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Brief Communication

Eschar and IgM ELISA in the Diagnosis of Scrub Typhus

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Abstract

Scrub typhus is one of the leading causes of acute febrile illness in India. This study aimed to determine the best diagnostic tool for the identification of scrub typhus and study the possible association between diagnostics and clinical characteristics. Patients with fever of ≤15 days admitted to the hospital satisfying the case definition of 47 kDa quantitative polymerase chain reaction (qPCR) positivity OR scrub typhus IgM ELISA positivity along with the presence of eschar OR Scrub typhus IgM ELISA positivity along with defervescence of fever within 72 h of initiation of specific therapy were recruited. Of the 116 patients satisfying the case definition, 47 kDa qPCR was positive in 43 (37%) patients, whereas IgM ELISA was positive in 104 (90%) patients and eschar was seen in 59 (51%) patients. The median duration of fever was 7.5 days (interquartile range 6–10 days). Multiorgan dysfunction syndrome (MODS) was described in 44 (37.9%) patients. Two patients (1.8%) succumbed to the illness. Presence of eschar and IgM ELISA positivity were detected in 106 (91%) cases. Scrub typhus, even with MODS, has low mortality because of immediate institution of specific therapy due to physician awareness. The presence of eschar and IgM ELISA positivity can be used to detect a majority of cases of scrub typhus.

Keywords: Acute febrile illness, eschar, mortality, multiorgan dysfunction syndrome, scrub typhus

Introduction

Scrub typhus caused by *Orientia tsutsugamushi* is one of the leading causes of acute febrile illness (AFI) in tropical regions and is transmitted to humans by chiggers. Clinical manifestations occurring approximately 5–15 days after chigger bite range from mild flu-like symptoms to shock and multiorgan dysfunction syndrome (MODS).[1] An eschar develops over the mite bite site and when this characteristic lesion is not present, it becomes difficult to differentiate it clinically from other conditions such as malaria, dengue and leptospirosis. A study from North India concluded that presence of eschar was associated with a poor prognosis,[2] while another study from South India concluded otherwise.[3] The former was a retrospective study using only serological tests and had missing data, whereas the latter was a prospective study based on serological diagnosis conducted exclusively in non-intensive care unit (ICU) patients. Doxycycline is the mainstay of treatment except in pregnancy. Macrolides can be used alternately. Fever defervesces in 24–48 h after initiating appropriate therapy. Serological tests are the mainstay of diagnosis. Though immunofluorescence assay is the serological reference standard, limited availability, inter- and intra-observer variation precludes its use as a readily available diagnostic test.[4] With more objective results and good sensitivity, it has been reported that scrub typhus IgM ELISA (ST IgM ELISA) is the preferred test for the diagnosis of scrub typhus in health-care settings.[5] Detection of nucleic acid by real-time polymerase chain reaction (PCR) targeting the 47 kDa gene yields good results and is being increasingly used for scrub typhus diagnosis.[6,7] In this study, we have taken into consideration a composite diagnostic tool comprising clinical, molecular and serological components to diagnose a case of scrub typhus and described its association with eschar and clinical outcome.

Materials and Methods

The study was conducted from August 2015 to December 2017 in a 2700-bedded tertiary care centre in South India. The study population included patients with AFI of ≤15 days with or without eschar who were negative for malaria and showed

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no growth in blood culture. In addition to scrub typhus IgM serology, 47 kDa quantitative PCR (qPCR) was performed in these patients.

IgM antibodies to scrub typhus were detected in serum using the Scrub Typhus IgM ELISA system (InBios International Inc., Seattle, WA, USA), with optical density (OD) values ≥1.00 being considered positive. *O. tsutsugamushi*-specific 47 kDa outer membrane protein gene qPCR was performed as described by Jiang *et al*.[8]

A composite case definition for scrub typhus was used in this study. A case of scrub typhus has to satisfy one of the following three criteria:

1. Positive for 47 kDa qPCR OR
2. Both eschar and ST IgM ELISA positive OR
3. Eschar negative but with a positive ST IgM ELISA and defervescence of fever within 72 h of initiation of therapy.

MODS was assigned to the respective cases based on the established criteria published in a previous study from the study centre.[9]

Results

Of the 701 inpatients with AFI, 237 patients satisfied the inclusion criteria of whom 116 were diagnosed as scrub typhus based on the case definition. A wide spectrum of age groups from 1 to 84 years was noted, with a median age of 42 years (interquartile range [IQR] 25.5–55). The number of females affected was 68 (58.6%) and 48 (41.3%) were males. The median duration of fever in days before presenting to the clinician was 7.5 (IQR 6–10 days). Along with fever, 57 (49%) patients had breathing difficulty, 25 (21.5%) had abdominal pain, 36 (31%) had headache and 15 (13%) had nausea and vomiting. Sixteen (13.7%) patients were admitted to the ICU and 9 (7.7%) required ventilatory support.

Eschar was observed in 59 (50.8%) of the total patients in the study. The distribution of eschar varied, with 13 (11.2%) cases having it on the abdomen, 11 (9.4%) on the back or shoulders, 10 (8.6%) in the axilla, 8 (6.8%) over the anterior chest wall, 7 (6%) in the groin region, 5 (4.3%) on the lower limbs and 5 (4.3%) in the head-and-neck region.

MODS was seen in 44 (37.9%) patients, of whom two patients (1.8%) died.

Contribution of eschar, IgM ELISA and 47 kDa qPCR to the case definition used in the study is summarised in Table 1.

IgM ELISA and qPCR were both positive in 31 (27%) patients. Poor agreement was observed between scrub typhus IgM ELISA and 46 kDa qPCR (kappa -0.21). Association between the presence of eschar and MODS was not statistically significant ($P = 0.316$).

Table 1: Distribution of cases with eschar, IgM ELISA and 47 kDa polymerase chain reaction

ST cases ($n=116$)	Eschar		IgM ELISA		47kDa qPCR	
	Positive	Negative	Positive	Negative	Positive	Negative
Yes	56	48	104	12	43	73
No	3	9	0	0	0	0

qPCR: Quantitative polymerase chain reaction

Discussion

Based on previous studies, appropriate algorithms for diagnosis and management of AFI have been devised at our centre. Though the MODS rates have been the same, a steady decline in mortality has been observed over the years at our institution. It was 14% in 2002–2003,[10] 7.8% in 2009,[11] 4.6% in 2013[12] and in the present study, it was 1.8%. A majority of patients (90%) were started on doxycycline or azithromycin empirically on clinical suspicion, even before the IgM ELISA results for scrub typhus were available. We believe that this has prevented mortality in our patients including those with MODS. In the current study, the presence or absence of eschar did not correlate with MODS or mortality.

The poor agreement between qPCR and IgM ELISA suggests that both of them are needed for diagnosis. Eschar biopsy is the best specimen for PCR, but it is not always seen or available for laboratory diagnosis.[6] IgM ELISA positivity and response to treatment with or without the presence of eschar diagnosed most cases (91.3%) of scrub typhus in this study. This is of use for centres which have ELISA capability but lack PCR instrumentation.

Use of blood clots and not eschar (which is a better specimen for PCR) and exclusion of outpatients are the drawbacks of the study. A prospective study design, molecular method for detection (47 kDa qPCR) in addition to serology, use of a robust composite case definition and no missing data are the strengths of this study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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