



Research Article

SCREENING OF SCRUB TYPHUS IN A CASE OF UNDIFFERENTIATED FEBRILE ILLNESS FROM A TERTIARY CARE CENTER

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Abstract- Introduction: Scrub typhus(ST) being a re-emerging zoonotic Rickettsial disease, presenting as undifferentiated febrile illness and goes under diagnosed due to low suspicion rate.

Objective: To study the incidence of Scrub typhus in samples meant for fever panel investigation in a tertiary care center. To compare and correlate the statistical significance of blood biochemical and blood counts.

Method: A cross-sectional study was conducted for 6 months. During which 122 samples were screened for scrub typhus using Immuno chromatographic test (ICT) and Enzyme Linked Immuno Sorbent Assay (ELISA). Both hematological and biochemical parameters were recorded for all the samples to compare their clinical significance. A detailed clinical history was collected and recorded for 14 Scrub typhus positive patients.

Results: Out of 122 samples screened 22 (18%) of them were positive for ST either by ICT or ELISA. Among the 14 ST patients 6(42.9%) of them presented with co morbidities viz., pulmonary tuberculosis 2(14.3%) followed by 1(7.1%) each with Multiple organ dysfunction, Acute Respiratory Distress Syndrome (ARDS), Acute kidney injury (AKI), and Chronic Kidney Disease (CKD). Incidentally 3(21.4%) of the patients had co infections with dengue, hepatitis A and B.

Summary and Conclusion: Our study shows ST to be considered as one of the common cause for febrile illness as the incidence was 18 % with male preponderance. Early diagnosis and treatment can bring down the morbidity and mortality. Hypoalbuminemia had a statistical significance (p-value: 0.004) than other parameters. Further studies like molecular assays can give more information.

Keywords- Hypoalbuminaemia, ARDS, Acute kidney injury, ELISA, Undifferentiated febrile illness.

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Introduction

Scrub typhus is a re-emerging vector borne zoonotic disease caused by obligate intra-cytoplasmic bacterium *Orientia tsutsugamushi* that is transmitted by the prolonged feeding of larval trombiculid mites. The clinical presentation ranges from mild (eschar, fever, headache and myalgia) to severe form (acute respiratory distress syndrome, meningoencephalitis and acute kidney injury causing death). Annually one million cases of Scrub typhus are reported globally with 10 % fatality rate [1, 2] and 6% mortality rate in untreated cases [3]. In India scrub typhus is the commonest among rickettsial diseases. Scrub typhus was thought to be prevalent in Himachal Pradesh, Jammu & Kashmir and southern parts of the country only (Tamil Nadu & Kerala) [4]. Since scrub typhus is evolving as an important etiological agent for undifferentiated febrile illness and very often under diagnosed or under reported, we screened for the incidence of scrub typhus in our tertiary care center in all the cases of undifferentiated febrile illnesses.

Materials and Methods

A cross sectional study was conducted, in the Central Clinical laboratory services, Department of Microbiology, Sri Ramachandra Medical College & Research Institute, a tertiary care center with 1800 bedded facility, after obtaining the approval of institutional ethics committee (CSP/15/MAR/40/09). A total of 122 blood sample sent for the fever panel investigation during the period of 1.05.2015 to 31.11.2015 was included in the study and samples representing proven disease

conditions were excluded from the study. The test samples were screened for scrub typhus using Immuno-chromatographic test (ICT) kit commercially procured from SD Bioline (standard diagnostics, INC, Korea, lot no: 18AD 15002) which detects the presence of IgG, IgM or IgA in the patient sample using major surface protein 56kDa antigen of *Orientia tsutsugamushi* and simultaneously Enzyme linked immuno sorbent assay (ELISA) using the kit Scrub typhus Detect TM IgM ELISA system procured from In Bios international, Inc. USA which detects the IgM antibodies to Recombinant major outer membrane protein antigen (r56). The tests were performed as per the manufactures instruction using standard controls.

