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Original Research Article

Baseline Weil Felix Titre among Healthy Population in Sub Himalayan Region of Kangra (H.P.) India

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ABSTRACT

Context: Rickettsial infections pose a serious threat to public health if not diagnosed properly. They remain under diagnosed due to lack of diagnostic facilities in developing world. Serology remains the mainstay of diagnosis and Weil Felix test has been widely used for presumptive diagnosis of rickettsial diseases. The interpretation of Weil Felix test depends upon baseline titre which is prevalent among the healthy population.
Aims: To determine the baseline titre of Weil Felix Test in healthy population of District Kangra and to define the significant titre for Weil Felix agglutination test for diagnosis of scrub typhus.
Settings and Design: A prospective cross-sectional study was conducted among healthy blood donors over a period of one year. Weil Felix test was performed on the sera obtained from the blood samples collected from the donors. Baseline titre for the Weil Felix test was thus determined.
Results: A total of 1122 blood samples were collected. Baseline titre for the OX2 antigen was ascertained to be 40 and a presumptive diagnostic titre was 80. For the OX19 and OXK antigens, the baseline titre was 80 and a presumptive diagnostic titre was 160.
Conclusions: Our findings show that the results of the Weil Felix test can be interpreted in the Sub Himalayan region with the baseline titre estimated among healthy population.

Key-words: Baseline titre, Rickettsial infections, Sub Himalayan Region, Weil Felix test.

Key Messages:
A baseline titre of 1:40 for OX2 and 1:80 for OXK and OX19 for the Weil Felix test in the Sub-Himalayan region of Himachal Pradesh is proposed.
A titre of 1:160 may be considered as a diagnostic titre for the OX19 and OXK antigens in this region and a titre of 1:80 can be considered as a diagnostic titre for the OX2 antigens.
There seems to be presence of scrub typhus as well as other rickettsial diseases in the region. Further entomological and epidemiological studies are required in the state for confirmation.

INTRODUCTION

Rickettsial infections are caused by a variety of obligate intracellular, gram-negative bacteria from the genera *Rickettsia, Orientia, Ehrlichia, Neorickettsia, Neoehrlichia,* and *Anaplasma,* belonging to the Alphaproteobacteria. [1] Infections caused by Rickettsia are divided into three main groups, the typhus group, the spotted fever group and the scrub typhus group. Typhus fever group includes epidemic typhus, endemic typhus and relapsing typhus or Brill Zinsser Disease. The Spotted fever group includes Rocky Mountain

spotted fever, Mediterranean spotted fever, Siberian tick fever, Indian tick typhus, Rickettsial pox etc. [2] Scrub typhus group includes only scrub typhus, the causative agent for which is *Orientia tsutsugamushi*. [3]

Rickettsial diseases are one of the important causes of fever of unknown origin. They pose a serious threat to public health if not diagnosed properly. Rickettsial infections are prevalent throughout the world except in Antarctica. [4] Mortality due to these infections is reported to occur in 1 to 30 % of untreated cases.

Scrub typhus is a Rickettsiosis caused by *O. tsutsugamushi*. The disease is transmitted by the bite of larval forms of the trombiculid mites. In India, the disease is endemic in the Shivalik range, Eastern and Western Ghats and the Vindhyanchal and Satpura ranges in Central India. [5] The disease is endemic in the state of Himachal Pradesh also, cases occurring every year more frequently in the months of July through November.

Various laboratory tests are available for the diagnosis of rickettsial diseases. Indirect immunoperoxidase assay (IPA) and immunofluorescence assay (IFA) are considered gold standards but are available in laboratories with higher level of facilities and expertise. PCR and ELISA techniques, particularly immunoglobulin M (IgM) capture assays are available at secondary level of health care like District hospitals and medical colleges. Weil-Felix test is helpful in establishing a presumptive diagnosis and can be considered at primary level with moderate level of infrastructure and expertise, in areas affected by scrub typhus. Titre of 1:80 is to be considered possible infection. However, baseline titres need to be standardized for each region. [1]

Diagnosis of scrub typhus presents a diagnostic challenge to both clinicians and laboratorians, especially in the resource constrained state of Himachal Pradesh. Serology remains the mainstay of diagnosis of scrub typhus. Weil Felix test has been widely used for presumptive diagnosis of rickettsial diseases. In endemic areas, healthy people may have antibodies which react up to a variable titre in Weil Felix test due to past exposure to rickettsial agents and cross reacting antigens of non motile strains of proteus species i.e. OX19, OX2 of *Proteus vulgaris* and OXK of *Proteus mirabilis.* Therefore the antibody titre varies from place to place and is referred to as the baseline titre of that area. The interpretation of Weil Felix test depends upon baseline titre which is prevalent among the healthy population in a particular geographical area.

