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Original Research Article

Comparative Analysis of IGM Elisa and Rapid Agglutination Test and Prevalence of Scrub Typhus in Tertiary Care Centre Warangal

Md. Rizwan Ansari[1], G.V. Padmaja[2], Juveria Sultana[3], Ajitha Reddy Edula[4]

[1]Assistant Professor, Department of Microbiology, Apollo Institute of Medical Sciences & Research, Jubilee Hills, Hyderabad, Telangana, India

[2]Professor & Head of the Department, Department of Microbiology, Kakatiya Medical College, MGM Hospital, Warangal, Telangana, India

[3]Assistant professor, Department of Microbiology, Prathima Relief Institute of Medical Sciences, Hanamkonda, Telangana, India

[4]Associate Professor, Department of Microbiology, Prathima Relief Institute of Medical Sciences, Hanamkonda, Telangana, India

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Corresponding Author: Dr. Md. Rizwan Ansari

Conflict of interest: Nil

Abstract:

In the Asia-Pacific region, scrub typhus is a major public health concern. It makes one million people sick every year and poses a hazard to one billion people worldwide. Scrub typhus is caused by Orientia tsutsugamushi and, if left untreated, can cause severe multiorgan failure with a 75-80% case fatality rate. O. tsutsugamushi's antigenic heterogeneity permits reinfection and prevents generic immunity. Scrub typhus is a neglected disease, and the little epidemiologic data and other relevant public health information on the disease in its endemic areas show that we still don't fully understand it. We studied the infection's clinical characteristics in 90 individuals who visited this tertiary care facility over a 16-month period. The ELISA's seropositivity rate was 11.11%, whereas the rapid test analysis revealed a rate of 22.22%. Scrub typhus was found to be substantially correlated with both eschar and animal exposure in this study. The PROGEN OXK antigen suspension (Weil-Felix) and the InBios scrub typhus IgM ELISA kit showed a significant difference in scrub typhus diagnosis ($p<0.05$). The results of the quick agglutination test showed that the sensitivity, specificity, positive predictive value, and negative predictive value were 60.0%, 82.5%, 30.0%, and 94.28%, respectively. Reducing morbidity and death from this condition will be greatly aided by raising awareness of it and managing it promptly.

Keywords: Scrub typhus, IgM, rapid, Weil-Felix & ELISA.

Introduction

Orientia tsutsugamushi, formerly known as Rickettsia tsutsugamushi, is the mite-borne infectious illness that causes scrub typhus. In the Asia-Pacific area, which includes, but is not limited to, Korea, Japan, China, India, Indonesia, Taiwan, Thailand, Sri Lanka, and the Philippines, scrub typhus is a severe public health concern. It poses a threat to about one billion people worldwide, and one million individuals get sick with it each year [1].

Scrub typhus has always been recognised as endemic in the region known as the "tsutsugamushi triangle". From Far East Russia in the north, Australia in the south, Pakistan in the west, and Japan in the east, this region spans more than 8 million km2 [2,3,4]. At least one billion people are at risk of infection due to the dense population in the endemic area. The disease has spread to non-endemic regions through infected individuals as a result of progressive globalisation and the resulting ease of travel [5]. Even though it is a prevalent ailment, it is frequently not well-reported. Scrub typhus symptoms can vary from a little disease similar to the flu to a serious and occasionally fatal condition. Additionally, it may mimic other illnesses including typhoid, dengue fever, or malaria. Scrub typhus is commonly underreported and underdiagnosed in India due to a lack of knowledge and inadequate diagnostic facilities. The identification of patients with scrub typhus can be greatly improved by combining clinical knowledge with test confirmation.

Humans contract O. tsutsugamushi by being bitten by an infected chigger, which is a larva of the parasitic mite Leptotrombidium deliense. The hexapod 0.2-mm larvae consume the host's lymph and tissue fluid for two to ten days. Wild rats are these chiggers' natural habitat. Instead of blood, the

chiggers consume tissue and lymph fluid. By consuming the bodily secretions of an infected rodent, these chiggers acquire the ability to spread the virus, which they carry with them throughout their lives. As adults, the infection is transferred to their eggs through the process of transovarial transmission. When the chiggers push their mouthparts down the hair follicles or skin pores to feed, they inject the O. tsutsugamushi, which they have in huge quantities in their salivary glands.

Accidental human infection happens when a person walks, sits or lies on an area infested with larval mites and takes up an infected chigger.

Among the risk factors for illness are: a) Exposure at work, such as farmers, ranchers, meat processors, trappers, veterinarians, loggers, sewage workers, rice growers, pet dealers, army personnel, and laboratory workers. b) Exposure in the household: infected rats infesting the house. Walking barefoot in farmlands with scrub vegetation, coming into contact with wild rats, and unintentional laboratory exposure are examples of low socioeconomic conditions.

