first observed in Japan where it was found to be transmitted by mites. The disease was, called tsutsugamushi (tsutsuga: meaning dangerous and mushi: insect or mite). This is found only in areas with a suitable climate, plenty of moisture and scrub vegetation. 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 typhus from Sikkim, Darjeeling, Nagaland & Manipur (unpublished data).²

The organism is transmitted through the bite of larval forms (chiggers) of trombiculid mites. Scrub typhus is widespread in the so called "tsutsugamushi triangle" which includes Japan, Taiwan, China and South Korea on the north, India and Nepal on the west and Australia and Indonesia on the south.³ In India, epidemics of scrub typhus have been reported from north, east and south India.⁴⁻⁹ Although the disease is endemic in our country, it is grossly under diagnosed owing to the non-specific clinical presentation, lack of access to the specific diagnostic facilities in most areas and low index of suspicion by the clinicians.¹⁰

The bacterium maintains itself in trombiculid mites and small mammals serve as reservoir hosts in the natural life cycle of the mites. Chiggers, the larval stage of mites, act as the transmission vector for *O. tsutsugamushi*.¹¹ Humans and small animals become infected following the bite of chiggers harboring *O. tsutsugamushi*. After an incubation period of 7–14 days, high fever, chills, headache, rash, and eschar usually develop in infected persons.¹²

The chigger bite is painless and may be noticed as a transient localized itch. Bites are often found on the groin, axilla, genitalia or neck. An eschar is often seen in humans at the site of the chigger bite. Eschars are rare in patients in countries of South-East Asia and persons of typhus-endemic areas commonly have less severe illness, often without rash or eschar.^{13, 14}

Worldwide more than one million cases occur annually and as many as one billion people living in endemic areas may have been infected at some time.¹⁵ Many studies from India particularly from South India^{5,6,7,10}, Haryana¹⁶, Himalayan region^{6,8}, Mumbai¹⁷ and Jammu¹⁸ have reported outbreak or isolated reports of scrub typhus in the past.

Differentiating scrub typhus from other forms of typhus as well as from fever, typhoid and meningococcal infections is often difficult during the first several days before the initial rash appears. The most common signs are similar to a variety of other infectious diseases (typhoid fever, murine typhus, leptospirosis and dengue fever, etc.) which should be taken into consideration¹⁹.

The diagnosis may be confirmed by a laboratory test such as serology. The cheapest and most easily available serological test is the Weil-Felix test, but this is notoriously unreliable. This test is now being replaced by a complement-fixation test. Each patient's serum is systematically tested against five *O. tsutsugamushi* serotypes. An IgM titer > 1:32 and/or a four-fold increase of titer between two sera confirm a recent infection. However, due to cross-reactions among serotypes, it is difficult to identify accurately a specific serotype.²⁰ The gold standard is indirect immunofluorescence antibody (IFA). Indirect immunoperoxidase (IIP) is a modification of the standard IFA method that can be used with a light microscope and the results of these tests are comparable to those from IFA.^{21, 22} Serological methods are most reliable when a four-fold rise in antibody titre is looked for.

Commercial rapid diagnostic kits provide reliable and well-accepted preliminary results within one hour, but the availability of these tests is severely limited by their cost.²³ However, other serological tests must be used in order to obtain confirmation of *O. tsutsugamushi* infection. ELISA provides more sensitivity and equal specificity when compared to commercial test kits.

The organism can be grown in tissue culture or mice from the blood of patients with scrub typhus but results are not available in time to guide clinical management. Molecular detection using polymerase chain reaction (PCR) is possible from skin rash biopsies, lymph node biopsies or ethylene diamine tetra acetic acid (EDTA) blood. *O. tsutsugamushi* can be demonstrated by standard and by nested PCR (24). Real time PCR assays are as sensitive as standard PCR but are more rapid and can give quantitative results.²⁵

The drug most commonly used is doxycycline; but chloramphenicol is an alternative. A combination therapy with doxycycline and rifampicin should be used in areas where there is poor response to doxycycline alone.²⁶ Azithromycin or chloramphenicol is useful for treating infection in children or pregnant women (doxycycline is relatively contraindicated in children).

