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Research Article

Relevance Of Orientia Tsutsugamushi Antibodies In Elevated Levels Of Liver Enzymes And Thrombocytopenia Among Patients In Tertiary Care Hospital

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ABSTRACT

Introduction and Aim: Scrub typhus is an endemic zoonosis in India and it has been categorized under non-malarial illness. Eschar is the prompt diagnosis of ST, but mostly we rely on the laboratory parameters like specific ELISA, Rapid Immunochromatographic tests (ICT) etc. This study is to identify the relevance of scrub typhus antibodies in patients with elevated levels of liver enzymes and thrombocytopenia in tertiary care hospital, Puducherry.

Materials and Methods: The investigative prospective study was conducted in tertiary care teaching institute, for the period of four months from July to November 2023. About 100 samples were collected from patients with elevated levels of liver enzymes and thrombocytopenia (n=50) and healthy voluntary blood donors (n=50). For these samples Rapid ICT and IgM/IgG ELISA was performed to confirm the disease.

Results: A total of 100 samples were performed using Rapid ICT and ELISA. Among 50 patient samples, five samples 10% were positive for ST RAPID ICT IgM and IgG antibodies which confirmed by IgM & IgG ELISA tests. Remaining 50 voluntary blood donor samples, two had (4.0%) positive for only ST IgG. There was no positivity was observed for ST IgM antibodies.

Conclusion: Standardization is required for non-specific markers viz., elevated levels of liver enzymes and thrombocytopenia after thorough evaluation we may consider as a tool for the diagnosis of ST.

Keywords: Scrub typhus; Immunoglobulin M; Immunoglobulin G; Immunochromatographic test; Thrombocytopenia

INTRODUCTION

Scrub typhus (ST) is an endemic as well as emerging/re-emerging zoonotic disease in nature and that is potentially spread to humans through the bite of chiggers Trombiculide family (*Leptotrombidium deliense* and *Leptotrombidium akamushi*), which is caused by a gram-negative intracellular bacterium *Orientia tsutsugamushi* (1-4). ST found in most of the eastern and southern parts of Asia called "Tsutsugamushi Triangle" viz., northern Australia, Japan, China, and India (1). Early clinical symptoms of ST are nonspecific and it could be mistaken for bacterial, viral, or malarial illnesses. Typical clinical symptoms of ST are high grade fever for more than a week, nausea, vomiting, headache abdominal pain, myalgia and swollen lymph nodes (5-11). It incubation period may takes up to 10 to 12 days. Eschar is the hallmark of ST, which also similar to some of spotted fever group rickettsiae. If ST is not treated, the complication may leads to acute renal failure, gastrointestinal haemorrhage, meningoencephalitis, myocarditis, and respiratory difficulty (11-12). Laboratory like pathology, biochemistry and microbiological parameters like differential count, platelet count, liver enzymes, CRP are non-specific, since they are highly helpful to support for an early diagnosis of ST in India

(11). ST mortality rates ranges from less than 1% to 50% when antibiotics are used appropriately (13). The cornerstone of treatment is still either chloramphenicol or doxycycline for adults and azithromycin for children (14). ST can be tested using different methods like Weil Felix Test (WF), Rapid Immunochromatographic test (RICT), Enzyme Linked Immunosorbant Assay (ELISA), Indirect Immunofluorescence assay (IFA), Polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) to detect the cases of ST in India, which was published literature on this disease (12-16). This study is to identify the relevance of scrub typhus antibodies in patients with elevated levels of liver enzymes and thrombocytopenia in tertiary care hospital, Puducherry.

MATERIALS AND METHODS

This investigative prospective study was conducted in Mahatma Gandhi Medical Advanced Research Institute, Puducherry for the period of four months from July to November 2023. About 100 serum samples which include 50 samples were already collected from the laboratory with elevated liver enzymes and thrombocytopenia and remaining 50 samples from the healthy blood donors for this study. These samples were tested by using Rapid ICT for IgM as well as IgG antibodies (ImmuneMed, Seoul, South Korea). All samples were included with elevated parameter like increased transaminases, Thrombocytopenia, and leucocytosis were highly suggestive of scrub typhus. Fever with normal level of liver enzymes and platelet count were excluded from the study. Scrub Typhus Rapid Card Method was performed as per the manufacturer's instructions. Briefly, 3µl Serum sample and 6µl sample diluted provided onto the hole of testing device, for the detection of IgM and IgG, the sample was first react with gold conjugated protein A. After that, each reactant diffuses on the membrane to the recombinant antigen marked as test lines. Wait for 15 minutes and till red line appear on the control line. Read and interpret the result exactly after 15 minutes, from the applying the diluted sample. If positive, test line turns to red colour because antigen-antibody -gold conjugate complex in formed on the test line. Ignore the red line appeared after 15 minutes interpretation. If red lines does not appear from at least one out of 2 lines, this result is interpreted as invalid, and do test again. After testing of ST Rapid ICT, the same samples were tested with the gold standard serological test ELISA to confirm the disease in patient with elevated liver enzymes and thrombocytopenia. ELISA was performed according to the previous study (15).