The hematological (total count, hemoglobin level, platelet count) and biochemical parameters (SGOT, SGPT, total bilirubin, alkaline phosphatase, total protein, serum creatinine, and albumin) of the enrolled patients were recorded and compared for their statistical significance. Statistical analysis was done using the T- test using SPS software. In all the Scrub typhus, positive patients a detailed clinical history was obtained and recorded.

Results

A total of 122 cases were enrolled in the study out of which 75 (61%) were males and 47 (39%) were females. Male female ratio is 1.6:1. Age and sex wise distribution of patient's sample is shown in [Table-1].

Among the 122 samples tested 22 (18%) of them were positive for Scrub typhus either by ICT or ELISA techniques, out of which 13 (59%) were males and 9 (41%)

were females. In the 22 positive patient samples, 15 (68.2%) were positive by both ICT and ELISA method and the remaining seven, 6 (27.3%) were positive by ICT only and 1(4.5) by ELISA only.

Table-1 Age and sex wise distribution of the study subjects enrolled in the study

AGE	MALE	FEMALE	TOTAL
1-10	0(0%)	1(0.8%)	1(0.8%)
11-20	7(5.7%)	4(3.3%)	11(9%)
21-30	14(11.5%)	13(10.7%)	27(22%)
31-40	11(9%)	9(7.4%)	20(16.5%)
41-50	9(7.4%)	2(1.6%)	11(9%)
51-60	14(11.5%)	8(6.6%)	22(18%)
61-70	8(6.6%)	3(2.5%)	11(9%)
71-80	9(7.4%)	6(4.9%)	15(12.4%)
81-90	2(1.6%)	1(0.8%)	3(2.5%)
91-100	1(0.8%)	0(0%)	1(0.8%)
Total	75(61.5%)	47(38.5%)	122(100%)

Out of the 22 Scrub typhus positive patients only 14 patient's clinical history could be collected and recorded. The signs and symptoms of the same is represented in [Table-2]. The analysis and clinical significance of laboratory parameters of the 22 Scrub typhus positive patients are shown in the [Table-3 & 4].

Among the 14 scrub typhus patients whose history could be elicited 6 (42.9%) of them presented with co morbidities in the form of Multiple organ dysfunction in 1

(7.1%) patient, ARDS in 1(7.1%)patient, acute kidney injury (AKI) in 1 (7.1%) patients, pulmonary tuberculosis in 2 (14.3%) patients and CKD in 1(7.1%). Co infections were also encountered in 3(21.4%) patients. One patient was co infected with Dengue and other the two patients with hepatitis B and hepatitis A virus respectively.

Table-2 Signs and symptoms of Scrub typhus positive patients

Symptoms n=14	Number of cases / percentage
Fever	12(86%)
Myalgia	3 (21.4)
Cough	3 (21.4)
Head ache	3 (21.4)
Breathlessness	4 (28.6)
Nausea	2 (14.3)
Vomiting	4 (28.6)
Abdominal pain	3 (21.4)
Loose stool	4 (28.6)
Edema	3 (21.4)
Signs n=14	Number of cases / percentage
Hepatomegaly	1 (7.1)
Splenomegaly	2 (14.3)
Eschar	Nil
Rash	Nil
Altered sensorium	1 (7.1)

Table-3 Blood parameters of Scrub typhus positive patients

Parameters	Total n=122	Scrub Typhus negative n= 100	Scrub Typhus positive n=22	p Value
Total leukocyte count				
Normal (4000-11000)	63(51.6%)	50 (%)	3(13.6%)	0.691
Lowered (<4000)	16(13.2%)	13(13%)	13(59.1%)	
Elevated (>11000)	43(35.2%)	37(37%)	6(27.3%)	
Total	122(100%)	100(100%)	22(100%)	
Platelet count				
Lowered (<1.5)	32(26.2%)	26(26%)	7(31.8%)	0.546
Normal (>1.5)	90(73.8%)	74(74%)	15(68.2%)	
Total	122(100%)	100(100%)	22(100%)	
Serum creatinine				
Normal (<1.4)	102 (83.6%)	67(67%)	18 (81.8%)	0.092
Elevated (>1.4)	20(16.4%)	33(33%)	4 (18.2%)	
Total	122(100%)	100(100%)	22(100%)	

Table-4 Liver function test results of Scrub typhus patients

Parameters	Total n=122	Scrub typhus negative n=100	Scrub Typhus positive n=22	p Value
SGOT				
Normal (<40)	64(52.5%)	55(55%)	9(40.9%)	0.559
Elevated (≥40)	58(47.5%)	45(45%)	13(59.1%)	
Total	122(100%)	100(100%)	22(100%)	
SGPT				
Normal (<40)	67(54.9%)	60(60%)	7(31.8%)	0.631
Elevated (≥40)	55(45%)	40(40%)	15(68.2%)	
Total	122(100%)	100(100%)	22(100%)	
Alkaline phosphatase				
Normal (<129)	95(77.9%)	21(21%)	16(72.7%)	0.104
Elevated (≥129)	27(22.1%)	79(79%)	6(27.3%)	
Total	122(100%)	100(100%)	22(100%)	
Albumin				
Lowered (<3.2)	26(21.3%)	21(21%)	9(40.1%)	0.004 (significant)
Normal (≥3.2)	96(78.7%)	79(79%)	13(59.9%)	
Total	122(100%)	100(100%)	22(100%)	
Total bilirubin				
Normal (<1.2)	95 (77.9%)	78(78%)	17 (77.3%)	0.484
Elevated (≥1.2)	27 (22.1%)	22(22%)	5 (22.7%)	
Total	122(100%)	100(100%)	22(100%)	

Discussion

Scrub typhus is one of the reemerging diseases with increased number of cases being reported from India [5]. The incidence of scrub typhus commonly occurs in rainy and hilly areas with moisture and scrub vegetation. However it can also occur in diverse habitats including sea shore, rice fields and semi dessert areas.[6]. Of late, the clinical presentation of scrub typhus lacking the classical

signs and symptoms (viz. - Eschars, Maculo-papular rash, lymphadenopathy, etc) and present as nonspecific symptoms, indistinguishable from other acute febrile illnesses. This unclear presentation makes it challenging for the treating physician to clench the diagnosis and treat.

In our study, the positivity rate of Scrub typhus was 18%. Various studies from different parts of the country have reported the positivity rate ranging from 14.2 %

to 49.2 % [Table-6]. The highest positivity rate of 49.1% was reported from Rajasthan [7].

While considering the relationship between the seasonal variation and Scrub typhus, our study was carried out from the month of May 2015 till the month of November 2015. The high positivity rate was seen in the month of October which is during the post monsoon period (n=22) 31.8%. Similar to our study, Bithu R, Narvencar KPS, and Gupta N reported a high incidence of Scrub typhus during the months of September to November [7-9]. Gupta N reported an incidence rate of 87% in the months of September to October, similarly Bithu R also reported an incidence of 49.1% during the same months. Increased incidence of Scrub typhus during this season, could be due to the harvesting actions taking place in this period and resulting increased risk of exposure to larval bites. [8] Scrub typhus incidence is not restricted to just monsoon seasons but may extend into the cooler months. Mathai in their study observed increase in Scrub typhus cases during the cooler months (October to February) [10]. Similarly, Parul Sinha reported with a positivity rate of 24.7% in the cooler months [11]. This seasonal increase in positivity rates should make the physicians to suspect Scrub typhus also as a causative agent in acute febrile illness as common as dengue and malaria in those seasons. However, in contrast, a study done by A Ramyasree et al., 2015 showed 39% Scrub typhus cases in the months of January- March [5].

