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Original Research Article

CLINICO-EPIDEMIOLOGICAL PROFILE AND COMPARATIVE EVALUATION OF SEROLOGICAL AND MOLECULAR METHODS IN DIAGNOSIS OF PEDIATRIC SCRUB TYPHUS IN A TERTIARY CARE CENTRE IN SOUTH INDIA

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Abstract

Background: Acute febrile illnesses, particularly scrub typhus, are common causes of tropical infections in children. Diagnosis is challenging owing to its ambiguous clinical presentation, and commonly used tests include ELISA and PCR. This main aim is to study the clinico-epidemiological features of scrub typhus among children presenting with acute febrile illness in a tertiary care institute and to compare the ability of nested PCR and IgM ELISA to detect scrub typhus infection. **Materials and Methods:** Children with fever for more than three days attending both outpatient departments who met the inclusion criteria were included in the study. A detailed history, clinical examination, anthropometric details with vital signs, and systemic examination were performed and documented. Under aseptic precautions, a peripheral venous blood sample was collected and sent for PCR and scrub serology. **Result:** Among 203 children, 111 were male, and 92 were female. The most frequent symptom was periorbital puffiness (52.2%); the most common was fever. Scrub typhus was strongly suspected in a child with an elevated total count, positive C-reactive protein, Hb, anaemia, hyponatraemia, and hypoalbuminaemia. Scrub PCR was positive in only 20 of the 98 cases of IgM ELISA positivity. However, the cases were negative for scrub IgM by ELISA or PCR. This indicates that the sensitivity of IgM ELISA for detecting positive cases was greater than that of nested PCR. **Conclusion:** Scrub typhus is a common disease in both rural and urban areas, with an incidence of 48.3%. Pathogenesis involves eschar and clinical features such as splenomegaly, hepatomegaly, and puffiness. IgM ELISA is more sensitive than nested PCR.

INTRODUCTION

Acute febrile illness constitutes most children attending the outpatient departments of most hospitals. Tropical infections remain a major cause of acute undifferentiated febrile illness. Dengue, enteric fever, scrub typhus, malaria, and leptospirosis were the most common diseases. Scrub typhus belongs to the group of rickettsial infections, and the incidence in Tamil Nadu is estimated to be 31% in a recent study.[1] The clinical presentation of scrub typhus varies from a simple undifferentiated fever to potentially fatal complications, including myocarditis, meningoencephalitis, and pneumonitis.[2]

Scrub typhus was first considered confined to rural areas, but our data suggest that scrub typhus is equally common in urban areas. The ambiguous nature of the clinical presentation makes diagnosing this disease clinically difficult.[3] Hence, the diagnosis mainly depends on molecular and serological diagnostic methods. Although indirect immunofluorescence assay (IFA) remains the gold standard, it is not widely available. Commonly used tests include the detection of IgM antibodies using Enzyme-linked Immunosorbent assay (ELISA) and polymerase chain reaction (PCR) for scrub antigens.[4-6] This main aim was to study the clinico-epidemiological features of scrub typhus among children presenting with acute febrile illness in a tertiary care institute and to compare the ability of

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nested PCR and IgM ELISA to detect scrub typhus infection.

MATERIALS AND METHODS

The institutional ethics committee approved this study before its initiation. Informed consent was obtained from all participants before their inclusion in the study.

Inclusion Criteria

Patients with an undifferentiated fever for >3 days, fever with thrombocytopenia, and fever with eschar were included in the study.

Exclusion Criteria

Immunocompromised patients with AIDS/lymphoma, secondary malignancy, neutropenia, bleeding disorders, and fever for more than four weeks duration (pulmonary tuberculosis, etc.) were excluded.

Methods: Children with a fever for more than three days attending both outpatient departments who met the inclusion criteria were included in the study. A detailed history, clinical examination, anthropometric details with vital signs, and systemic examination were performed and documented. Under aseptic precautions, a peripheral venous blood sample was collected and sent for PCR and scrub serology. All information obtained was recorded in a predesigned format, and the results were analysed.

For the IgM ELISA, serum from the venous sample was separated by centrifugation at 2500 rpm for 15 min and then stored at -20 °C. A scrub typhus detection kit (InBios International Inc., Seattle, USA), which utilises a recombinant antigen of Orientia tsutsugamushi to detect IgM antibodies by ELISA, was employed in this study. The dilution of the test sample was 100 µL of 1:100 diluted serum sample. The cut-off was fixed at 0.406, and this was calculated based on the Optical Density (OD) values from 300 samples of known scrub typhus infections, sera from normal healthy controls, and sera from patients with other infections.

For PCR - Blood collected in the anticoagulant-coated tube was centrifuged at 2200rpm for 10 min to separate the buffy coat and was stored at -70oC for future DNA extraction. Nested PCR was performed to identify the 56kDa type-specific antigen gene under the standard protocol.

Statistical analysis: Descriptive and Inferential statistics were analysed using IBM SPSS version 20.0 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp). The mean and SD were used to summarise quantitative data between the groups (Age and Fever). Frequency and percentage were used to summarise the qualitative data (clinical parameters of symptomology, presenting clinical values, IGM and PCR positivity, and complications). Where applicable, the chi-square test with Yates Continuity correction was used to compare qualitative and categorical data between the IGM-positive and IGM-negative groups and the IGM-negative and scrub PCR-positive groups. Statistical significance was set at $P < 0.05$.

RESULTS

Among the 203 children enrolled, the mean age group was 5.7 years, while the average duration of fever was 6.3 days (SD - 2.72). Among the 203 children, 111 were male and 92 were female.

The most frequently occurring symptom was periorbital puffiness (52.2%), followed by vomiting (42.9%), while the IgM ELISA for scrubs was positive in 98 children (48.3%) and negative in the remaining 105 children (51.7%).

Ninety-eight children tested positive for scrub serology using IgM ELISA. Among the enrolled patients, the most common symptom was fever, followed by vomiting (39.8%), abdominal pain (22.4%), cough (16.3%), and loose stool (12.2%) [Table 1].

The most common finding in children with scrub typhus was eschar, which was present in 69.4% of scrub typhus cases, followed by periorbital puffiness (52%), hepatomegaly (49%), and splenomegaly (27.6%).

Among children positive for scrub IgM ELISA, leukocytosis was found in 42.9%, whereas leukopenia was found in only 7.1%. The remaining 49% of the children had normal total counts. Thrombocytopenia was the predominant finding in children with scrub typhus (69.4%), while the remaining children had a normal platelet count (30.6%). Anaemia was found in 72 cases (73.5%) of scrub typhus. C-reactive protein was positive in 72 cases (73.5%) by scrub IgM ELISA.

Regarding Laboratory parameters, there was a higher incidence of hypoalbuminaemia (93.9%) and hyponatraemia (61.2%) in the children with scrub typhus. Urea was elevated in 7 children, but creatinine was normal in all children recruited in the study who were positive for scrub IgM ELISA, showing that pre-renal acute kidney injury can occur in scrub typhus. Hyperbilirubinemia was found only in 4.1% of children with scrub typhus. However, elevation of SGOT and SGPT enzymes was found in nearly 78% of the children with scrub typhus [Table 2].

39 out of 98 IgM scrub-positive children showed symptoms such as vomiting, loose stools, yellow urine discolouration, cough, breathlessness, bleeding, abdominal pain, abdominal distension, oliguria, and seizures. There was no significant difference in IgM scrub positivity symptoms ($p>0.05$).

Examination revealed a significant association between eschar and scrub typhus ($p=0.0001$). Eschar was present in 68 cases among the 98 IgM scrub-positive children. This confirmed that eschar is the pathognomonic of scrub typhus.

Splenomegaly ($p=0.004$) was found in 27 IgM scrub-positive children, while hepatomegaly ($p=0.0001$) was found in 48 of the 98 positive children. This

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establishes a significant association between splenomegaly and hepatomegaly with scrub typhus.

In the investigation, scrub typhus was strongly suspected in a child with elevated total count, positive C-reactive protein, Hb, anaemia, hyponatraemia, and hypoalbuminaemia [Table 3 and 4].

IgM scrub and nested PCR abilities were compared to detect positive scrub typhus cases. It is noteworthy that all 98 children who were positive for scrub IgM ELISA became afebrile within 48 hours of starting doxycycline treatment. Scrub PCR was positive in only 20 of the 98 cases of IgM ELISA positivity. However, the cases were negative for scrub IgM by ELISA or PCR. This indicates that the sensitivity of IgM ELISA for detecting positive cases was greater than that of nested PCR [Table 5].

Table 1: The incidence of 203 children and scrub typhus (IgM ELISA positive)

Symptoms	INCIDENCE (%)	
	Among children enrolled (n=203)	Scrub Typhus (IgM ELISA positive)
Vomiting	49.2	39.8
Loose stools	12.3	12.2
Cough	15.8	16.3
Breathlessness	3.4	5.1
Yellowish discoloration of urine	1	2
Bleeding manifestations	2	3
Abdominal pain	19.7	22.4
Abdominal distension	1.5	2
Rash	1.5	3.1
Oliguria	5.4	7.1
Seizures	5.9	4.1

Table 2: Incidence of various examination and lab findings with scrub typhus

		Incidence (%)
Examination finding	Pallor	7.1
	Icterus	1
	Periorbital puffiness	52
	Pedal oedema	3.1
	Lymphadenopathy	9.2
	Eschar	69.4
	Splenomegaly	27.6
	Hepatomegaly	49
	Tachypnea	5.1
	Poor perfusion	3.1
	hypotension	2
Laboratory parameter	Urea elevated	7.1
	Creatinine elevated	0
	Hyponatremia	61.2
	Hypoalbuminemia	93.9
	Hyperbilirubinemia	4.1
	SGOT/PT elevation	78.6

Table 3: Comparison of various parameters with IGM ELISA positive and negative

		IGM ELISA Positive (n=98)	IGM ELISA Negative (n=105)	P value
Vomiting	Absent	59(60.2)	57(54.3)	0.39
	Present	39(39.8)	48(45.7)	
Loose stools	Absent	86(87.8)	92(87.6)	0.97
	Present	12(12.2)	13(12.4)	
Cough	Absent	82(83.7)	88(83.8)	0.88
	Present	16(16.3)	17(16.2)	
Breathlessness	Absent	93(94.9)	103(98.1)	0.21
	Present	5(5.1)	2(1.9)	
Yellow discoloration of urine	Absent	96(98)	105(100)	0.14
	Present	2(2)	0	
Bleeding manifestations	Absent	95(97)	103(98.1)	0.93
	Present	3(3)	2(9)	
Abdominal pain	Absent	76(77.6)	86(81.9)	0.52
	Present	22(22.4)	19(18.1)	
Abdominal distension	Absent	96(98)	104(99)	0.52
	Present	2(2)	1	
Rash	Absent	93(94.9)	105(100)	0.068
	Present	3(3.1)	0	
Oliguria	Absent	91(92.9)	100(95.2)	0.3
	Present	7(7.1)	4(3.8)	
Seizures	Absent	94(95.9)	97(92.4)	0.28
	Present	4(4.1)	8(7.6)	
Eschar	Absent	30(30.6)	102(97.1)	0.0001
	Present	68(69.4)	3(2.9)	

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Pallor	Absent	90(91.8)	96(91.4)	0.89
	Present	7(7.1)	8(7.6)	
Icterus	Absent	95(96.9)	105(100)	0.29
	Present	1(1)	0	
Periorbital oedema	Absent	47(48)	50(47.6)	0.96
	Present	51(52)	55(52.4)	
Lymphadenopathy	Absent	89(90.8)	100(95.2)	0.12
	Present	9(9.2)	4(3.8)	
Pedal oedema	Absent	95(96.9)	103(98.1)	0.59
	Present	3(3.1)	2(1.9)	
Splenomegaly	Absent	71(72.4)	93(88.6)	0.004
	Present	27(27.6)	12(11.4)	
Hepatomegaly	Absent	49(50)	80(76.2)	0.0001
	Present	48(49)	25(23.8)	
RR	Absent	93(94.9)	104(99)	0.08
	Present	5(5.1)	1	
Perfusion	Absent	95(96.9)	104(99)	0.28
	Present	3(3.1)	1	
BP	Normal BP	96(98)	105(100)	0.14
	Hypotension	2(2)	0	
Total count	Low	7(7.1)	21(20)	0.005
	Normal	49(50)	57(54.3)	
	High	42(42.9)	27(25.7)	

Table 4: Comparison of various parameters with IGM ELISA positive and negative

		IGM ELISA Positive (n=98)	IGM ELISA Negative (n=105)	P value
CRP	Negative	26(26.5)	69(65.7)	0.001
	Positive	72(73.5)	36(34.3)	
HB	0	26(26.5)	51(48.6)	0.001
	1	72(73.5)	54(51.4)	
Platelet count	Normal	30(30.6)	43(41)	0.12
	Thrombocytopenia	68(69.4)	62(59)	
Sodium*	Normal	37(37.8)	61(58.1)	0.008
	Hyponatremia	60(61.2)	42(40)	
Potassium	Hypokalemia	4(4.1)		0.075
	Normal	93(94.9)	102(97.1)	
	Hyperkalemia	1	3(2.9)	
Urea*	Normal	90(91.8)	101(96.2)	0.33
	High	7(7.1)	4(3.8)	
Creatinine	Normal	98(100)	105(100)	1
	High		0	
Albumin*	Normal	6(6.1)	43(41)	0.001
	Hypoalbuminemia	92(93.9)	55(52.4)	
Total bilirubin	Normal	94(95.9)	100(95.2)	0.81
	High	4(4.1)	5(4.8)	
SGOT	Normal	21(21.4)	31(29.5)	0.18
	High	77(78.6)	74(70.5)	
SGPT	Normal	42(42.9)	52(49.5)	0.34
	High	56(57.1)	53(50.5)	
Myocarditis	Negative	97(99)	105(100)	0.29
	Positive	1	0	
Pleural effusion	Negative	92(93.3)	103(98.1)	0.12
	Positive	6(6.1)	2(1.9)	
Pulmonary infiltrates	Negative	97(99)	105(100)	0.29
	Positive	1	0	

Table 5: Comparison of IgM scrub and Scrub PCR in diagnosing scrub typhus

			Frequency	Percentage
Among IGM ELISA-positive (n=98)		Negative	78	79.6
		Positive	20	20.4
Among Scrub PCR	Negative	Negative	102	56.7
		Positive	78	43.3
	Positive	Negative	3	13
		Positive	20	87

DISCUSSION

In our study, the enrolled children had an average of 5.7 years and presented with fever for a mean duration of 6.3 days. Among the 203 children, 98 tested positive for scrub typhus via IgM ELISA testing. The incidence of scrubs was nearly 48.2%, which is higher than that reported in other studies. Behera et al. reported an incidence of 26.3% in their study.[7] In comparison, Jacob et al. reported an incidence of 23%.[8] This also proves that scrub is no

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longer endemic only to rural areas, as our study was conducted in children presenting to a tertiary care centre well within the city's limits. Among the clinical features, the most significant was the presence of eschar in 68 cases of scrub typhus. This significant association was in line with the observations made by Varghese et al., Karthikeyan et al., and Vivekanandan et al.[9-11] Leukocytosis was present in 42.9% of the children positive for scrub typhus. This was a similar observation was made by Varghese et al., who reported 46% leukocytosis.[9]

Thrombocytopenia was present in 68% of cases, similar to the observations made by Varghese et al. and Karthikeyan et al.[9,10] This shows that scrub typhus commonly presents as fever with thrombocytopenia. Anaemia was significantly associated with scrub typhus. C-reactive protein positivity was significantly correlated with scrub typhus. Hyponatraemia and hypoalbuminaemia are significantly associated with scrub typhus. Hyponatraemia and hypoalbuminaemia are significantly associated with scrub typhus. Hyponatremia was observed in 61.2% of cases, which was very high when compared with the observations made by Ganesh et al. (35%).[12] This observation explains the vasculitis component leading to capillary leakage in scrub typhus.

The incidence of acute kidney injury in our study was 7.1%, with all cases showing an increase only in urea and normal creatinine levels. This incidence is in line with the observations made by Varghese et al.[9] Our incidence of AST elevation was 78.6%, which is less than the observation made by Ganesh et al. (91%).[12] Positive C-reactive protein had a significant association with scrub typhus. Four children had probable scrub encephalitis, one had scrub myocarditis, and one had scrub pneumonitis. This study indicated that IgM scrub can effectively detect cases of scrub typhus with a sensitivity higher than nested PCR. Ninety-eight cases were positive by IgM ELISA, while only 20 cases tested positive by nested PCR. The baseline against which these investigations were confirmed was a criterion of probable scrub typhus defervescence within 48 h of starting doxycycline treatment. Thus, this study clarifies that IgM ELISA can be an alternative to IFA for detecting scrub typhus. Nested PCR has a lower sensitivity than IgM ELISA.

CONCLUSION

Scrub typhus is endemic not only to rural areas but also to urban areas. In the present study, the incidence of scrub typhus was 48.3%. The presence of eschar is pathognomonic for scrub typhus. Clinical features such as splenomegaly, hepatomegaly, and periorbital puffiness are significantly associated with scrub typhus. