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Original Research Article

Evaluation of two diagnostic methods of scrub typhus with Bayes rule

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ABSTRACT

Background: Scrub typhus or rickettsia, a zoonotic infection occurs due to the transmission of Gram-negative bacteria, *Orientia tsutsugamushi* and/or *Tsutsugamushi* sp. by the larval stage of the trombiculid mite, *Leptotrombidium deliense.* Infections at endothelial cells and phagocytes manifest clinically as vasculitis with an acute onset of fever, rashes, headache, myalgia, multiple organ dysfunction, eschar, respiratory morbidity, meningioencephalitis and sepsis syndrome on skin, whose severity varying with patients. Herein obtained data is used for evaluating the dependability of two determinative tests.
Methods: The prospective survey with 80 rickettsia patients was carried during the last 6 months of 2016. Patients with the occurrence of fever for more than two days and a clinical suspicion of rickettsia infection were promoted for Weil-Felix as well as, ELISA tests.
Results: The prevalence of rickettsia in the population of 80 patients was 17.5, and the sensitivity (the portion of the people with the rickettsia infection who will have positive Weil-Felix test results) value was 0.7143 and specificity (the people without the disease who will have negative Weil-Felix test results) value was 0.9697. Probability of Weil Felix test positive was 0.15, and probability of ELISA test positive was 0.175.
Conclusions: The dependability of each test independently or a posteriori probability was 0.857. Both tests are dependable for correctness of outcomes being 0.857 or 85.7%, in combination; a high specificity value of Weil Felix test is a valuable test.

Keywords: Bayesian analysis, ELISA test, Rickettsia, Scrub typhus, Weil Felix test

INTRODUCTION

As a re-emerging fiendish zoonotic bacterial infection, scrub typhus occurs in the 'tsutsugamushi triangle' at Asian Pacific rim, Northern Australia, South Asia and Southeast Asia.[1] The larval stage (chigger) of the trombiculid mite, *Leptotrombidium deliense* transmits Gram-negative obligate intracellular bacteria, *Orientia tsutsugamushi* and/or *Tsutsugamushi* sp. to humans through biting causing infection at endothelial cells and phagocytes, manifesting as acute vasculitis. Acute onset of fever, rashes, headache, myalgia, multiple organ dysfunction, eschar, respiratory morbidity, meningioencephalitis and sepsis syndrome at any body-site are the clinical manifestations, which can be mild to severe form varying with patients. If left untreated, fatality is seen in 30-50% cases. The main difficulty in the diagnosis and management of rickettsia infection is the lack of facilities for definitive diagnosis.[2] Nowadays, the Weil-Felix test is considered obsolete, but the better diagnostic technique enzyme-linked immunosorbent assay (ELISA) is the indirect fluorescent antibody (IFA)

assay is available at referral centers.3Additional complications, gastrointestinal manifestations, tinnitus and hepatitis syndromes from rickettsial infections have reported from Asian countries.[4] In India, the diagnosis of rickettsia infections is not dependable due to the lack of community-based data and non-availability of impeccable laboratory tests.[5,6] The disease has been documented from states, Jammu and Kashmir, Himachal Pradesh, Uttaranchal, Rajasthan, Assam, West Bengal, Maharashtra, Kerala and Tamil Nadu less recently in India.[7] In strongly suspected scrub typhus cases, while awaiting the report of diagnostic test, tetracycline 100 mg twice per day for 10 day is administered empirically, if there is no evidence of liver failure.

The objective of this study was to identify the clinical and laboratory characteristics in patients suspected of having rickettsia infections, presenting to a tertiary hospital in eastern India; and concomitantly, two diagnostic methods, the Weil-Felix and ELISA tests are considered for digital estimation of fallibility/ reliability in arriving at the correct conclusion at rickettsia infection status. This fixated analysis should help clinicians during circumspection of clinical features of patients suffering from scrub typhus with test reports on how much quantitatively each method is dependable. Bayes rule is used with the accumulated clinical data in resolving dependability of each test method during a correct diagnosis.

What Bayesian analysis can do?

Diagnostic tests are used for revealing the occurrence of randomly distributed scrub typhus such as rickettsia infections. The accuracy of a diagnostic test can be measured by comparing the test results with the true condition of patients individually. Herein, the ambivalence of ELISA test and Weil Felix test results for comparative accuracy could be resolved with the account of data as evidence, by an appropriate statistical analysis involving probability - as how much each test is dependable. A clinician would be always eager to know numerically about the errors of each test, at one's laboratory condition. Obviously, the Weil Felix test is assumed as the gold standard, since it reveals the true condition of a patient. Thus, it is an ideally based truth with which, the second test, being user-friendly and patient-friendly can be compared for digital values as a method.