The illness typically manifests clinically as a gradual onset of fever and chills, followed by a severe generalised headache, diffuse myalgias, rash, and eschar, or it can begin suddenly with headache, anorexia, and malaise. Cough, relative bradycardia, nausea, vomiting, diarrhoea, regional lymphadenopathy followed by generalised lymphadenopathy, and infrequently, meningitis are further symptoms (6,7,8,9). The incubation period ranges from 6 to 20 days, with an average of 7 to 10 days [9,10]. Rate of Case Fatality: 1.3% to 33.5%.[11]. Hepatitis (40.5% of scrub typhus patients), thrombocytopenia (28.4%), acute respiratory distress syndrome or ARDS (20.5%), acute renal damage (19.2%), meningitis (16.4%), shock (16.2%), and myocarditis (15.5%) were the most frequent sequelae.

Diagnostic Criteria:

Presumptive Diagnosis: Past farming exposure, mites and eschar presence. Disorganised Thrombocytopenia, hepatic enzymes, leucocytosis, or leukopenia with high bilirubin and creatinine are examples of non-specific laboratory values. The Weil-Felix test

Confirmatory Diagnosis: IgM ELISA

Indirect fluorescent antibody (IFA) Eschar biopsies and organism culture at certain, designated facilities. The 16S rRNA gene is the target of the polymerase chain reaction [12].

Study Time: In 2021, the study was carried out over a six-month period.

Study Type: The Weil-Felix (rapid) test and IgM ELISA are two frequently used diagnostic modalities for scrub typhus that are being compared in this prospective investigation.

Sample Size: Serum samples from 90 scrub typhus cases that were clinically suspected were gathered and prepared for IgM ELISA and the fast test.

Study Population: Prior to the study approval was acquired from the Institutional Ethics Committee of Kakatiya Medical College & Hospital Superintendent of Mahatma Gandhi Memorial Hospital (MGMH), Warangal, Telangana State, India.

The MGMH in and around Warangal city recognised patients with a clinical suspicion of scrub typhus from both in-patient and out-patient units across several departments. Prior to the study, all patients' consent was obtained. Every patient had a thorough medical history that included information on the onset of their illness, occupational exposure, contact with diseased animals, and exposure to mite-infested environments.

Criteria for Inclusion: More than five days of acute feverish illness linked to any of the following: chills and rigours, myalgia, and headache. Hepatosplenomegaly, rashes, jaundice, nausea, vomiting, diarrhoea, stomach pain, lymphadenopathy, and anuria or oligouria indications of inflammation of the meninges.

Criteria for Exclusion: Patients with a different definitive diagnosis and expectant mothers are excluded.

Sample Collection: Using sterile vacutainers that were solely equipped with clot activators and no anticoagulants, 5 millilitres of whole blood were drawn from patients. The resultant serum was separated, tagged, and kept after the blood samples were centrifuged for ten minutes at 3000 revolutions per minute. Following sample collection, an IgM ELISA test and a Weil-Felix test were used to analyse the serum.

The PROGEN antigen suspension, produced by Tulip Diagnostics (p) Ltd, Goa, was used for the Weil-Felix test. Any antibodies generated in the patient's serum due to Scrub Typhus Illness will mix with all of the stained PROGEN suspension; in other words, the antigen will cause an agglutination reaction. The absence of cross antibodies is indicated by no agglutination. The manufacturer's instructions were followed for conducting the testing.

IgM antibodies were found using the Scrub Typhus Detect TM IgM ELISA system, which is produced by InBios International, Inc., U.S.A. The manufacturer's instructions were followed for performing the test and computations.

Signification & Statistical analysis: A value of P <0.05 was deemed statistically significant. For all

computations, SPSS software (version 20.0) was utilized for analysis**.**

Serum samples from 90 patients who had a fever of unclear cause that lasted longer than five days were collected findings are shown in Figure I. These samples were tested using the fast agglutination (Weil-Felix) method and the IgM ELISA method to identify antibodies produced against scrub typhus.

Results of the analysis of the rapid agglutination (Weil-Felix) test: The PROGEN antigen suspension's rapid agglutination test findings are shown in Figure II Twenty (22.22%) of the 90 examined samples were positive (figure IIIa,b,c), while 70 (77.77%) were negative. Results of IgM ELISA analysis: Using the Scrub typhus Detect TM IgM ELISA system, made by InBios International, the 90 collected samples were subjected to ELISA IgM detection. ELISA and quick test comparison: The sensitivity, specificity, positive predictive value, and negative predictive value of fast agglutination, when using IgM ELISA as the standard method for identifying scrub typhus in this study, are 60.00%, 82.50%, 30.00%, and 94.28%, respectively.

Table 1: IgM ELISA Analysis

	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12
A	0.101 NC	0.086 NC	0.111	0.107	0.119	1.960	0.067	0.085	0.104	0.092	0.115	0.103
B	1.358 PC	1.541 PC	0.289	0.927	0.134	0.069	0.081	0.196	0.076	0.091	0.677	0.264
C	0.197	0.136	0.250	0.151	0.266	0.067	0.096	0.101	0.074	0.104	0.064	0.478
D	0.109	0.124	0.181	0.797	1.540	0.080	0.092	3.733	0.122	0.180	0.061	0.092
E	0.090	0.136	0.112	0.102	0.272	0.085	0.142	0.082	0.296	0.074	0.086	0.090
F	0.096	0.107	0.268	0.085	0.067	0.085	0.379	0.128	0.126	0.361	0.099	0.074
G	0.173	0.113	0.534	0.137	0.100	0.072	0.107	0.387	0.084	0.101	0.321	0.060 D1
H	0.140	0.096	0.140	0.165	0.108	0.094	2.019	0.083	2.768	0.099	1.815	0.061 D2

NC = Negative control; PC = Positive control; D = Dummy sample, Reactive and Non-reactive

Table 2: Result of IgM ELISA

Result	Number	Percentage
Reactive	10	11.11
Non-reactive	80	88.88

Table 3: Age and gender distribution of ELISA analysis

Age	Sex	Reactive	%	Non-reactive	%
Adult (>12 yr.)	Male	3	3.33	31	34.44
	Female	3	3.33	28	31.11
Children <12 yr.)	Male	2	2.22	12	13.33
	Female	2	2.22	9	10.0

.Table 4: Symptom wise distribution in ELISA analysis.

Symptoms	ELISA (Reactive)	Percentage	ELISA (non-reactive)	Percentage
Fever	10	11.11	80	88.88
Chills & Rigor	7	7.77	27	30.0
Headache	7	7.77	42	46.66
Myalgia	7	7.77	50	55.55
Nausea/Vomiting	5	5.55	1	1.11
Diarrhea	0	0	0	0
Abdominal pain	1	1.11	2	2.22
Cough	3	3.33	23	25.55
Anuria/Oliguria	0	0	0	0
Rashes	2	2.22	3	3.33
Travel History	0	0	7	7.77
Rain Exposure	4	4.44	33	36.66
Animal Exposure	6	6.66	20	22.22
Farmland Exposure	2	2.22	13	14.44
Insect Bite	2	2.22	14	15.55
Lymphadenopathy	4	4.44	31	34.44

Eschar	2	2.22	0	0
Icterus	0	0	0	0
Hepatosplenomegaly	0	0	0	0
Meningeal irritation	0	0	0	0
Relative bradycardia	0	0	0	0

Table 5: Rapid – ELISA comparison.

Result	Rapid	%	ELISA	%
Reactive	20	22.22	10	11.11
Non-reactive	70	77.77	80	88.88

Table 6: Symptom wise comparison in ELISA analysis.

Symptom	ELISA (Reactive)		ELISA (Non-reactive)		P value	χ^2
	Present	Absent	Present	Absent		
Fever	10	0	80	0	<0.75	NA
Chills & Rigor	**7**	**3**	**27**	**33**	**<0.05**	**4.96**
Headache	7	3	42	38	<0.5	1.09
Myalgia	7	3	50	30	<0.75	0.215
Nausea / Vomiting	**5**	**5**	**1**	**79**	**<0.001**	**33.95**
Diarrhea	0	10	0	80	<0.75	NA
Pain Abdomen	1	9	2	78	<0.25	1.552
Cough	3	7	23	57	<0.95	0.007
Anuria / Oliguria	0	10	0	80	<0.75	NA
Rashes	**2**	**8**	**3**	**77**	**<0.05**	**4.47**
Travel history	0	10	7	73	<0.5	0.949
Rain Exposure	4	6	33	47	<0.95	0.006
Animal Exposure:	**6**	**4**	**20**	**60**	**<0.025**	**5.30**
Farmland Exposure	2	8	13	67	<0.90	0.90
Insect Bite	2	8	14	66	<0.90	0.038
Lymphadenopathy	4	6	31	49	<0.95	0.006
Eschar	**2**	**8**	**0**	**80**	**<0.001**	**16.36**
Icterus	0	10	0	80	<0.75	NA
Hepatosplenomegaly	0	10	0	80	<0.75	NA
Meningeal Irritation	0	10	0	80	<0.75	NA
Relative Bradycardia	0	10	0	80	<0.75	NA

Table 7: Rapid agglutination – IgM ELISA comparison

	ELISA	Rapid (WF)	Total
Reactive	10	20	30
Non-reactive	80	70	150
Total	90	90	

Statistically significant at P < 0.05.

Table 8: Comparison with other studies

Studies	Seropositivity in Rapid agglutination	Sensitivity Rapid agglutination	Specificity Rapid agglutination	PPV Rapid agglutination	NPV Rapid agglutination	Seropositivity in ELISA IgM
Jacob AE et.al (2021)	-	40.0%	94.0%	87.0%	61.0%	-
Meerah et.al (2018)	4.2%	69.76%	99.85%	96.77%	-	6%
Seema Rani et.al (2016)	27.4%	72.5%	91.4%	-	-	29.46%
K.S. Roopa et.al (2015)	26.0%	-	-	-	-	47.0%
Usha K et.al (2014)	56.42%	-	-	-	-	58.21%
Present study	**22.22%**	**60.0%**	**82.5%**	**30.0%**	**94.28%**	**11.11%**

All values are expressed as mean ± SD; P value < 0.05

Figure 1: Total serum samples collected.