There are no effective vaccines for scrub typhus. It is now known that there is enormous antigenic variation in *Orientia tsutsugamushi* strains and immunity to one strain does not confer immunity to another.²⁷ This complexity continues to hamper efforts to produce a viable vaccine.²⁸

The Armed Forces Research Institute of Medical Sciences (AFRIMS) Bangkok, Thailand is the WHO Collaborating Centre (CC) for Emerging Diseases. The Department of Entomology, AFRIMS is the only laboratory in the world that has the ability to rear and colonize scrub typhus-infected *Leptotrombidium* mites, the vector of *O. tsutsugamushi*.²⁹ 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. Rickettsiae 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. Egg and animal inoculation methods have been replaced by faster and more sensitive cell cultures. Facilities for laboratory diagnosis of Rickettsial diseases are available at National Centre for Disease Control, Delhi where samples can be sent for confirmation. Prompt institution of effective antibiotic therapy against rickettsiae is the single most effective measure for preventing morbidity and mortality due to rickettsial diseases.²

Subjects and Methods:-

Study design:

This prospective study was conducted in the Department of Microbiology, Shri Guru Ram Rai Institute of Medical and Health Sciences during September to November 2013.

Material & Methods:-

A total of 294 samples were collected on clinical suspicion of febrile illness and assessed for the presence of antibodies against *Orientia tsutsugamushi*, IgM antibodies of Dengue and *Salmonella Typhi* and IgG antibody & NS1Ag of Dengue. Commercially available Immuno Chromatographic Tests (ICT) were used for this study. Samples showing a positive result for antibodies against *O. tsutsugamushi* were tested for IgM antibodies against *O. tsutsugamushi* using IgM ELISA.

Results:-

Of the 294 samples tested, 132/294 (44.8%) samples were found to be positive by rapid ICT test for *Orientia tsutsugamushi*. Since rapid ICT test detects all IgM, IgA and IgG antibodies, these samples were retested by ELISA for IgM antibodies against *O. tsutsugamushi*. It was observed that 61/132 (46.2%) samples were positive for IgM antibodies. Overall picture of febrile cases when observed, revealed that 61/294 (20.7%) cases were suffering from acute illness pertaining to scrub typhus.

Of the confirmed cases, 36/61 (62.1%) were found to be females. The clustering of cases was observed in age group of 31-50 years followed by 0-10 years. (Table 1) Scrub typhus cases presented with a myriad of symptoms of which gastrointestinal symptoms were the major ones followed by headache apart from the common presentation of fever. (Table2)

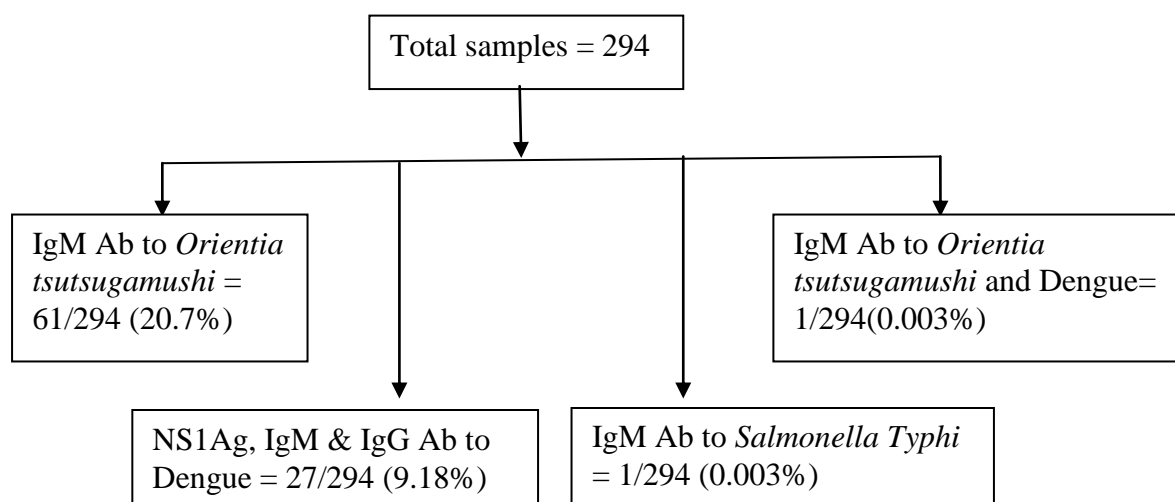
Although presence of ESCHAR is a pathognomic feature for diagnosis of scrub typhus but rarely observed in India and Indian subcontinent. Also, it was not observed in any of the cases, on thorough physical examination which correlates with the previous studies conducted in other parts of the country.