Thus, the ELISA test is the candidate to be assessed for its sufficiency as a diagnostic test. However, the Weil Felix test also has a degree of unreliability, not having representable or adequate rickettsia from the appropriate serum consequent to improper placement of guided needle tip due to technical error. Thus prudently, the Bayesian analysis based on obtained data as evidence could measure the degree of belief/ assumption, for what percent the Weil Felix test could be taken as gold standard, and concomitantly, how much numerically the ELISA test would be dependable. To evaluate the inherent probability of each, the prior probability (a priori probability or prevalence or the prevalence of rickettsia infection was determined before using data, prevalence= [(TP+FN)/N], with N = number of total number of serum masses. And both tests are independent by themselves, but are critical in determining the status of each test. Furthermore, there are several associated test statistics: the sensitivity (true positive rate) - this is the portion of the people with the rickettsia infection, who will have positive ELISA test results, computed by [TP/(TP+FN)], and the specificity (true negative rate) - this is the portion of the people without the disease, who will have serum in ELISA test results, computed by [TN/(FP+TN)]; these test statistics are bases of the Bayesian analysis.8

Furthermore, the false positive rate - it is the probability of errors of the Weil Felix test, computed by [FP/(FP+TN)], and the false negative rate - it is the probability of errors of the ELISA test, computed by FN/(TP+FN) are important. And the positive predictivity - it is the post-test probability of the disease that gave a positive test result or this is the portion of the people who actually have rickettsia, computed by [TP/(TP+FP)], predicted positivity by the ELISA test; and the negative predictivity - post-test probability of the disease that gave a negative test result or this is the portion of the people negative for serum, computed by [TN/(FN+TN)], predicted serum by the ELISA test. Also, the diagnostic accuracy (inherent validity or predictive validity) - it is the ability of the ELISA test to be correctly positive or negative, computed by [(TP+TN)/N].

Furthermore, the positive likelihood ratio is the ratio between TP rate and FP rate, computed by [sensitivity/(1-specificity)], when the ELISA test result is positive; and the negative likelihood ratio is the ratio between FN rate and TN rate, computed by [(1-sensitivity)/specificity], when the ELISA test result is benign. Admittedly, larger the positive likelihood ratio value, the greater is the likelihood of serum sample, and similarly, the smaller the negative likelihood ratio value, the lesser is the likelihood of serum, in a population. And a posteriori probability is the value from post-test arithmetic computation of the data for diagnostic efficiency, and it specifically analyses how much (numerically) good/dependable the test is, independently in arriving at the truth - the coveted conclusions from these tests on individual patients.

METHODS

The present survey work on rickettsia was carried out in during the 6-month period from July 2016 to December 2016. As a prospective study, patients with the occurrence of fever of more than two days and a clinical suspicion of rickettsial infection were promoted for ELISA test and Weil-Felix test. The case probability included patients with high intermittent fever and having at least five out of the following clinical features present headache, myalgia, regional lymphadenopathy,

generalized lymphadenopathy, hepatomegaly, splenomegaly, presence of an eschar, or presence of a maculopapular rash or any case of fever which could not be diagnosed within 2 days of basic work up in hospital. Demographic details of each patient, the clinical course of the illness, and complications of infection were recorded.

Basic hematological and biochemical tests and relevant imaging were carried out. More specific tests were done when indicated, especially when there were complications such as myocarditis and encephalitis. The confirmatory tests to identify the causative agent were performed on 2 ml of serum. These serum samples were stored at -20 ^{0}C until those were analyzed at the viral and rickettsial zoonoses branch for IgM by ELISA. Haemogram, liver function tests and renal function tests were done in all patients. Serum was collected for Weil Felix Test according to the standard procedures. The scrub typhus detecting instrument is manufactured in USA, Chemwell, model no. 2910, coy: Euroimmune.

RESULTS

The age of the total 80 patients presented with probable rickettsia ranged from 21 to 60 years with a female-male, ratio of 45-55% (Table 1).

Of the total 80 (100%) cases, 68 (85 %) positive cases were ELISA test and Weil Felix test detected in 12 (15 %) cases. Out of 68 (100%), initial presentation such as fever 58 (85), headache 42 (62.5), cough 36 (52.5), abdominal pain 21 (31), eschar 44 (55), skin rashes 20 (25.7), lymphadenopathy 24 (30.1), leukocytosis 24 (30.1), impaired liver function test 18 (25.8), hepatosplenomegaly 24 (34.7) were detected in ELISA test. However, in Weil Felix test 12 (100%) positive, fever 8 (65), headache 7 (55.5), cough 5 (45.5), abdominal pain 3 (25.8), eschar 6 (53.5), skin rashes 2 (20.3), lymphadenopathy 3 (25.7), leukocytosis 3 (25.7), impaired liver function test 1 (13.6) and hepatosplenomegaly 3 (25.7). Out of 68 (100%), complication such as meningitis/ meningoencephalitis 8 (11.8), carditis 7 (11), acute respiratory failure 4 (6.6), septic shock 7 (11), jaundice 5 (7.4), peptic ulcer 6 (9.6), acute pancreatitis 8 (11.8) and thrombocytopenia 8 (11.8) were recorded in ELISA test. While in Weil Felix test 12 (100%) meningitis/ meningoencephalitis 1 (0.8), congestive heart failure 4 (3.2), acute respiratory failure 1 (0.8), septic shock 4 (3.2), jaundice 1 (0.8), peptic ulcer 2 (1.6), acute pancreatitis 5 (4) and thrombocytoperia 4 (3.2) were recorded (Table 2).