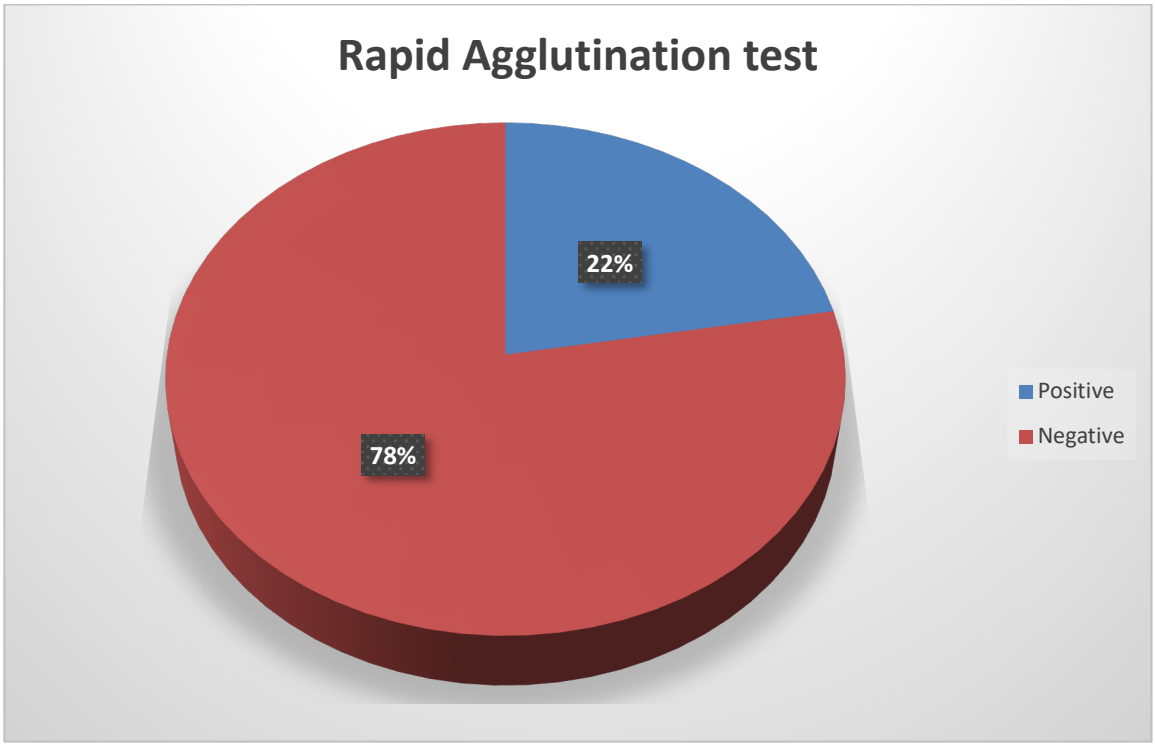

Figure 2: Rapid test results

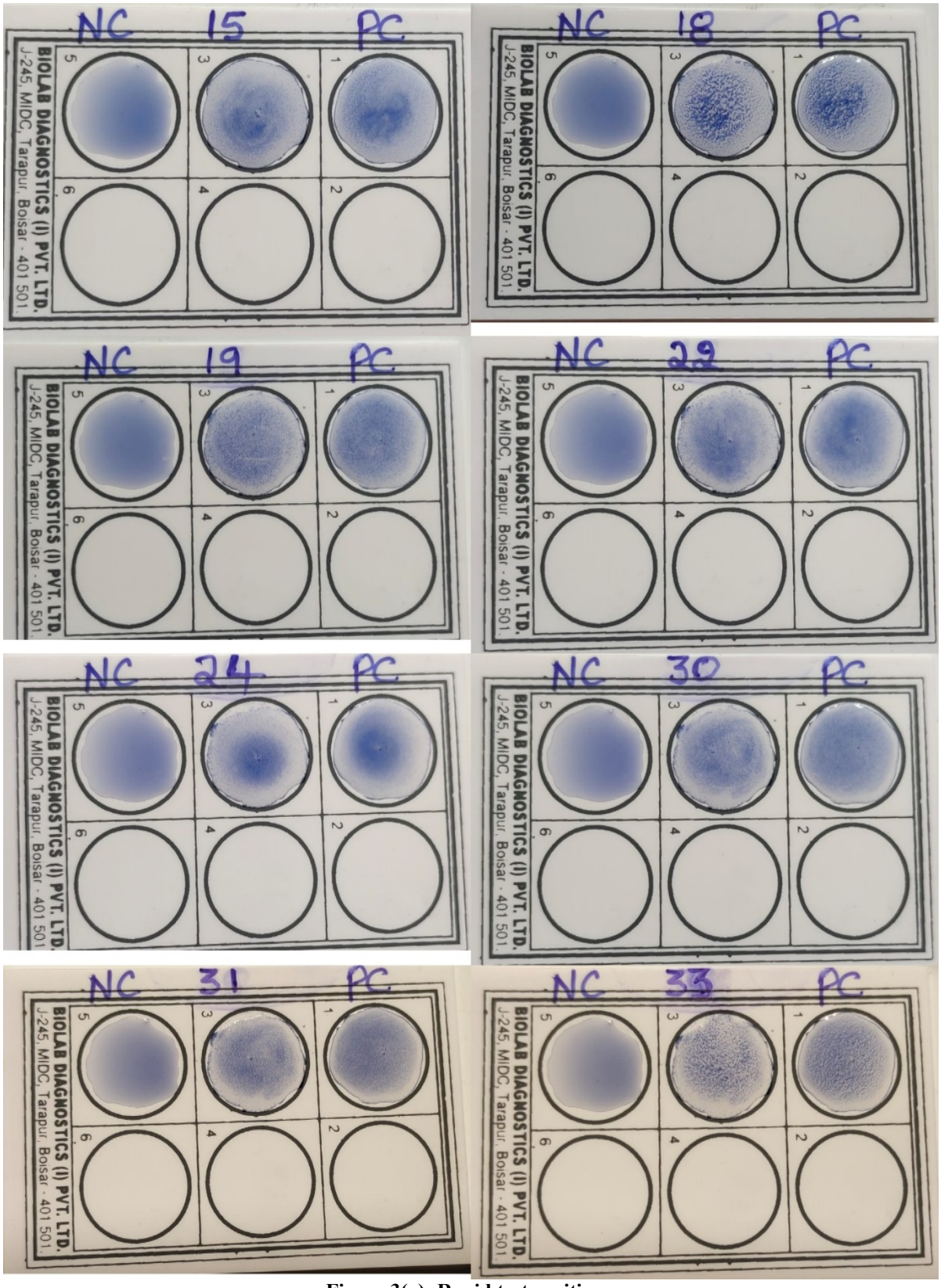

Figure 3(a): Rapid test positives.

