Original Article

Relevance of Weil-Felix Test in Diagnosis of Scrub Typhus in India

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Abstract

Objective : To study the relationship of Weil-Felix test and microimmunofluorescence test.

Methods : Sera of 21 patients with clinical diagnosis of scrub typhus were subjected to Weil-Felix and Microimmunofluorescence tests.

Results : On Weil-Felix test, 13 (62%) sera showed titers 1: ≥ 40-320. 7 patients showed titers 1 :≥ 320, 3 showed titers 1 : 160, 2 showed titers 1 : 80 and 1 patients showed titers 1 : 40, to *Proteus* OXK antigen. All 21 sera showed significant titers to *O. tsutsugamushi* on microimmunofluorescence.

Conclusion : Weil-Felix test is not a very sensitive test in diagnosis of scrub typhus but due to of lack of availability of definitive tests in India it can be a useful tool when used and interpreted in the correct clinical context. ©

Introduction

Scrub typhus, a rickettsial disease caused by *Orientia tsutsugamushi*, is spread by bite of larval trombiculid mites. The infection manifests as nonspecific febrile illness, which is sometimes accompanied by gastrointestinal, respiratory, or central nervous system symptoms. Illness can be inapparent or severe and death is reported to occur in 1% to 30% of untreated cases. Scrub typhus is endemic in the tropical and subtropical regions of the Asian continent.[1] The presence of rickettsial diseases in India have been has been documented in Jammu and Kashmir, Himachal Pradesh, Uttaranchal, Rajasthan, Assam, West Bengal, Maharashtra, Kerala and Tamilnadu. Small mammals trapped in a feral biotope of a public park area of south Delhi revealed presence of the known scrub typhus vector Leptotrombidium (L.) deliense, the suspected vector Gahrliepia (S.) ligula and Gahrliepia (Walchia) sp. of chigger mites, on them. Recently the disease has reemerged in many areas of India including our State.[2-8]

Diagnosis and surveillance of this disease can be challenging, particularly in the absence of advanced laboratory diagnostic techniques Rickettsial diseases may pose a serious public health problem when not diagnosed or misdiagnosed. Although rickettsiae can be isolated from or detected in clinical specimens, serological tests still remain the main tool for the diagnosis. Microimmunofluorescence, latex agglutination, indirect hemagglutination, immunoperoxidase assay, and enzyme-linked immunosorbent assay are various serological tests available.[9] Immunofluorescence assay (IFA) is the "gold standard" technique and is used as a reference technique in most laboratories. The sensitivity and specificity obtained by immunoperoxidase assay for the serodiagnosis of scrub typhus resemble those obtained by IFA. The commercially available dot blot immunoassay for the diagnosis of scrub typhus lacks both sensitivity and, especially, specificity. This test can be considered useful only as a first-line test, as an alternative to the Weil-Felix test, for the rapid diagnosis of acute cases of infection in areas with a high prevalence.[10]

The Weil-Felix (WF) test is based on the detection of antibodies to various *Proteus* species which contain antigens with cross-reacting epitopes to antigens from members of the genus *Rickettsia* with the exception of *R. akari*. Whole cells of *Proteus vulgaris* OX-2 react strongly with sera from persons infected with *SFG rickettsiae* with the exception of those with *Rocky Mountain Spotted Fever (RMSF)*, and whole cells of *P. vulgaris* OX-19 react with sera from persons infected with typhus group rickettsiae as well as with *RMSF*. Subsequently, the OX-K strain of *Proteus mirabilis* was demonstrated to agglutinate with sera from scrub typhus patients and was further used in the diagnosis of *O. tsutsugamushi*-related infections. The criterion for a positive result is either one determination of a titer of 1:320 or greater or a four fold rise in titer starting from 1:50. By the WF test, agglutinating

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antibodies are detectable after 5 to 10 days following the onset of symptoms, with the antibodies detected being mainly of the immunoglobulin M (IgM) type. However, the WF test may be positive without rising IgM antibody titers. The poor sensitivity of the WF test are now well demonstrated but a good correlation between the results of the WF test and detection of IgM antibodies by an immunofluorescence assay (IFA) is often observed.[10]

