Weil Felix test for detection of Rickettsial infections

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Abstract

Introduction: Rickettsial diseases though very commonly found in India, are often misdiagnosed due to lack of awareness and non-availability of diagnostic facilities.

Aim: The aim of this study was to determine the prevalence of rickettsial infections in patients attending our hospital with complaints of fever.

Materials and Method: A total of 150 serum samples were tested for rickettsial infections by Weil Felix test and the results were analysed. 25 serum samples from afebrile patients were also tested as controls.

Results: Of the 150 samples, 59 samples showed significant titres, out of which 29 showed significant titres to OX-K, 10 to OX-2, 11 to OX-19 and mixed titres seen in other 09 samples.

Conclusions: Clinical suspicion and routine testing for rickettsial diseases will be useful in patients presenting with fever for prompt diagnosis and management of these cases.

Keywords: Rickettsial diseases, Fever patients, Weil Felix test

Introduction

The human pathogens in the family Rickettsiaceae are small bacteria of the genera rickettsia, orientia, coxiella, and ehrlichia. They are obligate intracellular parasites and, except for Q fever, are transmitted to humans by arthropods. They do not stain well with Gram's stain but are readily visible under the light microscope when stained with Giemsa, Gimenez, acridine orange, or other stains.(1) Many rickettsiae are transmitted transovarially in the arthropod, which serves as both vector and reservoir. Rickettsiae are pleomorphic coccobacilli, appearing either as short rods (0.3 x 1–2 m) or as cocci (0.3 m in diameter). Rickettsiae, except for *C burnetii*, multiply in endothelial cells of small blood vessels and produce vasculitis. The cells become swollen and necrotic; there is thrombosis of the vessel, leading to rupture and necrosis. Vascular lesions are prominent in the skin, but vasculitis occurs in many organs and appears to be the basis of hemostatic disturbances. Disseminated intravascular coagulation and vascular occlusion may develop. In the brain, aggregations of lymphocytes, polymorphonuclear leukocytes, and macrophages are associated with the blood vessels of the gray matter; these are called typhus nodules. The heart shows similar lesions of the small blood vessels. Other organs may also be involved. The clinical manifestations of all the acute presentations are similar during the first 5 days: fever, headache, with or without nausea, vomiting, and cough. As the course progresses, clinical manifestations—including occurrence of a macular, maculopapular, or vesicular rash; eschar; pneumonitis; and meningoencephalitis—vary from one disease to another.(2) The vector responsible for transmission of the disease determines the geographical distribution of the disease. Given the climatic conditions and the geography of our country, these diseases are to be very much seen in India, but the data regarding the prevalence of the disease in various parts of India is very negligible. Rickettsial infections previously not considered very seriously, have now started to be a challenge for physicians. This has happened due to low index of suspicion and the non-availability of diagnostic facilities for confirmation of these cases. Also rickettsial diseases usually present with non-specific symptoms and can often be mis-diagnosed. The presence of characteristic eschar is often taken as a clue towards clinical diagnosis for these cases; unfortunately it is not present in all the cases. Failure to identify these cases at the right time can lead to inappropriate treatment and thus to significant morbidity. Sometimes these cases can also turn out to be fatal.(3) This study was carried out to determine the prevalence of rickettsial infections in patient's attending our hospital with complaints of fever.

Materials and Method

150 patients attending outpatient department from July 2016 to December 2016 at Basaveshwara Medical College and Hospital, Chitradurga with complaints of fever for more than 07 days were included for the study purpose. All the necessary clinical details were collected in a pre-designed and pre-tested proforma. After taking all proper aseptic precautions and informed verbal consent, the patient's blood samples was collected in a BD (Becton Dickinson) vacutainer without any anticoagulant. The serum was separated by centrifugation of the blood sample at 1000 rpm for three minutes. The serum samples were tested immediately. Whenever a delay in testing was

anticipated, the samples were stored at -20ºC. Then at the time of testing, samples were brought to room temperature before the test was carried out. Antigens for Weil Felix test were procured from PROGEN, Tulip diagnostics Pvt Ltd. Weil Felix test was done using standard protocol with doubling dilution of 1:20 to 1:160 for initial screening[4] followed by further dilutions (from 1:20 to 1:1280) to achieve end titre. The patient's samples were also tested to rule out other diseases like dengue, malaria, enteric fever and leptospirosis. 10ml of blood from different sites with an interval of 30 minutes duration were collected for blood culture from those who appeared very sick and required admission. Serum from 25 afebrile patients who had given their samples for other investigations in biochemistry laboratory was tested as controls for Weil Felix test.

Ethical considerations: The protocol for this study was approved by the Institutional Ethical Committee (IEC). The approval was on the agreement that patient anonymity must be maintained, good laboratory practice, quality control ensured, and that every finding would be treated with utmost confidentiality and for the purpose of this research only. All work was performed according to the International guidelines for Human Experimentation in Biomedical Research.[5] Approval was obtained from the subjects by taking the informed consent.