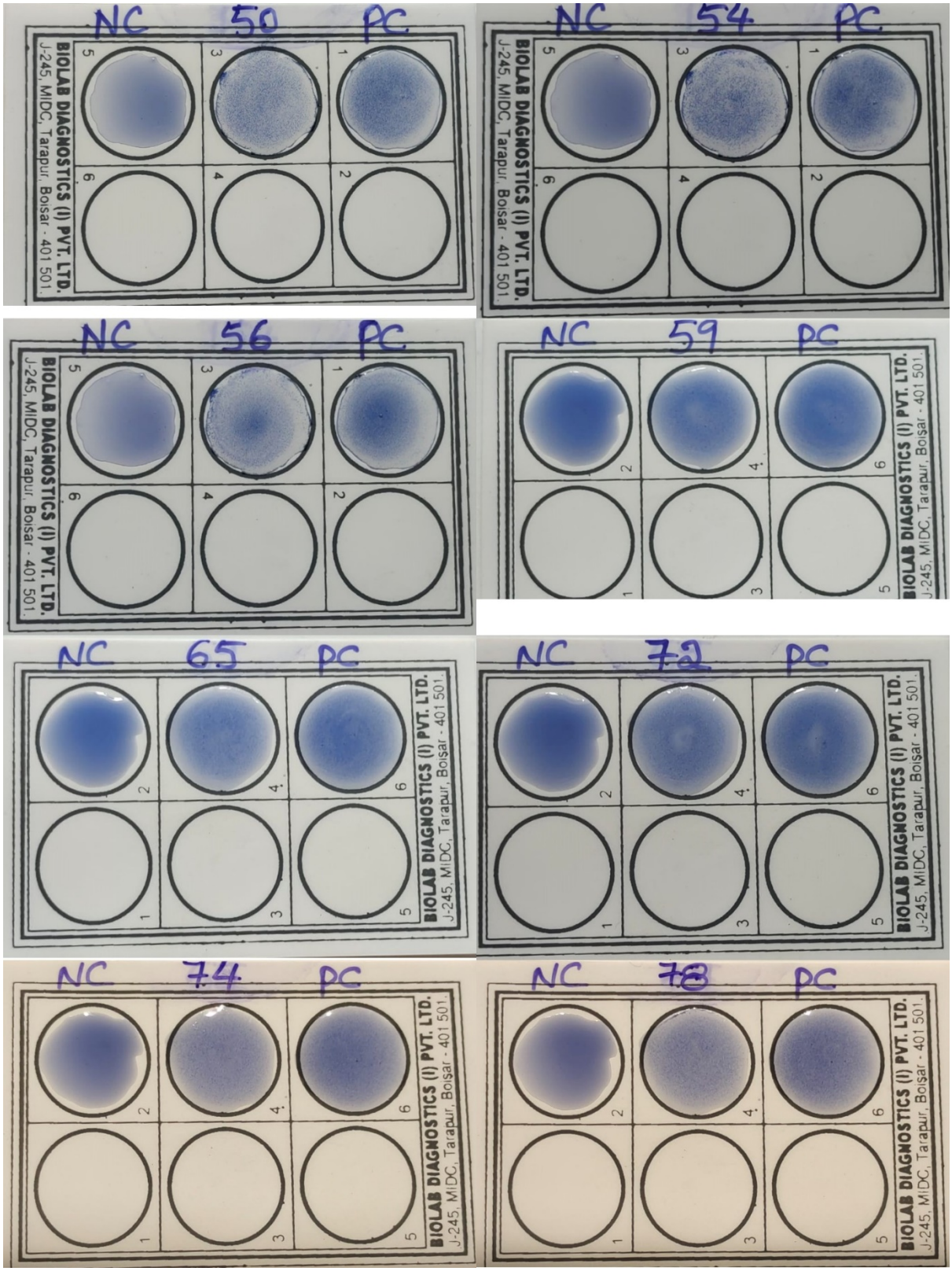

Figure 3(b): Rapid test positives.

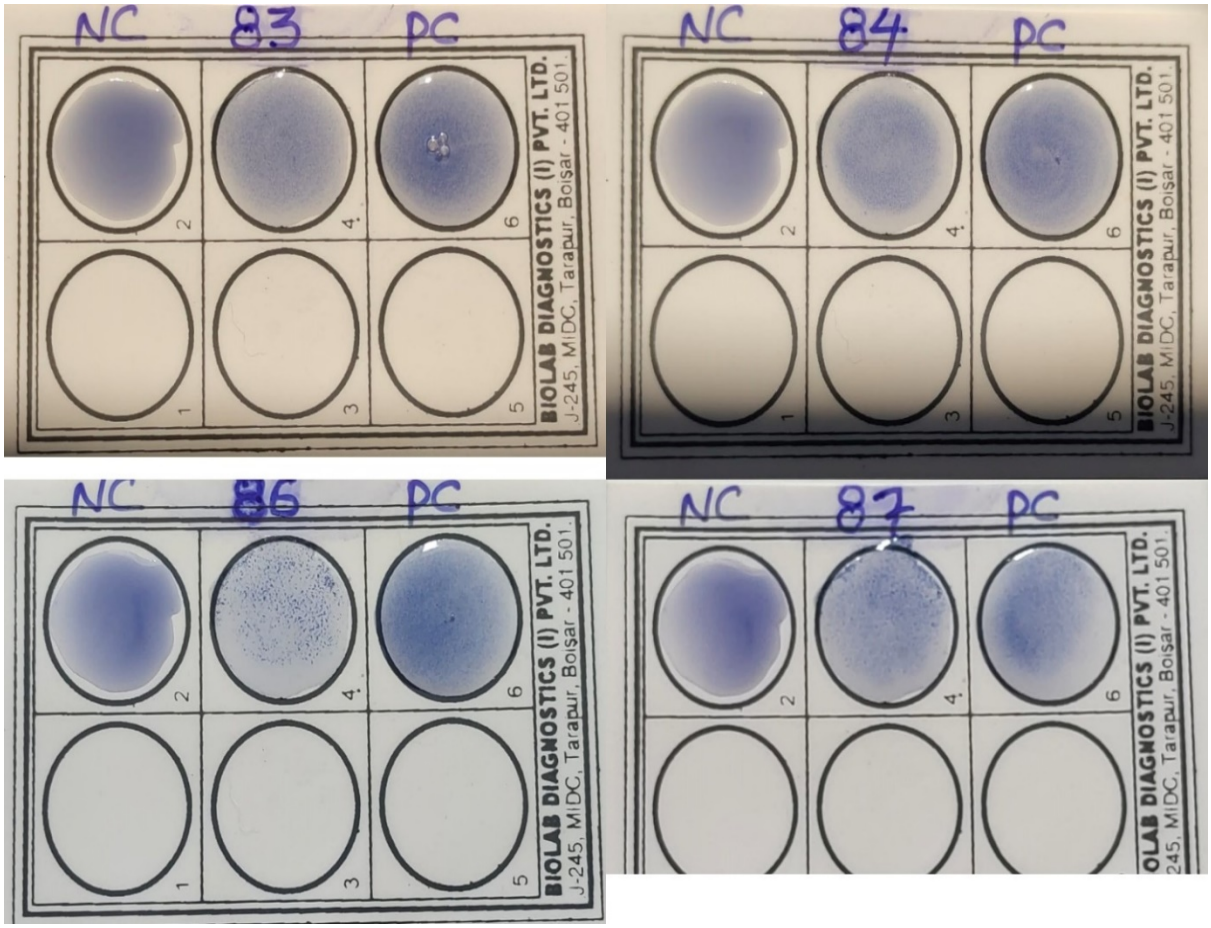

Figure 3(c): Rapid test positives.