The sensitivity value is the ability of the Weil Felix test to detect the rickettsia status, when it is truly present, i.e., it is the probability of a 'positive test result'. On the other hand, the specificity value is the ability of the Weil Felix test to give the negative result with rickettsia free individuals, i.e., it is the probability of a 'negative test result'. Additionally, the diagnostic accuracy value estimates accuracy of both Weil Felix test and ELISA tests together. And applying the Bayesian concept with these recorded data (Table 3), a bandwagon of other test statistics could be computed for additional probability values, along with their corresponding 95 % confidence interval (CI) values (Table 4).

Table 1: Age and sex distribution of cases of scrub typhus.

Sex	21-30 years	31-40 years	41-50 years	51-60 years	Total
Male	08	11	13	12	44 (55)
Female	06	09	10	11	36 (45)
Total	14 (17.5)	20 (25)	23 (28.75)	23 (28.75)	80 (100)

Note: y= years; Numbers in parenthesis are percent values.

Table 2: Comparison of characteristics in 80 scrub typhus patients with rickettsia positive findings in ELISA and Weil Felix test.

Characteristics	ELISA positive (N=68)	Weil Felix test positive (N=12)
Initial presentation		
Fever	58 (85)	8 (65)
Headache	42 (62.5)	7 (55)
Cough	36 (52.3)	5 (45)
Abdominal pain	21 (31)	3 (25.8)
Eschar	44 (55.6)	6 (52.3)
Skin rashes	20 (25.7)	2 (20.3)
Lymphadenopathy	24 (30.1)	3 (25.8)
Leukocytosis	24 (30.1)	3 (25.8)
Impaired liver function test	18 (25.8)	1 (13.6)
Hepatosplenomegaly	24 (34.7)	3 (25.8)
Complication		
Meningitis/meningoencephalitis	8 (11.8)	1 (0.8)
Congestive cardiatis	7 (11)	4 (3.2)
Acute respiratory failure	4 (6.6)	1(0.8)
Septic shock	7 (11)	4 (3.2)
Jaundice	5 (7.4)	1(0.8)
Peptic ulcer	6 (9.6)	2 (1.6)
Acute pancreatitis	8 (11.8)	5 (4)
Thrombocytopenia	8 (11.8)	4 (3.2)

Table 3: The generic 2x2 table with number of cases assigned to, based on ELISA test and Weil.

Weil Felix test	ELISA test		Total
	Positive	Negative	
Positive	TP = 10 (0.125)	FP = 02 (0.025)	(TP+FP) = 12 (0.15)
Negative	FN = 04 (0.05)	TN = 64 (0.8)	(FN+TN) = 68 (0.85)
Total	(TP+FN) = 14(0.175)	(FP+TN) = 66 (0.825)	N = 80 (1.0)

TP=10 cases were true-positives (ELISA test-positive, Weil Felix test - positive); FP=02 cases were false-positives (Weil Felix test-positive, ELISA test- negative); FN=04 cases were false-negatives (ELISA test- positive, Weil Felix test-- negative); and TN=64 samples were true-negatives (ELISA test- negative, Weil Felix test- negative); N=population size or total number of cases. Corresponding fraction values are given in parentheses. Prevalence of rickettsia in the population= 0.175. FP cases are the type I errors, while FN cases are the type II errors.

Table 4: Computed probability values of different Bayesian test statistics of diagnosis of rickettsia.

Test statistic	Formula	Value	95% CI
Prevalence or a priori probability	(TP+FN)/N	0.1750	0.7560-1.1390
Sensitivity (true positive rate)	TP/(TP+FN)	0.7143	0.8388-1.0612
Specificity (true negative rate)	TN/(FP+TN)	0.9697	0.9139-1.9860
Diagnostic accuracy	(TP+TN)/N*	0.9250	0.8930-1.0010
Positive predictivity	TP/(TP+FP)	0.8333	0.8651-1.0349
Negative predictivity	TN/(FN+TN)	0.9412	0.8996-1.0040
False positive rate	FP/(FP+TN) = (1- specificity)	0.0303	0.7451-1.1549
False negative rate	FN/(TP+FN) = (1-sensitivity)	0.2857	0.7741-1.1259
Positive likelihood ratio	sensitivity/ (1-specificity)	2.3573	0.7090-1.1910
Negative likelihood ratio	(1sensitivity) / specificity	0.2946	0.7752-1.1248
A posteriori probability	$P(E_1\|E)$	0.5647	0.8127-1.0873

For abbreviations see Table 3; CI=confidence interval. For the detailed formula of a posteriori probability see text;* alternately = (sensitivity) (prevalence) + (specificity) (1 - prevalence).

A posteriori probability

A posteriori probability or 'P (E1|E)', the probability value of a rickettsia truly positive, could be calculated by the Bayesian formula,

P(E1|E) = P(E1)×P(E|E1)/[P(E1)×P(E|E1)+P(E'1)× P(E|E'1)], where,

E is the event that the ELISA test result is rickettsia; E1 is the event that the same case has the Weil Felix test result positive; E'1 is the partition of the space for all cases from healthy (without any positive in the probability rickettsia) and it is a recorded value.

Here several probability values are,

P (E) = probability of Weil Felix test positive=0.15 from Table 3;

P (E1) =probability of ELISA test positive (TP+FN) =0.175 from Table 3.

Thus, P (E|E1) = 0. 0.15/0.175=0.857;

P (E'1) = probability of TP=0.125; P (E|E'1) = probability of (TP+TN) = 0.125+0.8= 0.925.

As we seek a posteriori probability value, substituting above values in its formula,

P(E1|E) = P(E1)×P(E|E1)/ [P(E1)×P(E|E1)+P(E'1) ×P(E|E'1)], we get,

P(E1|E) = (0.175×0.857)/[0.175×0.857)+(0.125×0.925)] = 0. 14997/[0.14997+0.11562] = 0.564667.

Thus, P (E1|E) or a posteriori probability value = 0.564667 or 0.5647.

DISCUSSION

Here all patients responded to tetracycline 100 mg twice/ day for 10 days confirming infection of scrub typhus without any fatal outcome. Outbreaks of scrub typhus from other parts of India have been recorded in the recent past.[9] Moreover; scrub typhus has been recognized as an emerging infectious disease for the last four to five decades.[10] In the past, the clinical diagnosis of scrub typhus was dependent on detecting eschar and rashes in addition to the history of outdoor activity.[11] However, the diagnosis of a rickettsia illness has been confirmed mostly by serologic testing with gold standard techniques, immunofluorescence antibody test (IFA), indirect immunoperoxidase (IP) test, ELISA and polymerase chain reaction (PCR).[12] The isolation of

causative bacteria in cell culture is limited by the lack of containment facility as well as, the lack of expertise in handling these high risk group pathogens in all hospitals always in India.[13] The Weil-Felix test is based on the detection of antibodies to an alkali based carbohydrate antigen, which are shared by rickettsiae and certain strains of Proteus sp., Proteus vulgaris OX19, and OX2 and P. mirabilis OXK. The OX-K strain of P. mirabilis was demonstrated to agglutinate with sera from patients with scrub typhus and was further used in the diagnosis of *O. tsutsugamushi* related infections. Many reports of scrub typhus and other rickettsia diseases from the Indian sub-continent are based on clinical findings and Weil-Felix test. Fever was the most common clinical feature seen in the study.[14] A necrotic eschar at the bite site of the mite is the common pathognomonic feature of scrub typhus. However, it is rarely seen in south East Asia and Indian subcontinent. Eschar was seen in 44 (55.6%) patients herein, while most studies recorded prevalence as high as 65% eshcar as the clinical index for diagnosis of scrub typhus without any reason.[13] ELISA technique is highly sensitive and reproducible, allowing the differentiation of IgG and IgM antibodies; which too is corroborated by Bayesian analysis herein. Crew JW et al. proposed for the detection of anti- *O. tsutsugamushi* antibodies by ELISA.[15] In present study out of 80 suspected 80 % cases were found IgM ELISA positive. Similar findings were seen in the studY.[14] Weil Felix test findings were correlated with ELISA reports to determine its sensitivity and specificity values.[8]

CONCLUSION

In cases of febrile illnesses clinicians should keep scrub typhus as one of the differential diagnosis especially in particular geographical region in all cases of fever with clinical manifestations, which do not fit into any common diagnosis after 2 days of common base line investigations. If diagnosis of scrub typhus is delayed, it would lead to complications; hence guidance from early diagnosis is important. For rapid diagnosis ELISA test for IgM against scrub typhus the gold standard. The sensitivity or the portion of the people with the rickettsia infection, who will have positive Weil-Felix test results with the value 0.7143, and specificity or the people without the disease who will have negative Weil-Felix test results value 0.9697 strengthen this test, statistically.

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