Determination of Cutoff of IgM ELISA for Diagnosis of Scrub typhus in Hilly Northern State of Himachal Pradesh

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Abstract

The aim of the study was to determine geographically relevant cutoffs for IgM ELISA in Himachal Pradesh. A total of 392 individuals including 292 patients of all age groups with clinically suspected scrub typhus and 100 healthy people were enrolled after taking a proper consent at Indira Gandhi Medical College, Shimla. An indirect ELISA that detects IgM antibodies to Orientia tsutsugamushi was performed in all serum samples. The serum samples from patients were subjected to IgM immunofluorescent antibody (IFA) (Fuller Laboratories, USA). A receiver operating characteristic (ROC) curve was drawn for ELISA to generate cutoff for the test. A total of 192 patients were found to be positive for scrub typhus. A set of 100 sera samples collected from healthy volunteers from Shimla were used to establish region specific cutoff for IgM ELISA. The cut off calculated from healthy volunteer was mean OD +3 standard deviation = 0.5. The ROC curve analysis of 392 participants revealed a cutoff-OD value of 0.46 with sensitivity and specificity of 91.7% and 99.5% respectively. Hence, a cutoff-OD value of 0.46 or 0.5 can be used as the cutoff for the diagnosis of scrub typhus for Himachal Pradesh based on our findings.

Keywords: Geographically relevant cutoffs, indirect ELISA, IgM antibodies, receiver operating characteristic (ROC) curve.
INTRODUCTION

Scrub typhus is an acute febrile illness caused by an obligate intra-cellular gram negative bacterium, Orientia tsutsugamushi. It is transmitted to humans by the bite of chiggers. It is a public health problem in Asia, where about 1 million new cases are identified annually and 1 billion people may be at risk for this disease. Mortality occurs in 30% to 50% of untreated cases (Diprabhanu et al., 2007). Diagnostic techniques like IgM ELISA and IgM IFA help in early diagnosis and treatment. Though, IFA is the serological gold standard (Manuj et al., 2015), IgM ELISA have remained the mainstay of diagnosis in various laboratories as it provides an objective result and has sensitivity similar to IFA (McDade JE, 1998). A four-fold rise in serum antibody is desired for diagnosis (Gavin et al., 2010). However, for guidance of initial treatment, there is a need for rapid diagnosis at the time of admission. One of the biggest challenges in the diagnosis of scrub typhus in this region is the unavailability of a standardized cutoff for these serological tests. The study was conducted to determine geographically relevant cutoffs for IgM ELISA in Himachal Pradesh.

MATERIALS AND METHODS

A prospective observational study was conducted over a period of 1 year, where 292 patients of all age groups with clinically suspected scrub typhus were enrolled in the study after taking a proper consent. 100 healthy people were also studied as control group. The study was conducted at Indira Gandhi Medical College, Shimla.

Serum was collected from all the patients using standard precautions. An indirect ELISA that detects IgM antibodies to Orientia tsutsugamushi was performed in all serum samples. Serum samples from 100 healthy controls were also subjected to IgM ELISA. The absorbance was measured at 450 nm. The serum samples from patients were subjected to IgM immunofluorescent antibody (IFA) (Fuller Laboratories, USA). The IFA slides were precoated with four scrub typhus strains (Gilliam, Karp, Kato, and Boryong) propagated in L292 cells. Serum samples of all the patients were put at dilutions of 1:64, 1:128, 1:256, and 1:512. The antigen-antibody reaction was visualized using a fluorescent microscope. A positive reaction was visualized as small green fluorescent rods in the background of counterstained red cells. The time taken for the fever to resolve after the initiation of specific antibiotic therapy was recorded. Patients were classified as positive and negative based on the following criteria: Any patient with response to antibiotics within 48 h accompanied by either presence of an eschar or positivity by IgM IFA was taken as positive. A receiver operating characteristic (ROC) curve was drawn for ELISA to generate cutoff for the test. The cutoff for ELISA was also determined using recommendations from kit literature. It was calculated by adding three standard deviations to the mean optical density (OD) value of ELISA runs on the samples of healthy volunteers.

Statistical analysis

ROC curves were generated using Medcalc Version 12.7 (MedCalc Software, Ostend, Belgium). 95% confidence interval was calculated for sensitivity and specificity of the cutoff.

RESULTS AND DISCUSSION

A total of 192 patients were found to be positive for scrub typhus. A set of 100 sera samples collected from healthy volunteers from Shimla were used to establish region specific cutoff for IgM ELISA. The cut off calculated from healthy volunteer was mean OD +3 standard deviation = 0.5. The ROC curve analysis of 392 participants revealed a cutoff-OD value of 0.46 with sensitivity and specificity of 91.7% and 99.5% respectively (Figure 1).

Fig.1. ROC curve analysis of the scrub typhus positive cases.

Most diagnoses of scrub typhus infections are made by serology. In primary scrub typhus infection, a significant IgM antibody titer is mounted by the end of the first week, whereas IgG antibodies typically appear by the end of the second week. With reinfection, however, IgM antibody titers may be variable and IgG detectable by the sixth day of infection (Navinchandra, 1982). The rapid progression of the disease makes it difficult to wait for 2 weeks before giving the final result. Such a diagnosis remains retrospective and is of little practical relevance for guiding initial management. Besides, in most cases, patients are not available for the paired samples. In such a case, there
is a need to establish baseline cut-off titre keeping in view the regional variations. ELISA is currently the preferred method of serological diagnosis (Manuj et al, 2015). The 56-kDa type-specific antigen (TSA) is best suited for diagnostic testing, as it is the major immunodominant surface protein, containing both group- and strain-specific epitopes, and it is abundant in patient sera. The 56-kDa TSA is also highly variable, accounting for strain differentiation and genetic diversity in O. tsutsugamushi. IFA uses fluorescence-labeled anti-human immunoglobulin to detect antibodies in the serum of the patient that have bound to immobilized bacterial antigen on a slide. Most centers in India use ELISA for the diagnosis. The recently published ICMR guidelines recommend a cutoff OD value of 0.5 (Manuj et al, 2015). Using uniform cutoff values all across the nation can be a problematic as many of our healthy controls had OD values above 0.5. A wide geographical variation has been noticed in different studies and therefore, a nationalized cutoff OD cannot be determined (Nitin et al, 2016). Several studies from India have used a cutoff-OD from 0.5 to 1 (Munegowda et al, 2015). We propose a cutoff-OD value of 0.46 or 0.5 for Himachal Pradesh based on our findings. The IgM ELISA for scrub typhus is more sensitive than IFA for low level antibody detection and post vaccination. Studies have suggested low sensitivity of IFA if high specificity is required (Bernard and Didier, 1997). Hence, for the serological diagnosis of scrub typhus, IgM ELISA may be used as an alternative reference test to the IgM IFA.

CONCLUSION

The samples were collected from different regions in Himachal Pradesh. Hence, a cutoff-OD value of 0.46 or 0.5 can be used as the cutoff for the diagnosis of scrub typhus for Himachal Pradesh based on our findings.

CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES


