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Microbiology Section

Serological Evidence of Scrub Typhus among Cases of PUO in the Kashmir Valley- A Hospital Based Study

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ABSTRACT

Introduction: Rickettsial infections are being increasingly recognized as a cause of acute febrile illnesses and should be considered a distinct possibility in patients presenting with suggestive clinical features. Their diagnosis remains a challenge in a country like ours where tests like immunofluorescence assay cannot be routinely done. Results of serological tests, when correlated with patients clinical profile can aid in the timely diagnosis of scrub typhus.

Aim: To find out the extent to which scrub typhus contributes to Pyrexia of Unknown Origin (PUO) in patients admitted to or attending the OPD of our hospital using simple tests like Weil-Felix Agglutination Test (WFT) and Enzyme Linked Immunosorbent Assay (ELISA).

Materials and Methods: A cross-sectional study was carried out in the Department of Microbiology, Government Medical College and Hospital, Srinagar, over a period of eight months (1st March to 31st October 2015). Serum samples from patients suffering from Pyrexia of Unknown Origin (PUO) were processed for the detection of Scrub typhus. A total of 162 samples were included in the study. These were subjected to WFT using OX-K strain. The serum samples were diluted 1/20 to 1/640 and a titre of ≥ 1:160 was considered as positive. The samples were also tested for IgM and IgG antibodies for scrub typhus by ELISA and tube agglutination test was done to detect typhoid fever and brucellosis.

Results: Of the 162 serum samples tested 22.8% tested positive scrub typhus by WFT. IgM ELISA and IgG was positive in 8 (4.9%) and 15 (9.3%) samples respectively. Sensitivity, specificity, positive and negative predictive values of WFT; taking IgM ELISA as a reference standard were 75%, 79.9%, 16.2% and 98.4% respectively.

Conclusion: Scrub typhus is prevalent in our state and the results of WFT supplemented by those of ELISA can aid in its diagnosis. However the results of these tests should always be regarded in light of the clinical condition of the patient.

Keywords: IgM ELISA, Indirect immunofluorescence test, *Orentia tsutsugamushi*, Rickettsia, Weil Felix Test

INTRODUCTION

Scrub typhus, caused by a small (0.3 to 0.5 by 0.8 to 1.5μm) intracellular Gram negative bacterium *Orientia tsutsugamushi* of the family *Rickettsiaceae*, is a potentially life threatening infectious disease that presents mostly as an acute undifferentiated febrile illness. The disease is transmitted to humans and other vertebrates by the bite of the larval trombiculid mites (Chiggers) and is endemic to Afghanistan, Pakistan, Russia, Korea, Japan, Indonesia, Papua New Guinea, northern Australia and some smaller islands in the western Pacific [1].

The National Centre for Disease Control (NCDC) has played pivotal role in providing serological evidence of rickettsioses in India in states like Rajasthan, Jammu and Kashmir, Himachal Pradesh, Uttaranchal, Haryana, Assam, Sikkim, Manipur, West Bengal, Maharashtra, Kerala and Tamil Nadu in the last decade. Rickettsial infections are an important cause of pyrexia of unknown origin (PUO) and need to be differentiated from other febrile illnesses like enteric fever, malaria, dengue, brucellosis, leptospirosis etc. [2]. However, the burden of rickettsioses is often under-estimated, due to low index of suspicion, non-specific signs and symptoms and the absence of widely available sensitive and specific diagnostic tests [3].

