Original Research Article

Serological evidence of rickettsial infection by Weil-Felix test in tertiary care hospital in Bangladesh

Abstract

Introduction: Rickettsial infections are re-emerging diseases and are major causes of febrile illnesses throughout the Asia-Pacific region. It is difficult to diagnose due to the non-specific clinical manifestations, absence of reliable and affordable diagnostic tests threrby contributes to increasing the acute febrile burden and preventive illness in many populations. Undiagnosed or late-diagnosed cases are associated with high morbidity and mortality. **Objectives:** The study aimed to determine rickettsial disease by Weil-Felix test and to know the frequency of rickettsial diseases in febrile patients presenting to tertiary care hospitals in Dhaka, Bangladesh. **Methods:** In this study, a total of 135 peripheral blood samples were taken and tested by Weil Felix test from clinically suspected patients of rickettsial fever. **Results:** Weil- Felix test was positive in 33((24.4%) cases. Of Weil- Felix test-positive cases, OX-2 was positive in 87.87% cases, followed by OX-K (6.06%), OX-19 (3.03%), and both OX-2 & OX-K (3.03%) cases. OX-2 positive cases are suggestive of spotted fever group, OX-K of scrub typhus group, OX-19 of typhus group, and OX-2 & OX-K are suggestive of both spotted fever group and scrub typhus group. This finding suggests that most cases were infected with spotted fever group rickettsiae (SFGR). C**onclusion:** Analyzing the present study's findings, it may be concluded that rickettsial infection is not uncommon in Bangladesh. Weil Felix test can be used in laboratories to diagnose rickettsial diseases where specific reliable serological or molecular test is not available.

Keywords: Rickettsial disease, Weil-Felix test. Febrile illness, Spotted fever group, Typhus fever group, Scrub typhus

1. INTRODUCTION

Rickettsial infections are re-emerging arthropod born worldwide zoonosis caused by the genus Rickettsia in the order Rickettsiales. Rickettsia is small obligate intracellular Gram-negative bacteria that reside in the host cell cytoplasm, transmitted by arthropod vectors such as ticks, fleas, mites, and lice [1] and responsible for spotted fever and typhus fever, having a predilection for vascular endothelium. The family Rickettsiaceae comprises two genera Rickettsia and Orientia[2]. A modern classification based on whole-genome analysis divides the species of the genus Rickettsia into four groups: spotted fever group (*R. rickettsii*, *R. conorii*, *R. parkeri*, and several others,) typhus group (*R. prowazekii and R. typhi*), ancestral group (*R. bellii and R. canadensis*, not known to be pathogenic), and transitional group (*R. akari, R. australis, and R. felis*) [3]. Throughout the Asia-Pacific region significant causes of febrile illnesses are Rickettsia. [4]. Rickettsial infections are re-emerging in the Indian subcontinent [5]. Acute rickettsial infection generally presents with non-specific flu-like symptoms [6].

Significant symptoms and signs in rickettsial infections include fever, rash, and headache, myalgia, and inoculation eschar, localized lymphadenopathy [7]. More characteristic symptoms are a rash and an inoculation eschar but are not always observed [8]. Various complications are associated with rickettsial illness, such as neurological (encephalopathy 15%, meningitis 5%, meningoencephalitis 5%, encephalitis 3 %), pneumonia (21%), gangrene (11%), renal failure (7%), shock, myocarditis, gastrointestinal hemorrhage, DIC, ARDS (5% each) due to underdiagnosis or late diagnosis [9]. ~~Dependiding~~ Depending on the causative agent

and the host, severity of these diseases varies. Some Rickettsia species such as *R. rickettsii and R. prowazekii*, and *O. tsutsugamushi* frequently cause more severe forms of the diseases [7]. Due to the non-specific clinical manifestations of rickettsial disease, the diagnosis is often missed, delaying treatment, and consequently may progress into severe forms [6].

Several methods for diagnosing rickettsial disease are available, including serology, molecular techniques, or culture. Serological tests are the most commonly used diagnostic approach for rickettsial disease worldwide [8,10]. Serological methods include the Weil-Felix assay, Immunofluorescence assays (IFA), indirect immunoperoxidase (IIP) assay, Enzyme-linked immunosorbent assay (ELISA), and Western blot analysis. Immunofluorescence assay (IFA) is the "gold standard" technique and is used as a reference technique in most laboratories [11]. Weil-Felix assay is the most easily used, cheap, and widely available serological method to test for rickettsial infection. This conventional agglutination assay is based on the cross-reactivity of rickettsial species with antigens in strains of *Proteus spp* [12]. Antibody reaction to ~~0X-19~~ OX-19 identifies typhus group (TG) rickettsiae (*R. prowazekii* and *R. typhi*) and *R. rickettsii*, whereas reaction to ~~0X-2~~ OX-2 identifies spotted fever group (SFG) rickettsiae and reaction to OX-K identifies *Orientia tsutsugamushi.* It is simple to carry out and continues to be used in many developing countries as a first-line diagnostic test [6,11]. The study aimed to identify the frequency of Rickettsial infection among the study population using the Weil-Felix test and analyze their demographic profiles.

2. MATERIALS AND METHODS

2.1. Patient selection and test strategy. We conducted a cross-sectional study among 135 clinically suspected patients of rickettsial infection with inclusion criterias (fever ≥10 days along with one or more of the following: rash, eschar, headache, myalgia, and patient with fever using doxycycline, tetracycline, chloramphenicol, azithromycin, clarithromycin, and fluoroquinolones. Febrile patients diagnosed as other than rickettsial disease were excluded**).** A total of 135 peripheral blood samples were collected with proper aseptic precautions from patients attending the department

of Internal Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU) and Dhaka Medical College Hospital DMCH from March 2014 to February 2015. Sera were obtained for performing the Weil-Felix test. Laboratory works were performed in the Department of Microbiology and Immunology, BSMMU, Dhaka, Bangladesh. The study was approved by the institutional review board (IRB) of BSMMU. Informed written consent was taken from the patients for research and publication.

2.2. Weil-Felix tests were performed for agglutination assay to see the anti rickettsial IgM antibody, which cross-reacts with the *Proteus vulgaris* OX-19 and OX-2 and *P. mirabilis* OX-K strains (PROGEN, Proteus antigen suspension for Weil-Felix test, 058-83/1110/AE/VER-01, Tulip Diagnostic (P) Ltd.Goa-403 722, India). According to standard protocol, the test was performed on each (135) sample by qualitative slide agglutination, and quantitative tube agglutination test with a double dilution of 1:20-1:160 for initial screening followed by further dilutions achieve end titer according to manufracture's instruction. A single Weil-felix titer 1:80 was considered as a positive result.

3. RESULTS

Out of 135 patients, Weil- Felix test was positive in 33 ((24.4%) cases and negative in102(75.56%) cases (Figure-I). Among 33 positive cases, 7 cases were positive at titre 1:80 ,24 cases were positve at titre 1:160 ,1 case positive at titre 1:320 and 1 case positive titre at 1:640. The prevalence of antibodies to spotted fever group was highest 29(87.87%), followed by scrub typhus 02(6.06%) and typhus fever group 01(3.03%), respectively. more than one rickettsiosis was seropositive in 01(3.03%) cases (Table-1). Among 33 Weil-Felix positive cases, 22(66.67%) were males, and 11 (33.33 %) were females, and the age of the patients ranged from 0-75 years, with 42 (42 %) of patients falling in the age group of 16-30 years followed by 36.37% (31-45 years), 9.09%(0-15 and 46-60 years) and 03-03%(61-75 years) (Figure-II). Table 2 explains the relationship between clinical presentation and Weil-Felix test positivity. Fever was present with long duration (>10 days) in all Rickettsia positive 33(100%) cases followed by headache 24(72.72%), bodyache 25(75.76%), rash 10(30.30%), cough 04

(12.12%) cases, nausea 03(9.09%) cases, and no patient was present with eshcar or history of the tick bite.