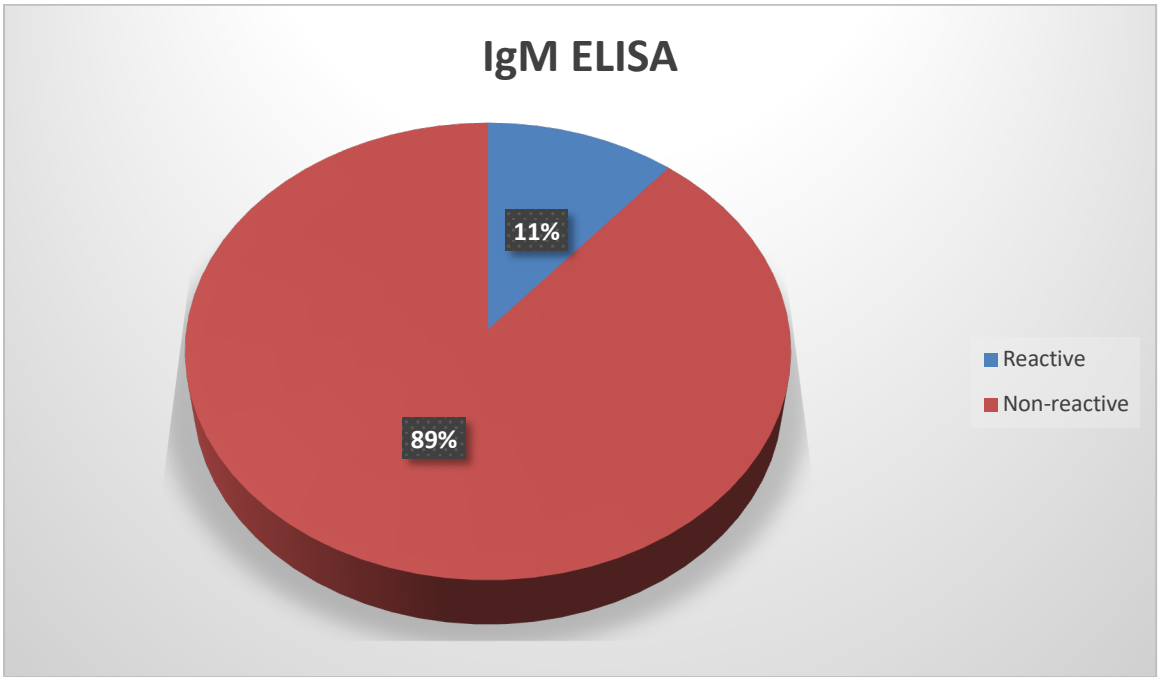

Figure 4: Result of IgM ELISA

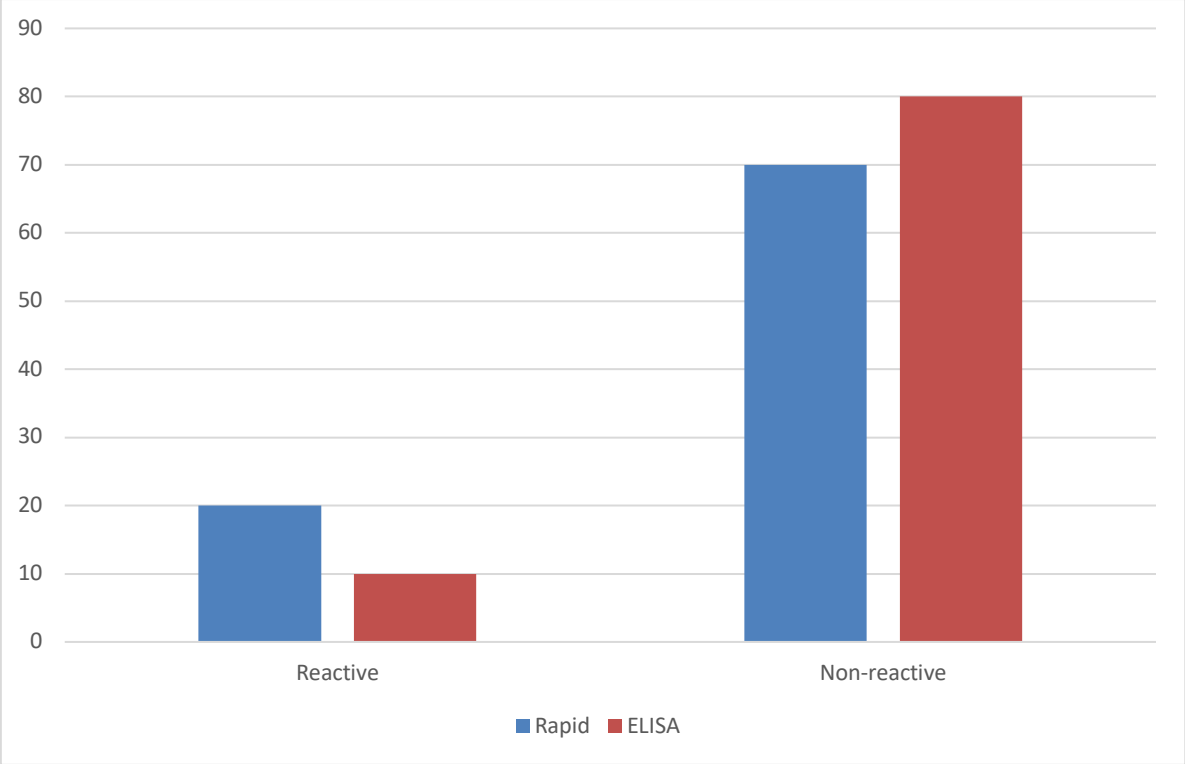

Figure 5: Rapid-ELISA comparison

Discussion

Scrub typhus's resurgence in recent years has led to its recognition as a significant public health concern [13]. Patients with scrub typhus may develop severe problems and ultimately pass away if treatment is not received. The mortality rate is between 3% to 60%. Either the original infection or its subsequent consequences, like pneumonitis, encephalitis, or cardiovascular failure, might cause death. By the end of the second week of untreated infection, the majority of deaths had taken place. Combining the clinical expertise, confirmatory test evidence, and high clinical suspicion can significantly reduce the hazard posed by this reemerging disease. Since there is a dearth of information on scrub typhus in Warangal, this study was conducted to determine the disease's incidence and seroprevalence as well as to compare the IgM ELISA method and the rapid agglutination (Weil-Felix) test in patients visiting Mahatma Gandhi Memorial Hospital in Warangal.

According to our study, fever (100%) was the most common clinical feature, lasting an average of 10 days. Myalgia (63.33%), headache (54.44%), chills and rigours (37.78%), cough (28.89%), nausea and vomiting (6.67%), rashes (5.56%), and abdominal discomfort (3.33%) were the next most common clinical features. Forty-two (46.6%) and forty-eight (53.3%) of the 90 serum samples that were taken were female. Of them, 65 (72.22%) were adults (>12 years old) and 25 (27.78%) were children (<12 years old). Of them, 41.11% had been exposed to rain, 28.89% to animals, and 16.67% to farming. 17.78% of cases had a history of insect bites, while 7.78% had a history of travel. Of the patients, 2.22% developed eschar and 38.89% had lymphadenopathy. Rapid test analysis revealed that 70 (77.77%) were negative and 20 (22.22%) were positive. Of the twenty positive samples, eleven were male, nine were female, and there were six children and fourteen adults.The current study's fast IgM analysis seropositivity is lower than that of Usha K et al. [15] and Meerah et al. [14]. IgM ELISA Analysis: Out of 80 (88.88%) non-reactive samples, 10 (11.11%) were reactive. IgM ELISA's seropositivity contrasts with research conducted by Jacob AE et al. [16]. IgM ELISA's sensitivity and specificity were determined to be 30.0% and 94.28%, respectively. In ELISA, there was no discernible significance difference between males and females.