The main stay of diagnosing scrub typhus has been serology, with Weil-Felix (WF) OX-K agglutination reaction being the oldest test in vogue. Although the test is inexpensive and easy to perform with rapid turn around time, it lacks sensitivity and specificity [4]. On the other hand the Indirect Fluorescent Antibody (IFA) test generally regarded as the gold standard, is more sensitive and specific, with results being available in a couple of hours, but it is costly and requires expertise [5]. In addition, the results of IFA are also influenced by the antigenic strains used, the specific antibody isotype measured and the choice of cutoff limits for diagnostic and epidemiological purposes [6]. IgM ELISA for scrub typhus is another serological diagnostic modality. Studies comparing the performance of IgM ELISA with IFA have found it to be quite satisfactory [3]. Also a good correlation between the results of the WF test and the detection of IgM antibodies by ELISA has been observed [7]. Isolation of *O. tsutsugamushi* by cell culture and/or animal inoculation is laborious, requires biosafety level III facilities and has a high turn around time (median time to positivity being 27 days) [6,8]. Molecular technique like Polymerase Chain Reaction (PCR) despite being rapid as well as sensitive and specific is beset with problems including high cost and the requirement of trained staff; attributes that make it impractical for many resource constraint areas where scrub typhus is endemic [6]. Delay in diagnosing scrub typhus is associated with high morbidity and mortality. Timely diagnosis of the disease can aid the clinician in instituting proper treatment in patients suspected of suffering from scrub typhus [3].

Although there have been some reports of this disease being present in this part of the country [8,9], none have assessed the magnitude to which it is prevalent in our population. The current study was thus designed to find out the extent to which scrub typhus contributes to PUO in patients admitted to or attending the OPD of our tertiary care hospital by using simple affordable tests like Weil-Felix agglutination Test (WFT) and Enzyme Linked Immunosorbent Assay (ELISA).

MATERIALS AND METHODS

This cross-sectional study was carried out in the Department of Microbiology, Govt Medical College/Hospital, Srinagar. A total of 162 serum samples from patients; suffering from PUO; were processed for the detection of scrub typhus from 1st March to 31st October 2015 (eight months). Patients who did not conform to the definition of classical PUO [10], those who were on antibiotics or immunosuppressive and/or immunomodulatory drugs, those with immunodeficiency were excluded from the study.

The patients had no history of travel to an area where scrub typhus is endemic. Common presenting symptoms were fever with rigors and chills, pain abdomen, vomiting, headache, jaundice, rash and lymphadenopathy. The samples were simultaneously tested for typhoid fever (Widal tube agglutination test) and brucellosis (tube agglutination test).

The samples were subjected to Weil-Felix agglutination assay using OX-K strain (Omega Diagnostics) as per manufacturer's instructions [11]. The serum samples were diluted 1/20 to 1/640 and a titre of ≥1:160 was taken as positive. Base line titre for Weil-Felix test was ascertained by screening 100 blood samples taken from healthy donors from the blood bank at our hospital. A value of 1:20 was seen in 8, 1:40 in 53 and 1:80 in 39 samples from healthy donors. The samples were also tested for IgM and IgG antibodies by ELISA (In Bios International Inc. USA) which was performed as per the instructions on the kits. Patient details were recorded on a pre-designed proforma. Ethical clearance for the study was sought from the hospital's ethical clearance committee.

STATISTICAL ANALYSIS

Data was entered in a Microsoft Excel spreadsheet. Continuous variables were summarized as mean and standard deviation. Categorical variables were summarized as percentage. For calculating sensitivity, specificity, positive predictive value and negative predictive value, IgM ELISA was as gold standard. Analysis was done using OpenEpi and SPSS version 16 software.

RESULTS

Out of a total of 162 serum samples, 37 (22.8%) tested positive and 125 (77.2%) were negative for scrub typhus by WFT. The samples were also tested for both IgM and IgG antibodies using ELISA. Positivity of WFT, IgM ELISA and IgG ELISA is shown in [Table/Fig-1]. Sensitivity, specificity, positive and negative predictive values of WFT taking IgM ELISA as a reference standard and IgG are given in [Table/Fig-2].