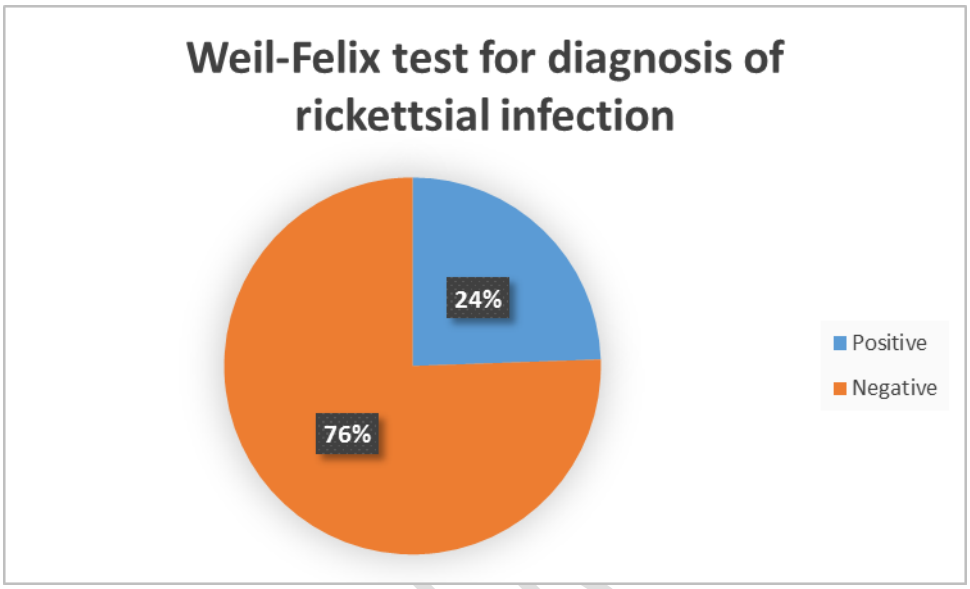

Figure I: Results of Weil-Felix test for diagnosis of rickettsial infection in study population (n=135)

Table- 1: Distribution of Weil-Felix antigens positivity with titer (n=33)					**Total (n=33)** **No %**
Antigen suspension	**Titer**				
	1:80	**1:160**	**1:320**	**1:640**	
OX 2	**06**	**21**	**01**	**01**	**29** (87.87%)
0XK	**01**	**01**	**00**	**00**	02 (6.06%)

OX 19	**00**	**01**	**00**	**00**	**01 (3.03%)**
OX2 +OX 19	**00**	**01**	**00**	**00**	**01 (3.03%)**

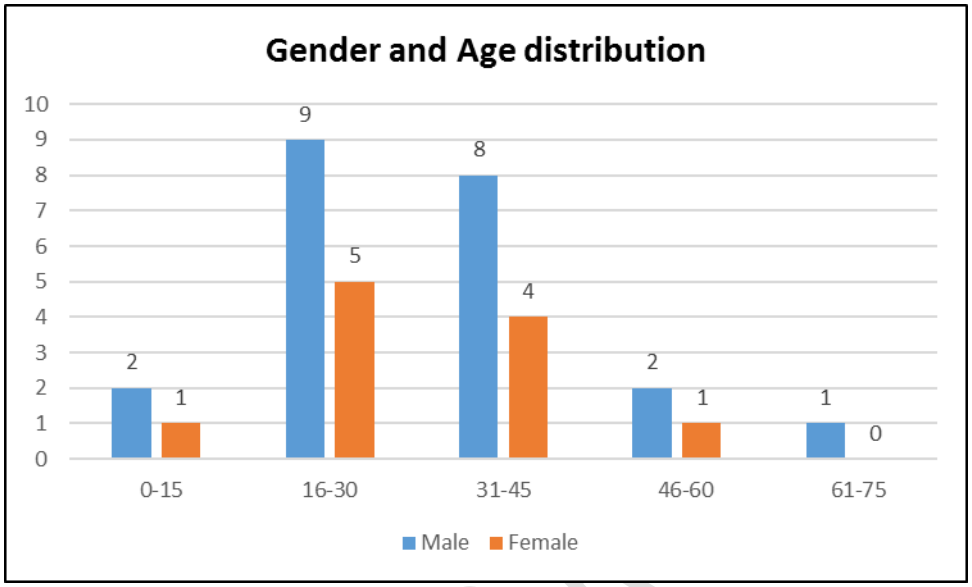

Figure II: Gender and Age distribution of Weil- Felix positive cases (n= 33)

Table-2: Clinical features of Weil-Felix positive cases (n= 33)

Clinical features	No.	%
Fever	33	100
Headache	24	72.72.
Bodyache	25	75,76
Cough	04	12.12
Rash	10	30,30
Nausea	3	09.09

Inoculation eschar	00	00
Tick bite	00	00

Note: The total number and percentage exceeded the positive cases due to the presence of more than one symptom.