Material And Methods

The Weil-Felix *Proteus* agglutination assay *(P. vulgaris OX-19, OX-2, OX-K* strain agglutination), (Wellcome Diagnostics, Dartford, England) was performed on each sample according to the manufacturer's instructions by diluting each serum 1/20 to 1/640. A single Weil-Felix titer 1: ≥ 320 or rise of four fold or more in titers on repeat testing starting from 1:40, were accepted as positive results. The serum specimens were tested by Microimmunoflorescense assay (MIF) using a panel of eleven rickettsial antigens, including *Spotted Fever Group (SFG)* rickettsiae (*R. japonica, R. helvetica, R. slovaca, R. conorii* subsp.*indica, R. honeï, R. heilongjangensis, and R. felis), R.typhi* and *O. tsutsugamushi* (*Gilliam, Kato and Kawasaki strains*). An IFA test was considered positive if antibody titers were at a cut off of 1/128 for IgG and 1/64 for IgM.[10] Sera of healthy persons were also subjected to both of these tests as control.

Results

Total 21 patients were studied. Age ranged from 3 years to 60 years (13 males, 8 females). All had fever (100%) ranging from 5-25 days. Chills and rigors (72%), vomiting (43%), headache and myalgias (38%), lymphadenopathy (53%), jaundice (53%), congested eyes (34%), hepato-splenomegaly (43%), pain abdomen (29%), altered sensorium (24%), seizures (19%), abnormal bleeding (14%), rash (10%) and eschar (10%) were main clinical features present. Investigations like Widal agglutination test, blood and urine culture, x-ray chest and peripheral smear could not reveal the cause of fever. On Weil-Felix test, 13 (62%) sera showed titers 1: ≥ 40-320. On WF test, 7(33%) patients showed titers 1 :≥ 320, 3 showed titers 1: 160, 2 showed titers 1: 80, and 1 patient showed titers 1: 40, to *Proteus* OXK antigen. On MIF, 21 cases showed significant antibodies titers (IgG / IgM) to *O.tsutsugamushi*, and all were negative to *SFG Rickettsioses.* 57% of patients were suffering from scrub typhus if titers of 1:80 were taken as minimum positive titers for diagnosis. Details of titers on WF test and their relationship to MIF tests and duration of symptoms are shown in Table 1. Ten patients in our study did not show titers to IgM antibodies on MIF and 7 of these did not show titers on WF test also. None of sera of control groups for both tests showed false positive reaction. All 21 patients received anti-rickettsial antibiotic (15 doxycyclin / 6 azithromycin). Pediatric patients received tablets and 2 adults with altered sensorium received injection azithromycin. Eight patients, who showed titers 1 :< 40 on WF test, were given ceftriaxone also in addition to doxycyclin. Eighteen (86%) improved and 3 (14%) died. The titers shown on WF test did not correspond with severity of the disease.

Table 1 : Serological titers on weil-felix and microimmunofluorescence

Sr. No.	OX2	OX19	OXK	MIF *O. tsutsugamushi* IgG / IgM	Duration of symptoms in Days
1.	1:<20	1:<20	1:>320	1:512 / 1: 256	5d
2.	1:<20	1:<20	1:>320	1:512 / 1:64	5d
3.	1:<20	1:<20	1:<20	1:512 / 0	7d
4.	1:<20	1:<20	1:>320	1:512 / 1:256	7d
5.	1:<20	1:<20	1:>320	1:128 / 1:256	7d
6.	1:<20	1:<20	1: >320	1:1024 / 0	8d
7.	1:80	1:<20	1:160	1:2048 / 0	8d
8.	1:<20	1:<20	1:160	1:128 / 1:128	8d
9.	1:<20	1:<20	1:>320	1:64 / 1:64	8d
10.	1:<20	1:<20	1:<20	1:256 / 0	10d
11.	1:320	1:80	1:80	1:256 / 1:64	10d
12.	1:<20	1:<20	1:<20	1:512 / 0	10d
13.	1:<20	1:<20	1:320	0 / 1:128	10d
14.	1:<20	1:<20	1: <20	1:512 / 0	12d
15.	1:<20	1:<20	1:<20	1:2048 / 0	12d
16.	1: 40	1:<20	1:>40	1:1024 / 1:128	12d
17.	1:<20	1:<20	1:<20	1:512 / 0	12d
18.	1:<20	1:<20	1:<20	1:2048 / 1:1024	14d
19.	1:<20	1:<20	1:80	1:1024 / 1:256	15d
20.	1:<20	1:<20	1:<20	1:2048 / 0	18d
21.	1:<20	1:<20	1:160	1:2048 / 0	25d