Table-5 Scrub typhus positivity rate in various studies

Name of study	Year of study	Place of study	Positivity rate
Sinha P et al.,	2012	Jaipur	24.7%
Bithu R et al.,	2012	Rajasthan	49.1%
Aggarwal H K et al.,	2012	Haryana	25%
Gupta N et al.,	2015	New Delhi	14.4%
Mittal G et al.,	2013	Dehradun.	14.42%
Our study	2015	Chennai	18%

In the present study among the 22 Scrub typhus positive patients, the highest positivity rate (91%) was found in the months of May to October.

Males are commonly affected more by scrub typhus than the female's due to their outdoor Occupational activities. In our study, the male female ratio is 1.4:1 (Male 59% & Females 41%). Similarly, Ramyasree A and Oberoi A also had male predominance

in their study [5,12]. In contrast Bithu R in his study from Rajasthan had female predominance (62.7%), which could be due to the dynamic involvement of females in field and farm works. Narvencar KPS (67%) and Sinha P (66.7%) had also similar findings in par with Bithu R [7, 8, 11].

In our study scrub typhus was found positive most commonly in the age group of 31-45 years (40.9%) followed by 46-60 years (27.3 %). On contrary, Bithu R in his study has reported a maximum of 47.4% of Scrub typhus positive cases in the age group of 16-30 years, followed by 21.8 % in the age group of 0-15 years [7].

Out of the 22 positive Scrub typhus patients, only 14 patient's clinical history could be retrieved as the remaining 8 were treated as out-patients.

The commonest clinical presentation was fever (85.7%) which was high grade associated with chills and Rigor. Similar presentation of high fever associated with chills and rigor was observed by Ramyasree A [5] and Dass R [13] in their study group. The mean duration of fever in our study was 15.4 days, which was almost in concordance with a study by Oberoi A (14.71 days) [12].

The [Table-6] shows the comparison of clinical symptoms of the present study with other studies. Presence of eschar in the earlier stages of the disease is a hint for making the diagnosis of Scrub typhus. The frequency of eschar varies from 7-97% as shown in various studies. 14 Some studies from South East Asia and Indian subcontinent have reported very few eschars or total lack of them which is comparable to our study as no single patient presented with eschar [5, 7, 8, 10].

The Laboratory parameters of the patients with febrile illness and Scrub typhus positive patients were compared for its statistical significance using t- test [Table-7].

In our study, 27.3% n=22 of the Scrub typhus positive patients had leucocytosis and 40.1% n=22 had hypoalbuminaemia. However, Hypoalbuminaemia alone had a significant p-value (0.004) when analyzed statistically [Table-4]. Narvencar KPS in their study reported a high rate of 67% of cases presenting with leukocytosis and 60% of cases presenting with Hypoalbuminaemia [8] Similarly Kim SW and Lee CS also had Leucocytosis or hypoalbuminaemia as associated laboratory findings in their patients who presented with severe Scrub typhus. [15, 16] Elevated SGOT and SGPT levels were in concordance with other studies [6, 17, 18].

O. tsutsugamushi antibodies showed focally scattered positive immunoreactions in the cytoplasm of some hepatocytes. This suggests that scrub typhus hepatitis.

causes mild focal inflammation due to direct liver damage [19].

Table-6 Comparison of clinical symptoms of our study with others

Parameters	Our study (%) 2015	Aggarwal H K 2014 (%) [13]	Sinha P 2014 (%) [22]	Narvencar K PS 2012 (%) [18]
Duration of study	6 months	5 months	3 months	16 months
No. of cases	14	25	42	15
Fever	83	88	100	80
Myalgia	21.4	64	47.6	80
Cough	21.4	-	14.2	46.7
Head ache	21.4	60	38	-
Breathlessness	28.6	48	83.3	60
Nausea/ vomiting	42.9	52	40.4	100
Abdominal pain	21.4	48	14.2	46.7
Loose stool/ diarrhea	28.6	24	11.9	26.7
Oedema	21.4	-	-	40
Splenomegaly	14.3	36	35.7	26.7
Hepatomegaly	7.1	40	59.5	60
Altered sensorium	7.1	16	19	6.7
Eschar	Nil	12	Nil	13.3
Mortality	Nil	12	Nil	33.5

Table-7 Hematological parameters of Scrub typhus positive patients

Laboratory parameters	Our study 2015	Sinha P 2014 [22]	Bithu R 2014 [16]	Narvencar K P 2012 (%) [18]	Mittal G 2015 [25]	Aggarwal H K 2014 [13]
TLC <4000	59.1	11.9	6	-	12.2	44
TLC 4000-11000	13.6	71.4	32.3	-	-	24
TLC >11000	27.3	16.6	39.1	66.7	-	32
Thrombocytopenia	31.8	85.7	63.9	39.1	29.9	-
Thrombocytosis	68.2	14.2	-	-	-	-
Elevated SGOT	59.1	94.6	69.1	80	51.4	84
Elevated SGPT	68.2	94.6	51.8	80	27.2	84
Elevated ALP	27.3	60	48.8	60	42.5	72
Hypoalbuminaemia	40.1	-	-	60	48.2	-
Elevated creatinine	18.2	60	15	33.3	-	24
Elevated bilirubin	22.7	33.3	27.1	46.7	48.2	-
Anaemia	77.3	-	-	-	86.1	36

Hepatitis in patients with Scrub typhus has been reported with the range from 58.5%-95% in recent review articles [20]. In our study, out of 22 Scrub typhus positive patients, 16 (73%) had altered liver enzyme parameters (SGOT and SGPT), 6(27.3%) had elevated alkaline phosphatase, 5 (22.7%) had elevated total bilirubin and 9(40.1%) had lowered albumin levels, which could be due to the inflammation of the liver following the Scrub typhus infection. Gupta N in his study has reported 54.5% of mild hepatitis. Reasonable elevation of hepatic transaminases which may rise till 90% had been reported from a study from Taiwan by Yang CH [20], which had been documented, from certain studies. Hu ML in their study showed elevated levels of SGOT, SGPT, ALP and total bilirubin with a percentage of 89.3% of, 91.7% of, 84.2% of and 38.5% respectively [21].

Acute respiratory distress syndrome (ARDS) is one of the life threatening complications following Scrub typhus infection [7]. Gupta N in his study had a significant preponderance of pulmonary manifestations in 69.7% of patients and in which 18.2% of them progressed to ARDS [9]. Two recent Indian studies from Himalaya's and Pondicherry performed by Mahajan SK[22] and Vevekanandhan M [18] respectively has observed 8-10% incidence rate of ARDS. Whereas only 1(7.1%) of our patient had ARDS.

Another frequently faced clinical problem with scrub typhus positive cases are Acute kidney injury (AKI) which is often mild and non oliguric [7]. Two (14.3%) of our patients presented with AKI. Gupta N [9] and Oberoi A [12] had 18.2% and 31.6% of the patients respectively with AKI which was more in number when compared with our study. Sinha P, in his study had reported about 60% of their patients who presented with renal impairments [16].

Co-viral infections were reported in 3 (21.43%) of our patients among which one was positive for dengue and 2 others were positive for hepatitis A and B virus respectively. Yeniden KH had four of his patients suffering from co-infections (2 with dengue and 2 with malaria) [23].

Multi-organ dysfunction is another common crisis faced in severe Scrub typhus infection. A study from Chennai by Ramyasree A had reported 6.25% of their patient's mortality with multi organ dysfunction [5]. In our study, mortality due to multi organ dysfunction was seen in one patient (7.1%). Indian studies have documented the mortality rate in Scrub typhus ranging from 2-12% and our study's mortality rate falls within this range. There are studies which shows higher incidence of mortality range going up to 33.5% [23].

Conclusion

As evident from our study Scrub typhus has to be considered as one of the common etiological agent in a case of undifferentiated acute febrile illness as, early diagnosis and treatment can bring down the morbidity and mortality. Biochemically hypoalbuminemia had a statistical significance (p-value: 0.004) More samples and molecular correlations would have thrown light to our study.

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