The baseline titre for Weil Felix test has not been determined in this region. [6] Hence this study was conducted to determine the baseline titre of Weil Felix Test in healthy population of District Kangra in special reference to scrub typhus and to define the significant titre for Weil Felix agglutination test for diagnosis of scrub typhus fever in a single serum sample.

MATERIALS AND METHODS

This study was conducted in the Department of Microbiology, Dr. Rajendra Prasad Government Medical College & Hospital Kangra at Tanda (H.P.) over a period of one year from March 2013 to February 2014. A total of 1122 blood samples were collected from adult healthy blood donors from Blood Banks at Dr. Rajendra Prasad Government Medical College Kangra at Tanda, Zonal Hospital Dharamshala and Sub Divisional Hospital Palampur to ascertain the baseline titre of Weil Felix Test. Written informed consent was taken from the study participants before enrolling them in the study.

Weil Felix test was performed on the sera obtained from the blood samples collected as per the standard technique. [7] Antigens Proteus vulgaris OX2, OX19 and Proteus mirabilis OXK were obtained from Central research Institute, Kasauli, Himachal Pradesh, India. The test was carried out using doubling dilution from 1:20 to 1:160 for initial screening. Those showing positive titres of 160 were screened further till end titre dilution. Weil Felix Test

was performed using the microtitre plate agglutination test method.

RESULTS

A total of 1122 blood samples were collected. Out of these, 618, 261 and 243 samples were from blood banks of DRPGMC & Hospital Kangra at Tanda, SDH Palampur and ZH Dharamshala respectively. The mean age of the study participants was 29.53 ± 8 years. Majority of the participants were males in the age group 21 – 40 years. [Table I]

A total of 1122 serum specimens were tested. For OX2 antigen, 100% donors had a titre of ≤ 80 while for OX19 antigen, 99.7% of the donors had a titre of ≤ 80. For the OXK antigen, 98.9% of the donors had a titre of ≤ 80. For the OX2 antigen, more than 95% of the donors had titres of ≤ 40. [Tables II & III] Hence, a baseline titre for the OX2 antigen is taken to be 40 and a presumptive diagnostic titre for the OX2 antigen is 80. Similarly, for the OX19 and OXK antigens, 95% of the donors had titres of ≤ 80. Thus, the baseline titre for the OX19 and OXK antigens is 80 and a presumptive diagnostic titre for these antigens is 160. [Tables II & III]

Table I: Age & Sex distribution of blood donors

Age Group (years)	Male		Female		Total	
	No.	%	No.	%	No.	%
1 -10	0	0	0	0	0	0
11-20	140	13.05	9	18.37	149	13.28
21 – 30	490	45.67	27	55.1	517	46.08
31 – 40	332	30.94	8	16.33	340	30.3
41 – 50	98	9.13	4	8.16	102	9.09
51 – 60	13	1.21	1	2.04	14	1.25
Total	1073	100	49	100	1122	100

Table II: Geographical variation of WFT Agglutinin titres in District Kangra, HP

Blood Bank	Total samples	Antigen	< 1:20(%)	1:20(%)	1:40(%)	1:80(%)	1:160(%)
Tanda	618	OX2	219 (35.4)	259 (41.9)	109(17.6)	31 (5)	0
		OX19	214 (34.6)	190 (30.7)	169 (27.3)	44 (7.1)	1 (0.1)
		OXK	51 (8.2)	165 (26.6)	265 (42.8)	130 (21)	7(1.1)
Palampur	261	OX2	81(31)	118 (45.2)	54(20.6)	8 (3)	0
		OX19	99(37.3)	97 (37.1)	48(18.3)	17 (6.5)	0
		OXK	12(4.5)	48(18.3)	135 (51.7)	63 (24.1)	3 (1.1)
Dharamshala	243	OX2	110 (45.2)	94 (38.6)	32 (13.1)	7 (2.8)	0
		OX19	74(30.4)	73 (30)	70 (28.8)	24(9.8)	2 (0.8)
		OXK	24 (9.8)	60 (24.6)	96 (39.5)	57(23.4)	6 (2.4)