Discussion

Amano *et al*[11] tested sera from 17 patients of scrub typhus in the acute and convalescent phases by indirect immunoperoxidase test, WF test, enzyme-linked immunosorbent assay (ELISA), and immunoblotting. In the comparison of antibody titers between acute- phase and convalescent-phase sera, a parallelism of increment was noted between the titers in WF test and titers of immunoglobulin M (IgM) in ELISA against *Proteus mirabilis* strain OXK-whole cells and OXK-lipopolysaccharides (*Proteus* OXK-LPS). Furthermore, IgM antibodies from almost all of WF test-positive sera recognized LPS from *Proteus* OXK in immunoblotting. Based on these results, it was concluded that IgM antibody may participate in WF test, and that *Proteus* OXK-LPS may have one of antigenic epitopes common to the components of *R. tsutsugamushi.*[11]

Amano *et al*[12] observed that of the sera which were positive to *Rickettsia tsutsugamushi* by indirect immunoperoxidase test, approximately 80% sera were positive to a Proteus OXK antigen by WF test at 10 or more days after the onset of fever. In ELISA using the OXK antigen, almost all of the paired sera of tsutsugamushi disease patients increased on the IgM

antibody titers with the rise of their titers by WF test, whereas the IgG antibody titers of these sera were unrelated with the titers of WF test.[12] In cases of acute infections caused by *SFG rickettsiae* or primary infection with *O. tsutsugamushi*, a significant antibody titer is observed at the end of the first week, concomitant with the detection of IgM antibodies, whereas IgG antibodies appear at the end of the second week. In the case of reinfection with *O. tsutsugamushi*, IgG antibodies are detectable by day 6, with IgM antibody titers being variable.[10] This can explain the absence of IgM antibodies in 10 sera on MIF and 7 (70%) of these sera did not show significant titers on WF test thus highlighting involvement of IgM in WF test. On WF test, the highest titers to OXK (1: > 320) were reached by 8 days and titers of IgG had risen after 14 days of onset of symptoms, on MIF. However it very difficult to determine the exact day of infection especially when the disease has an incubation period of 6 to 21 days and the infection manifests as a lengthy (5–36 days) disease with nonspecific symptoms.[13] The problem is further complicated by the fact that all patients in this study belonged to rural background and majority with low literacy rate. In this study, only single WF test has been done at admission of patient to hospital, 8 (38%) patients did not show significant titers and repeat testing subsequently might have shown significant titers even in these patients also. This could be a limitation in our study, but it is very difficult to call back a patient for follow up in a hilly area like ours where patients have to travel long distances, many times on foot, to reach hospital. Some of patients in our study might have suffered from reinfection with variable response of IgM leading to a problem in interpretation of results of serological tests, in relation to duration of symptoms. It is known that WF test results may be negative during the early stages of the disease because agglutinating antibodies are detectable only during the second week of illness moreover treatment in early stages of the disease may blunt or delay the serological response.[14]

At present, scrub typhus is rarely diagnosed because of its nonspecific clinical presentation, because of a low index of suspicion and the lack of diagnostic facilities in India. In a study conducted in south India,[15] the sensitivity for OX-K was 30% at a titer breakpoint of 1:80, but the specificity and positive predictive value were 100%. At a breakpoint of 1:20, the sensitivity was 61%, the specificity was 94%, and positive predictive value was 84%. At a breakpoint of 1:40, the sensitivity was 49%, the specificity was 96%, and positive predictive value was 88%. Accurate and early diagnosis of scrub typhus remains a challenge in India because of its nonspecific presentation and the paucity of confirmatory diagnostic resources. In our study also 57% of patients showed titers 1: $\geq$ 80 on WF test. However, as per *Isaac*[15] *et al* specificity of this test was high even at lower titers, so patients with low titers should also be evaluated for scrub typhus. The disease remains largely under-recognized and a lot of wasted resources may be directed towards expensive PUO work-up if this condition is not considered. However, the availability and the cost of other serological methods are major problems in India and because of current circumstances, it is suggested that the diagnosis of scrub typhus should be largely based on a high index of suspicion and careful clinical, laboratory, and epidemiological evaluation. Use of empiric treatment should also be considered to reduce the high mortality observed with the disease. Introduction of improved diagnostic methods would allow greater appreciation for the prevalence of the disease.

Though Weil-Felix agglutination test is not a very sensitive test but when positive, it is rather specific test.[14] The use of WF test is acceptable in conditions where definitive investigations are not possible and it is still not entirely obsolete but has to be interpreted in the correct clinical context.

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