A hospital based survey for rickettsioses in Shimoga, Karnataka using the Weil-Felix test

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Abstract

Background: Members of the genus Rickettsiae are transmitted by ticks, mites and lice to humans. The rickettsial agents are important causes of fever. Clinical diagnosis of rickettsial fevers is difficult and laboratory support is necessary to confirm the etiology. Mortality is 30-40% in untreated cases. The Weil-Felix test has been traditionally used to detect rickettsial antibodies in sera. However, the test has poor sensitivity. Despite availability of newer modalities of diagnosis, many laboratories offer only the Weil-Felix test for diagnosing rickettsial fevers.

Objectives: To obtain information about the types of rickettsial fevers present in Shimoga, Karnataka, using the Weil-Felix test.

Methods: 277 patients with undiagnosed fever of seven days or more were included in the prospective study. Sera were subjected to a panel of tests to screen for common infectious etiologies including rickettsiae. Only the (exclusively) Weil-Felix positive sera were considered for the study. Serum samples testing positive for any other infectious disease with/ without detectable rickettsial antibodies were not studied further. Results of the Weil-Felix test were analyzed.

Results: Of the total 277 samples, 87 (31.4%) were positive for rickettsial antibodies. Spotted fevers (34.50%) and the scrub typhus (25.30%) are the commonest rickettsioses. Typhus fevers are also present (17.25%). Younger males were more frequently seen to have rickettsial fever, but this could be a fallacy. Weil-Felix test could not detect antibodies in 71 (27.8%) clinically suspected rickettsial fever samples.

Conclusions: In Shimoga, Karnataka, spotted fever, scrub typhus and typhus fevers are present. The Weil-Felix test despite having limitations remains useful at present. More studies need to be conducted at the community level in Shimoga district, Karnataka to get a clearer picture of the types of rickettsia and the magnitude of the problem.

Keywords: Karnataka, Rickettsia, Scrub typhus, Shimoga, Spotted fever, Typhus, Weil-Felix

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Introduction

Members of the family Rickettsiaceae are important causes of fever. These are Gram-negative small obligate intracellular organisms and are transmitted by arthropod vectors such as lice, fleas, ticks and mites, either by their bite or feces. Clinical diagnosis of rickettsial fevers is difficult in most cases due to the varied presentation and clinicians need to remain alert always to diagnose the etiology of fevers correctly. Laboratory support becomes essential despite having a high index of suspicion for the rickettsiae because of the often nonspecific presentation and the 35 to 40% mortality in untreated cases.[1]

Of late, several new methods such as the 'gold standard' immunofluorescence assay, indirect Immunoperoxidase Assay (equivalent to the 'gold standard'), commercial rapid detection kits like scrub typhus RCT and scrub typhus IgM and IgG Rapid Immunochromatographic Assay (PanBio, Brisbane, Australia) and Multies Dip-S-Ticks Scrub Recombinant Assay (Integrated Diagnostics, Baltimore, Maryland, USA) have become available for the laboratory diagnosis. But, most of these are very expensive and therefore the best tests are often unavailable to the clinicians.[2]

Traditionally, rickettsial fevers have been diagnosed in the laboratory using the Weil-Felix heterophile agglutination test. But this test is known to be unreliable and has poor sensitivity and specificity. This study was undertaken to define the relevance of the Weil-Felix test in our set up and to generate information about the rickettsial diseases in Shimoga town, Karnataka State.

Objectives

To obtain information about the types of rickettsial fevers present in Shimoga, Karnataka, using the Weil-Felix test.

Material and Methods

For this prospective study, we included patients of all ages who came with undiagnosed fever of more than seven days to our Medical College hospital located in Shimoga town, Karnataka state. The study was conducted over a period of one year, from June 2015 to May 2016. Permission from the Institute Ethical Committee was taken for undertaking the study.

A total of 277 patients were entered into the study. 5ml of blood was collected in a plain vial and allowed to clot. Serum was separated and used for the various tests as per their manufacturers' instructions. The serum samples were screened for malaria, dengue, enteric fever, brucellosis, leptospirosis by using commercially available kits based on the lateral flow immunochromatography principle. The serum samples that tested positive for any other infectious disease mentioned above (with or without detectable rickettsial antibodies) were not studied further.

To detect rickettsial antibodies, the Weil-Felix test was performed and interpreted as per manufacturer's instructions using commercially available antigens (Tulip Laboratories, Goa, Progen OX 2, OX 19 & OX K). Titers of more than 1:160 for OX-K and more than 1:80 for OX-2 and OX-19 were considered significant. The demonstration of a fourfold rise in titer was not possible in our study.

Result

Results were collected, tabulated and analyzed using Microsoft Excel 2013.

Table 1

Total number of samples included (1 sample per person)	277 (=*n*)
Only Weil-Felix positive	87 (31.41%)
Sera that were negative for rickettsial antibodies but positive for other infectious etiologies*	81 (29.24%)
Sera that were positive for rickettsial antibodies along with other infectious etiologies*	38 (13.72%)
Sera that were negative by all 'fever panel' screening tests	71 (25.63%)

* Enteric fever, malaria, dengue, chikungunya, leptospirosis, brucellosis

Table 2: Weil-Felix test results (above and below the cut-off levels)

Weil-Felix positive : 87	
OXK >1:160 , OX 2 & OX 19 <1:80	22 (25.30%)
OX19 > 1:80, OX 2 < 1: 80 & OX K <1:160	15 (17.25%)
OX2 > 1:80, OX 19 < 1: 80 & OX K <1:160	30 (34.50%)
OXK, OX2 & OX19 positive (OX 2 & OX 19 < 1:80; OX K < 1:160)	20 (23%)

Table 3: Age distribution

Age group	Number of patients
0-18 years	75 (86.21%)
19- 60 years	10 (11.49%)
61 years and above	02 (2.30%)

Table 4: Sex distribution

Sex	Number of patients
Male	57 (65.52%)
Female	30 (34.48%)

Discussion

In our country there is a large number of possible causes of fever. Most of the time empirical treatment suffices. However, when fever becomes prolonged, people seek medical attention. One of the important yet poorly diagnosed causes of fever is the rickettsioses. It is well known that rickettsioses present in a very nonspecific manner and carry a mortality of about 35-40% if untreated.[1]

Our town Shimoga, in Karnataka state, India, has primarily an agricultural economy and is surrounded by vast paddy fields, arecanut plantations, scrub lands and forests. Rickettsial fevers are commonly included as differential diagnoses by clinicians in and around this town. However, it is seen that they do not usually ask for any laboratory test for diagnosing rickettsia. There could be several reasons for this : presentation of the various rickettsial fevers is not very distinctive, treatment of all the rickettsial fevers remains the same-doxycycline or azithromycin, the response to the appropriate medication is quick and positive, antibiotic treatment is started based on clinical suspicion alone, without waiting for laboratory confirmation of the rickettsial etiology and only a few laboratories offer a test that detects the rickettsiae in the early days of the illness.[2]

The other common infectious diseases are enteric fever, malaria, dengue, chikungunya, leptospirosis and brucellosis. We have adequate information here about the various other infectious diseases mentioned above, except the rickettsioses. We have been relying on the general information that scrub typhus is the most extensive rickettsiosis in India and the whole of South-east Asia.[3,4,5,6,7] We wanted to clarify whether this assumption is appropriate. This is part of the reason for this study. Besides, to our knowledge, there is no other study that has been done from this area.

At our Medical College Hospital, blood samples of patients presenting with undifferentiated fever of more than seven days duration are subjected to a panel of screening tests for the above mentioned diseases including the rickettsioses.

For the diagnosis of rickettsial disease several laboratory tests are available. Immunofluorescence Assay (IFA) is the serological 'gold standard'. Indirect Immunoperoxidase Assay (IPA) is comparable to the 'gold standard' test. But these are only available at laboratories with higher level of facilities and expertise.[2]

Commercial rapid detection kits like Dip-STicks, scrub typhus RCT and scrub typhus IgM and IgG Rapid Immunochromatographic Assay (PanBio, Brisbane, Australia) and Multies Dip-S-Ticks Scrub Recombinant Assay (Integrated Diagnostics, Baltimore, Maryland, USA) are available but are still expensive. There is also the need to include local serotypes in the tests for them to be relevant in our country.[8] The Department of Health Research, Government of India and the Indian Council for Medical Research do not recommend any rapid test for diagnosis of scrub typhus at the present stage of development of these tests as they need further evaluation.[2] IgM ELISA has been evaluated and found to be quite satisfactory in comparison to the gold standard, but samples need to be pooled for ELISA which can lead to delayed diagnosis thus influencing the overall outcome.[9] Polymerase chain reaction has been used to diagnose rickettsial infection.[8]

None of the local laboratories in Shimoga including ours have access to the reference tests and the only test that is available at present is the Weil-Felix test.

The Weil-Felix test continues to be in use due probably to its advantages that score over its disadvantages. Pros of this test include easy availability of the test reagents from reputed manufacturers, minimal requirements of laboratory-ware, simplicity of test procedure and its relative economy. The most important cons being the retrospective nature of the serological test and inability to satisfactorily differentiate between the various rickettsial etiologies. The Weil-Felix test also has poor sensitivity. The test may be negative in up to 50% of cases and it can show false positive results as well.[10,11,12] Rita Issac and others found that the Weil-Felix agglutination test had only 30% sensitivity at a titer of 1:80. However, the specificity of this test was high even at lower titers.[13] Therefore, when there is a clinical suspicion of rickettsiosis, the Weil-Felix test result may be expected to be reasonably specific.

Taking the above into consideration we have used the Weil-Felix test to detect the rickettsial antibodies in suspected cases and to obtain a picture of the distribution of rickettsioses in and around Shimoga town in Karnataka.

The Department of Health Research and the ICMR both recommend that baseline titer be determined for each geographical area in order to correctly interpret the test.[2] However, since we had no data about the baseline titers here in Shimoga, we adopted the baseline titers recommended by the manufacturer of the test reagents and investigators in the nearby urban areas.[14]

Our data showed that of the total 277 samples included in the study, 125 were found positive with the Weil Felix test. 38 of these 125 results were positive for other infectious etiologies as well and were not considered further. 87 (31.4%) sera were positive for rickettsial antibodies. Important to note that the Weil-Felix test could not detect any antibodies in 71(27.8%) sera and these samples were of those patients who were clinically suspected to have rickettsial infection.

We note from our data that spotted fevers (34.50%) spread by tick bites and the scrub typhus (25.30%) are the commonest rickettsioses in this area. Typhus fevers are also present here (17.25%). In contrast, a study from a nearby geographical area detected scrub typhus (50%), followed by spotted fevers (7.14%) and typhus fevers (7.14%).[14]

It is apparent from the tables, that the males of younger age group are more commonly affected. We think this might be a fallacy and there might be a cultural or other angle to it. The younger males are brought to the hospitals by their parents and this gets recorded as data and creates bias in the hospital-based studies such as ours. The girl children, the parents themselves and the senior citizens might be getting treated at their village level itself. The local practitioners in the peripheral areas usually treat their fever patients with antibiotics, possibly leading to improvement in the patients' symptoms or even obtaining a cure. However we need to go out into the community and perform the study to substantiate this hypothesis.

We accept that there are deficiencies in our study: it is a hospital-based study; using a poorly sensitive serological test; the sample size is small and we have been unable to demonstrate rising or falling titers.

Conclusion

Several tests are available to diagnose rickettsioses accurately. But they are not routinely used, possibly due to economic or other reason. Weil Felix test, despite having poor sensitivity remains useful for the time being till the other tests become affordable and commonplace. Using this test, we find that spotted fevers and scrub typhus are the commonest rickettsioses in Shimoga, Karnataka state. Typhus fevers are also found to exist here. Further study needs to be done at the community level to assess whether the conclusions drawn from this particular study are valid in the field.

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