Table III: Baseline titres for Weil Felix test

	< 1:20 (%)	1:20 (%)	1:40 (%)	1:80 (%)	1:160 (%)
OX2	410 (36.54)	471 (41.98)	195 (17.38)	46 (4.1)	0
OX19	387 (34.49)	360 (32.09)	287 (25.58)	85 7.58)	3 (0.27)
OXK	87 (7.75)	273 (24.33)	496 (44.2)	250 (22.28)	16 (1.42)

DISCUSSION

Scrub typhus is transmitted by trombiculid mites and often presents as fever of unknown origin. Clinically, there is little to distinguish it from co-endemic diseases such as typhoid fever, leptospirosis and dengue fever. The mainstay of diagnosis remains serology and a high degree of clinical suspicion. The oldest test in current use is the Weil Felix OXK agglutination test. It is inexpensive, easy to perform and results are available overnight. However, it lacks sensitivity and specificity. [7,8]

Scrub typhus is a re-emerging Disease. IFA is the gold standard for diagnosis of scrub typhus but not easily available and is expensive. In resource constrained areas, Weil Felix test remains the modality of choice for diagnosis. The Weil Felix titres among healthy populations of different areas differ and depend upon the endemicity of scrub typhus in an area. It is mandatory to know and update the baseline Weil Felix titre for proper interpretation of the test. Kangra region (H.P.) in Sub Himalayan region is endemic for scrub typhus and no indigenous titre of Weil Felix Test in this region has been determined. Knowledge of local baseline titre in healthy individuals helps in correct interpretation of this commonly done test. A fourfold rise in titre in paired sera is generally considered diagnostic of scrub typhus. [9] However, such a diagnosis is retrospective and cannot be used as a guide for initial treatment.

Diagnosis is based on a single acute serum sample, using a cut off antibody titre. Cut offs ranging from 1:10 to 1:400 are often quoted with little corroborating evidence and without establishing titres in healthy local populations. [10] A policy document jointly brought out by DHR and ICMR in 2015 also recommended a titre of 1:80 to be considered as possible infection. However, the policy document also recommends standardization of baseline titres for each region. [1]

Veena Mittal et al [11] in a study in Delhi (2004) tested 700 blood samples by Weil Felix test. They reported the baseline titres 40 for the OX2, OX19 and OXK antigens. These results are in accordance with our findings for the OX2 antigen, but discordant in relation to the OX19 and OXK antigens.

In the studies by Mahajan et al [12] in Shimla and M Vivekanandan et al [13] in Pondicherry a titre of > 80 was considered as diagnostic titre for scrub typhus which is in accordance to our study. In another seroprevalence study by K Usha et al [14] in Tirupati, a titre of 40 was considered as baseline titre, which is discordant with our study results.

CONCLUSION

Scrub typhus agglutinins are common among apparently healthy blood donors in Kangra with wide variations in baseline Weil Felix agglutination titres. The OXK antibody titre >1:80 could be diagnostically significant in the presumptive diagnosis of scrub typhus in Kangra region. A titre of 1:40 and 1:80 has been considered as baseline titre for OX2 and OX19 respectively. When ELISA facilities are not available, Weil Felix test can be used provided the results are interpreted with clinical findings and prevailing OXK, OX19 and OX2 agglutination titre in the local population.

We propose a baseline titre of 1:40 for OX2 and 1:80 for OXK and OX19 for the Weil Felix test in this region of Himachal Pradesh. A titre of 1:160 may be considered as a diagnostic titre for the OX19 and OXK antigens in this region. A titre of 1:80 may be considered as a diagnostic titre for the OX2 antigens in this region. Based on our observations there seems to be presence of scrub typhus, as well as other rickettsial diseases. Further entomological and epidemiological studies are required in the state for confirmation.

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