Table 1: Age wise distribution of scrub typhus positive cases

| Age group (in years) | Males | Females | Total |
|----------------------|-----------|-----------|------------------|
| 0-10 | 4 | 4 | 8 (13.4%) |
| 11-20 | 4 | 8 | 12 (20%) |
| 21-30 | 4 | 5 | 09 (15%) |
| 31-40 | 3 | 8 | 11 (18.3%) |
| 41-50 | 6 | 5 | 11 (18.3%) |
| 51-60 | 2 | 5 | 07 (11.7 %) |
| 61-70 | 1 | 1 | 02 (3.3%) |
| Total | 24 | 36 | 60 (100%) |

Table 2: Predominant symptoms observed in scrub typhus positive cases

| Symptoms | Number of cases | Percentage(%) |
|-------------------|-----------------|---------------|
| Body rash | 3 | 5 |
| Breathlessness | 3 | 5 |
| Bodyache | 3 | 5 |
| CNS | 5 | 8.3 |
| Cough | 8 | 13.3 |
| Fever with chills | 8 | 13.3 |
| Headache | 10 | 16.7 |
| GI symptoms | 28 | 46.7 |
| Fever | 60 | 100 |

Figure 1: Summary of test results**Discussion:-**

Scrub typhus caused by *O.tsutsugamushi* is known to be endemic to Asia-Pacific region including the tropical countries like India. Many outbreaks have been reported in the past particularly from South India, Himachal Pradesh, Mumbai, Karnataka & Jammu.^{5, 32, 17, 8} Scrub typhus still remains under diagnosed due to low index of suspicion, lack of diagnostic facilities, limited awareness and nonspecific clinical presentations. Most of the cases belong to low socioeconomic status which implies poor hygiene and lack of awareness leading to exposure to the vector.

This study was conducted from September to November 2013. Of the total samples received in laboratory, with a history of febrile illness and clinical suspicion of scrub typhus, only 61/294 (20.7%) were found to be positive for *O.tsutsugamushi*. In our study, most of the cases belonged to age group of 11-50 years which is almost similar to the study conducted by Mahajan et al.⁶

Thirty seven (37) females against 24 males were found to be affected in our study which is similar to study done by Mahajan et al.⁶ however the study done by Kedareshwar P S et al.³¹ from Goa did not mention any predilection for sex. Predominance in women may be due to their extensive involvement in farm work. Ogawa et al.¹⁵ have stressed that age and sex are known to influence the occurrence of scrub typhus mainly due to exposure and inclination to outdoor activities whether occupational or recreational. A closer association between human and rodent population during monsoon months and greater land area covered by scrub vegetation could be a reason for an increase in cases in monsoons.

Of the total number of positive cases studied from, 25/61 (40.98%) were from Dehradun. The higher positivity from Dehradun district could be due to the fact that number of patients coming from nearby areas may not represent the accurate disease burden in those areas. Our hospital is a tertiary care referral centre where patients are referred by clinicians from neighbouring areas possibly after a prolonged period of sickness. Milder cases that responded to treatment at peripheral level may have gone undiagnosed. An extensive multicentre study for exact prevalence of scrub typhus in this region is necessary.

The clinical picture in the cases studied varied from the classical presentation. Although the presence of ESCHAR is pathognomonic feature for diagnosis of scrub typhus but rarely observed in India and Indian subcontinent.⁸ The eschar was not observed in any of the cases studied despite thorough physical examination which correlates with the previous studies conducted in other parts of the country.^{4, 7, 18}

In the present study 28/61 (46.6%) of the cases predominantly presented with gastrointestinal symptoms such as nausea and vomiting, abdominal pain and loose motions. Similar observation was also reported from a study conducted by Kedareshwar et al.³¹ It also becomes necessary to differentiate between the number of recent or past infections on the basis of presence of IgM and/or IgG antibodies which would give a better picture of endemicity and burden of disease in a particular geographical area. Also, there is limited data on the relationship between scrub typhus and gastrointestinal dysfunction.³¹

Conclusion:-

Many other diseases present with fever, rashes, myalgia, headache such as typhoid, dengue and leptospirosis. In cases of pyrexia of unknown origin, gastrointestinal symptoms in the absence of eschar, should allow for suspicion and screening of the patient for scrub typhus.

In the present study, the rapid kits available in the market were found to detect broadly IgA, IgG and IgM classes of antibodies against *O.tsutsugamushi*. Therefore, it is difficult to pinpoint whether the cases are being diagnosed in acute phase or in the later part of illness if not confirmed by ELISA. It is the need of the moment to conduct research and devise rapid tests which can detect IgM and IgG antibodies separately for better classification of the cases in the endemic area.

This study implies emergence of scrub typhus in our region. However, a more extensive multicentric study would project the exact burden of the disease in our area.

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