Results

A total of 150 patients were included in the study. The age of the patients' ranged from 03 -78 years. Of the 59 cases showing significant titres, 55 (93.2%) were also in the age group of 20 -50 years. (Table 1). Our study included 97 male and 43 female patients. Significant titres were in seen 42 male and 17 female patients (Table 2). In our study, we have taken fever more than 07 days duration as the primary marker of the disease, since eschar and rashes may not be present in many cases. So all the 150 cases had fever included in the study gave history of fever more than 07 days duration. Next most common complaint of the patients in our study was headache 122 cases (81.3%) followed by nausea and vomiting in 116 cases (77.3%). Rashes were seen in 62 (41.3%) of our patients. The various clinical features seen in our patients are shown in Table 3. Literature search revealed no data with respect to baseline titre in rickettsial diseases in this region. However the 25 controls tested in this study showed a titre of less than 1: 40 for all the three antigens. Mittal et al[6] in their study have considered suggestive clinical findings along with titre of 1:80 as significant. Based on both these results, we have taken titre of 1: 80 as significant. Of the 150 samples tested, 59 (39.3%) samples showed a titre of 1:80. Out of these 59 samples, 29 (49.2%) showed significant titres to OX-K suggestive of scrub typhus, 10 (16.9%) to OX-2 suggestive of spotted fever group, 11 (18.6%) to OX-19 suggestive of tick typhus.[7,8] 09 (15.2%) samples showed significant titres to more than one antigen making it difficult to interpret the results (Table 4). Of the 59 samples showing significant titres, 11 (18.6%) also tested positive for dengue serology, 02 (3.38%) to enteric fever, 01(1.69%) to malaria, 02 (3.38%) to leptospirosis and 05 (8.47%) samples were positive for blood culture. The various co-infections seen in rickettsial diseases in shown in Table 5.

Table 1: Age wise distribution of cases and their positivity rate

Distribution (years)	No. of cases	No. showing significant titres
0 – 9 yrs	02	00
10 – 19 yrs	06	01
20 – 29 yrs	39	26
30 – 39 yrs	34	18
40 – 49 yrs	41	11
50 – 59 yrs	16	03
60 yrs and above	12	00
Total	150	59

Table 2: Clinical features of the patients included in the study

Clinical features	No. of Patients
Fever	150
Headache	122
Rashes	62
Myalgia	30
Nausea/vomiting	116
Hepatomegaly	72
Splenomegaly	52

Table 3: Weil Felix tests results

Antigen	No. showing significant titre
OX-2	10
OX-19	11
0X-K	29
OX-2 with OX-19	05
OX-K with OX-19	01
OX-K with OX-2	03

Table 4: Showing co-infections in rickettsial diseases

Significant titre to (n)	Tested positive for				
	Dengue	**Enteric fever**	**Malaria**	**Leptospirosis**	**Blood culture**
OX-K (29)	06	00	01	02	03
OX-2 (10)	01	00	00	00	01
OX-19 (11)	02	01	00	00	01
Mixed titres	02	01	00	00	01

Discussion

Weil Felix is a heterophile agglutination test where we use antibodies to various proteus antigens which have cross reactivity to certain epitopes of rickettsial antigens. The main drawback of this test is it lacks sensitivity and specificity. Better investigations like immunofluorescence, western blot and polymerase chain reaction for rickettsial infections have been developed, but they are neither cost effective nor widely available. Especially, in a place like ours where the exact prevalence of disease is not known and also the patient affordability in not high, Weil Felix is the only test that can serve as an initial investigation for these cases and guide the clinician for starting appropriate treatment. Ours is the first study of its kind in this region. The age group between 20-50 years and males were found to be susceptible to have rickettsial infections. Kamarasu et al[9] in their meta-analysis of from Tamil Nadu and Udayan et al[10] from coastal Karnataka also had similar findings in their studies. It may be because rickettsial infections being arthropod borne and males in the 20 -50 year age group are more prone to go out for outdoor activities are exposed to the transmitting vectors. The most common presenting symptom apart from fever in our study was found to be headache 122 cases (81.3%) followed by nausea and vomiting in 116 cases (77.3%). Kamarasu et al[9] had reported to have headache (93.8%) and myalgia (71.9%) in their cases. Out of the 59 samples showing significant titres, 29 (49.2%) were positive for OX-K, 10 (16.9%) for OX-2 and 11 (18.6%) to OX-19. Our study results are in comparison with a study by Mittal et al[5] from Delhi which showed 42.6% positive for OX-K, 39.3% for OX-2, and 8.1% for OX-9 with a titre of 1:80 taken as significant. A study by Udayan et al[9] showed higher positivity rate (88.2%) to OX-2 compared to OX-K (21.5%) and OX-19 (19.6%) where a titre of 1:160 was considered to be significant. The findings in our study has encouraged us to include Weil Felix test in routine evaluation of any case presenting with fever of more than 07 days duration and to go for scrub typhus detection by ELISA method. 11(18.6%) out of these 59 cases were co-infected with dengue virus. Malaria, Enteric fever, Leptospirosis and malaria co-infection was seen 01, 02 and 02 cases respectively. 16 out of these 59 cases required admission and blood culture was done in those cases of which 05 yielded Gram negative bacteria. Udayan et al[10] in their study had 05 and 02 cases co-infected with enteric fever and dengue virus respectively. Borkakoty et al[11] et al from Arunachal Pradesh had reported to have Leptospira co-infection in 08 (25.8%) out the 30 cases of positive for scrub typhus and none had co-infection with malaria. In our study we had 02 (6.89%) of our cases positive for scrub typhus had leptospira co-infection and 01 (3.44%) had malaria co-infection. These finding puts more emphasis on looking for co-infections in fever cases rather than being satisfied with a finding a single aetiogical agent for fever.

Limitations of the study

It would have been much better, had we compared our positive results with ELISA, immunofluorescence or other confirmatory tests in reference laboratories.

Conclusions

We need to keep in mind the possibility of rickettsial infections in patients presenting with fever of more than 07 days duration. It's high time now that all microbiology laboratories start Weil Felix test as an initial diagnostic test for detection of rickettsial infections. Further studies comparing the results of Weil Felix test with other specific tests are required for proper diagnosis of these cases. Also further studies to determine the significant titre in that particular area using controls with serum samples from afebrile patients would further help in better interpretation of the results of Weil Felix test. Also we need keep the possibilities of co-infections while evaluating cases of fever with more a week duration.

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