RESULTS

A total of 100 samples were performed to attempt the relevance of ST IgG antibodies against the patients with elevated liver enzymes and thrombocytopenia followed by an equal number of voluntary blood donors were employed as a control for this study. Among 50 patient samples, five samples 10% were positive for ST RAPID ICT IgM and IgG antibodies which confirmed by IgM & IgG ELISA tests. Remaining 50 voluntary blood donor samples, two had (4.0%) positive for only ST IgG were showed in Table – 1. There was no positivity was observed for ST IgM antibodies. Previously, none of them were diagnosed as ST patients.

DISCUSSION

Rapid Immunochromatographic Tests for scrub typhus diagnosis offer a quick and accessible method for detecting the presence of antibodies associated with *Orientia tsutsugamushi* infection, facilitating timely clinical decisions and patient management (18-19). ST Rapid ICT kit is quite cost-effective and now its essential for detection of cases and to treat early as possible. Previous studies from Pondicherry have highlighted the prevalence of scrub typhus by the application of various Immunochromatographic testing kits. Most of the rapid kits are adhered to the detection of IgM and/or IgG antibodies (15). Hence, there was no rapid kit available for *O. tsutsugamushi* antigen in India as well as other developing or developed countries. Few Indian researchers from Pondicherry, New Delhi etc., have evaluated commercially available molecular diagnostic kits for Scrub typhus in India. InBios ST IgM Rapid kit has been introduced in Indian market only from 2015 onwards (20). These kits are quite helpful in resource poor and remote rural areas as Point of care (POC) tests and can significantly reduce the morbidity and mortality of ST. IFA the 'gold standard' test for serological diagnosis of ST and other rickettsioses and molecular diagnostic tests are routinely performed by overseas researchers but only by few Indian rickettsiologists (15). IFA tests are technically demanding and highly subjective mandating a good observer skill (1). Many studies from India, have recorded that the majority of the children showed an elevated levels of liver enzymes and the low platelet counts ranges from 26% to 72% (21). In the present study, only five samples were positive for scrub typhus rapid ICT and ELISA IgM and IgG antibodies. Among five samples, two were positive patients had both ST IgM and IgG antibodies in the elevated levels of liver enzymes and low platelet count. In voluntary blood donors, we found two samples were positive for ST IgG antibodies. Since, the healthy donors had antibodies may exposure to the ST infection previously. Serious complications of ST are preventable by an early and accurate diagnosis followed by treatment. In most of the time, thrombocytopenia is one of the important diagnoses for scrub typhus as well as other viral diseases like dengue etc. We recruited only a limited number of patients as well as healthy donors for this study. Hence,

to know the exact role of scrub typhus in the patients with elevated liver enzymes and thrombocytopenia, the study should screen a greater number of samples for the reliability of the disease.

CONCLUSION

To conclude, that standardization may require for non-specific markers viz., elevated levels of liver enzymes and thrombocytopenia may consider as a tool for the diagnosis of ST.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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Nil

AUTHOR'S CONTRIBUTION

1. Dr Pradeep designed the work and performed ST rapid ICT card test, IgM and IgG ELISA; drafted the manuscript
2. Dr Divya had done the Data analysis and Manuscript writing
3. Dr Ramani and Dr Selvakumar has edited and modified the manuscript

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Table – 1: Results of Rapid Immunochromatographic test for Scrub typhus diagnosis (n=100).

SI. NO	SAMPLE ID	Rapid Immunochromatographic test for ST		SI. NO	SAMPLE ID	Rapid Immunochromato-graphic test for ST	
		IgG	IgM			IgG	IgM
1	SAMPLE 1	-	-	34	HS63	-	-
2	SAMPLE 2	+	+	35	HS74	-	-
3	SAMPLE 3	-	-	36	HS69	-	-
4	SAMPLE 4	-	-	37	HS42	-	-
5	SAMPLE 5	-	-	38	HS65	-	-
6	SAMPLE 6	-	-	39	HS72	+	+
7	SAMPLE7	-	-	40	HS5B	-	-
8	HS41	-	-	41	HS46	-	-
9	HS42	-	-	42	HS60	-	-
10	HS43	-	-	43	HS56	-	-
11	HS44	-	-	44	HS48	-	-
12	HS21	-	-	45	294	+	+
13	HS-46 p	-	-	46	HS50	-	-
14	HS-46	-	-	47	290	-	-
15	HS-B	-	-	48	299	-	-
16	HS-B	-	-	49	HS41	+	+
17	HS-62	-	-	50	HS61	-	-
18	300	-	-	51	HS64	-	-
19	HS50	-	-	52	292	-	-
20	HS52	-	-	53	298	-	-
21	HS53	-	-	54	HS58	+	+
22	HS75	-	-	55	HS59	-	-
23	299	-	-	56	HS60	-	-
24	54	-	-	57	HS61	-	-
25	HS71	-	-	58	HS62	-	-
26	HS26	-	-	59	HS53	-	-
27	HS27	-	-	60	HS64	-	-
28[	HS55	-	-	61	BD1	-	+
30	HS52	-	-	62	BD2	-	+
31	HS70	-	-	63-100	BD63-100	-	-

Foot Note: ST – Scrub typhus; HS – Hospital sample (patient serum); BD – Blood donors