Age of the patients ranged from 5 to 70 years (93 males, 69 females). Fever (3-22 days) was the presenting symptom in all the patients (n=162, 100%). This was followed by rigors and chills in 135 (83.3%), pain abdomen in 79 (48.8%), lymphadenopathy in 77 (47.5%), vomiting in 52 (32.1%), headache in 25 (15.4%), hepatosplenomegaly in 23 (14.2%) and jaundice in 17 (10.5%) patients. Also rash was present in 14 (8.6%) of the 162 patients. None of the patients had an eschar on their body. Of the 162 patients, typhoid fever and brucellosis was diagnosed in 16.1% (n=25) and 8.6% (n=14) respectively on the basis of tube agglutination tests. None of these patients tested positive for scrub typhus by either WFT or IgM/IgG ELISA.

WFT	IgM		IgG		Total
	Positive	Negative	Positive	Negative	
Positive	6 (3.7%)	31 (19.1%)	10 (6.2%)	27 (16.7%)	37 (22.8%)
Negative	2 (1.2%)	123 (75.9%)	5 (3.1%)	120 (74.1%)	125 (77.2%)
Total	8 (4.9%)	154 (95.1%)	15 (9.3%)	147 (90.7%)	162 (100%)

[Table/Fig-1]: Depicts the number of cases that tested positive by WFT, IgM and IgG ELISA.

	IgM ELISA	IgG ELISA
Sensitivity	75%	66.7%,
Specificity	79.9%,	81.6%,
Positive predictive value	16.2%	27.0%
Negative predictive value	98.4%	96%
Diagnostic accuracy	79.6%	80.3%.

[Table/Fig-2]: Depicts the sensitivity, specificity, PPV and NPV of WFT as compared to IgM and IgG ELISA.

Majority of the patients in whom WFT was positive were in the age group of 30-49 yrs (n=24, 64.7%). Although a higher number of serum samples from male patients (n=21, 56.8%) were positive for scrub typhus by WFT than the female patients (n=16, 43.2%); the difference was not statistically significant. Likewise serum samples from male patients (n=13, 56.5%) were positive for scrub typhus by IgM/IgG ELISA, than female patients (n=10, 43.5%); the difference again being non-significant. Maximum number of patients presented with fever 37 (100%), followed by headache, 22 (59.5%); pain abdomen, 18 (48.7%); rash and lymphadenopathy, 8 (21.6%). Twenty two (59.5%) of the 37 patients hailed from a rural setting whereas 15 (40.5%) were from urban areas. The difference between the two was not significant. Farming was the major occupation of most of the patients (n=19, 51.4%). Rest were housewives (n=9, 24.3%), school teachers (n=4, 10.8%) and businessmen (n=5, 13.5%).

A titre of 1: 320 was seen in 15 (40.5%) serum samples on the WFT, whereas a titre of 1: 160 was seen in 18 (48.7%) and 1: 640 in 4 (10.8%) serum samples. In all the other samples the titres ranged from 1:20 (n=16), 1: 40 (n=46) and 1:80 (n=63). All the patients whose sera tested positive for scrub typhus on IgM ELISA were treated with doxycycline 100 mg twice daily for 7 days. Clinical response was seen 5 patients where as in 3 patients fever and other signs and symptoms continued to persist.

DISCUSSION

Rickettsial infections are re-emerging and should be considered in the differential diagnosis of PUO with every possible effort made to differentiate them from other more common causes [12]. Accurate and timely diagnosis of scrub typhus remains a challenge in India due to resource constraints and the limitations of the widely used diagnostic tests. Even the gold standard test, Indirect Immunofluorescence Assay (IFA) is riddled with drawbacks most important being cost and the technical expertise required [6,13]. Given such a scenario the use of tests like WF and ELISA is justified in conditions where more expensive diagnostic tools are not available; albeit the results are interpreted in correlation with the clinical condition of the patient.

We performed WFT, the results of which were confirmed by IgM and IgG ELISA, on 162 serum samples of patients who presented to the OPD of our hospital and were labeled as cases of PUO. A single acute phase serum sample was tested and a break point titre of 1:160 or above was seen in 22.8% of the patients. This is somewhat lower than what has been reported from other parts of the country [Table/Fig-3]. IgM and IgG ELISA positivity (4.9% and 9.3% respectively) was lower in the samples tested in our study which is in contrast to what has been reported by other authors [Table/Fig-3] [2,3,14-18].