4. DISCUSSION

Due to the non-specific clinical manifestations of rickettsial disease and the absence of available specific laboratory tests, the diagnosis is often missed, delaying treatment with consequent progression into severe forms [6]. Rickettsial infections are the causes of significant morbidity and mortality in undiagnosed or late diagnosed cases. [13]. In developing countries undiagnosed or late diagnosed cases thus significantly increasing financial burden on many populations [4]. In our region due to lack of advanced laboratory techniques diagnosis of rickettsial diseases is difficult. Biosafety level 3 containment is required for isolation of Rickettsiae from clinical specimens, and clinical management become difficult due to unavailability of the results in time. Hence serology remains the main tool for diagnosis. Among the different specific serological test for diagnosis of Rickettsial infection, gold-standard diagnostic tests like indirect immunofluorescence antibody (IFA) and indirect Immunoperoxidase (IIP) requires highly trained persons to perform and is expensive [14] and not available in our country. The Weil-Felix assay is the most easily used, cheap, and widely available serological method to test for rickettsial infection and continues to be used in many developing countries as a first-line diagnostic test [6,11].

This study was conducted to see the frequency of rickettsial diseases among clinically suspected febrile patients of Rickettsial fever by Weil-Felix test. We used single acute-phase sera from patients with febrile illness attending tertiary level hospitals. In the present study, Weil- Felix test was positive in twenty-four percent of cases. A similar finding was observed in a study in India from 2005 to 2009, which showed seropositivity as thirty-three percent [15]. Another study in south India observed that thirty-six percent of the patients were reactive to the WF test [16].

The reactivity of Weil-Felix test depends only on the titers of the IgM antibody. In secondary rickettsial infection, there is the rapid rise of IgG antibody, and IgM

antibody persist for a short period. So, it may give false-negative results for the Weil-Felix test [17]. Weil-Felix test has low sensitivity as it gives a high percentage of false-negative results, which is common in the case of scrub typhus and shows low specificity as false-positive results in leptospirosis and relapsing fever, *Proteus* infections, brucellosis, and acute febrile illness [18].

Among Weil-Felix positive cases, the distribution of Weil-Felix antigens revealed that most of the cases (87.87%) were positive for OX-2 (suggestive of spotted fever group) followed by OX-K (suggestive of scrub typhus group), OX-19 (suggestive of typhus group), and both OX2 & OXK (suggestive of both spotted fever group and scrub typhus group). This finding suggests that most cases were infected with spotted fever group rickettsiae (SFGR). A similar study was performed in India, where 88.23% were positive for OX- 2, 19.60% were positive for OX- 19, and 21.56% were OX- K positive [19]. In this study, most of the patients were between the age group of 16-60 years. Similar findings have been observed in a study in India which showed the most affected age group (79.3%) was between 11-60 years [15]. There was a male preponderance than female in this study, and the male: female ratio was 2:1. Several studies were conducted which showed similar findings regarding male: female ratio. A similar study in Malaysia reported a male and female ratio was 1:1, and another study in Malaysia showed male and female ratio was 2:1 [20,21]. Findings of the current study reflect that male are more infected than females. Reasons for this difference are not established still now. A study carried out by Walker in USA, mentioned that it might be due to increased exposure to infection through their occupation or pleasure in taking them outdoors in the foothills and mountains [22]. Fever was the most common finding, and all patients had a fever for a long duration (>10 days) in this study. Other associated features were headache followed by bodyache, rash, cough, and nausea. There was no inoculation eschar present. Rash was present only in thirty percent of cases as rash may not appear in all cases, or some patients may have a few cutaneous lesions that are only detectable after a meticulous examination [23]. A study was conducted on Thai-Myanmer border among 7 febrile patients, of which 4 were without any rash or eschar [24].

5. CONCLUSION

The prevalence rates could not be assessed, due to limitation of time, budget and resource. Moreover, all cases with fever occurring in the different area throughout the country could not be tested and large number of samples could not be included in this study.

This study revealed that most of the cases were SFG Rickettsia. However, it is clear that rickettsial diseases are not uncommon in Bangladesh. In conclusion, the Weil-felix test was promising as a screening test for diagnosing rickettsial diseases in correlation with clinical features in a hospital setting where gold standard tests are not available, like Bangladesh. This will guide the clinicians to provide early and precise treatment and help reduce morbidity and mortality due to rickettsial diseases. Further studies by specific test for Rickettsia has to be done to ~~dertermine~~ determine the prevalance of rickettsial infection and to understand epidemiological aspects and strain variability of this re-emerging infection throughout Bangladesh.

ETHICAL STATEMENT: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by institutional review board (IRB). Written informed consent was obtained from the patients for research and publication of this study.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any

litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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