Comparison of the IgM Rapid test and IgM ELISA: According to the current study, the seroprevalence of Scrub Typhus in Warangal was 11.11% while the seropositivity of the rapid agglutination was 22.22%.when using IgM ELISA as a standard for diagnosis. The findings of the IgM ELISA analysis and the IgM fast test showed a substantial difference, as shown in Table 7. Based on the aforementioned data, we discovered that the Progen OXK antigen suspension kit's Sensitivity of Rapid Agglutination (Weil-Felix) test was 60.0%. In the current investigation, the sensitivity of the quick agglutination test is lower than that of Meerah et al. and Seema Rani et al., but

it is higher than that of Jacob AE et al. [16] The current study's quick agglutination test analysis's sensitivity and specificity are on par with Seema Rani et al. [17] which has similar significant fatality rate**.**

Conclusion

Our goal is to present a thorough examination of the epidemiology, prevention, and management of scrub typhus in both its historically endemic regions and newly identified areas of infection. Scrub typhus has emerged as a major cause of infectious fever and a major infectious illness in India. Scrub typhus case fatality rates have varied from 9.8 to 68.8%, depending on the complications, according to data from another research. Other studies from south India have found almost identical death rates, which the authors ascribed to a lack of knowledge about this illness.

According to an observational research from the same area of south India, mortality has decreased as people's awareness of this infectious disease has grown. It is evident from our current investigation that Scrub typhus is quite prevalent in the community. However, it is usually moderate, but in a small number of cases, it can cause major complications or even death.

Therefore, in order to prevent the disease and its deadly complications, as well as to lower the morbidity and mortality linked to the disease, clinicians should develop a high degree of clinical suspicion and consider scrub typhus as an important differential diagnosis when dealing with cases of pyrexia of unknown origin.

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