Taking IgM ELISA as the reference against which the results of WFT were compared, the sensitivity, specificity, PPV and NPV of WFT was found to be 75%, 79.9%, 16.2% and 98.4% respectively. Although the sensitivity of WFT in our study was higher the PPV was only 16.2% as mentioned earlier. The PPV is based on sensitivity of a given test and prevalence of the disease for which it is used. A lower prevalence of scrub typhus in our study could be the reason behind the low PPV. However, what is noteworthy in this study is that the NPV of WFT was found to be 98.4%. Thus, this test in our setting could be helpful in ruling out the presence of scrub typhus

Parameters	Present study	Other studies
Sero prevalence of scrub typhus	22.8%	26 %; Roopa KS et al., 2015 [8] 56.4%; Usha K et al., 2014 [15] 46.2%; Mittal V et al., 2012 [2]
IgM/ IgG ELISA positivity	4.9% / 9.3%	58.2%; Usha K et al., 2014 [13] 30.9%; Gurang S et al., 2013 [16]
Sensitivity and specificity of WFT*	75% and 79.9%	87% and 98% Prakash JA et al., 2006 [17] 30% and 100% Isaac R et al., 2004 [12]
Age group	30-49 yrs	40-60 y; Munilakshmi P et al., 2015 [3] 25-65 y; Usha K et al., 2014 [15]
Gender	Males	Females; Munilakshmi P et al., 2015 [3] Males; Kawoosa Z et al., 2012 [14] Males; Lee N et al., 2008 [18]
Common presenting symptom	Fever	Munilakshmi P et al., 2015 [3] Usha K et al., 2014 [14] Kawoosa Z et al., 2012 [14]
Eschar present	No	Usha K et al., 2014 [14] Yes; Kawoosa Z et al., 2012 [14]

[Table/Fig-3]: Comparison of the present study with different studies across India.
*Sensitivity and specificity of WFT as compared to IgM ELISA

and would be a helpful test in triaging the patients in an outbreak.

In the present study most of the patients in whom WFT, IgM and IgG ELISA or both were positive were in the age group of 30-49 years. Our results revealed that the incidence of infection was higher in young adults, male patients and farmers; likely due to increased involvement in outdoor activities like working in the fields, collecting firewood and recreation. Comparison of our results with those seen by other investigators is shown in [Table/Fig-3].

WFT has been widely used as an aid in diagnosing rickettsial diseases around the globe, even though the results of the test maybe negative in the initial stages of the disease due to absence or sub optimal levels of agglutinating antibodies in blood of the affected patients; the antibodies being usually detected around the second week of infection [7]. National Centre for Disease Control (NCDC) in their survey used WFT to detect the sero-prevalence of rickettsial diseases in PUO cases in Delhi from 1999 to 2004. They concluded that although WFT lacks sensitivity and specificity it can still be used as a preliminary test in a country like ours where the exact magnitude of the disease is not known [2].

This is the first study from the Kashmir division of our state to report the prevalence of scrub typhus among PUO cases to the best of our knowledge. Large scale studies are warranted to find out the exact magnitude of not only scrub typhus but other rickettsial disease as well in our population and understand better the demographics associated with them.

LIMITATION

Our study had certain limitations as well. The results are based on a single sample collected from patients where as recommendations are to test two paired serum specimens collected at least 14 days apart. Also, we did not compare the results of ELISA or WFT with the gold standard IFA or any other comparable molecular method (e.g. PCR), owing to which we might have missed some cases.

CONCLUSION

WFT can be used as an initial screening tool in patients of PUO with features suggestive of scrub typhus due to its low cost. Supplemented with other tests like IgM/IgG ELISA it can prove helpful in either making a diagnosis of or excluding the disease. This is especially important in settings like ours where more expensive and technically demanding tests like IFA cannot be routinely done.

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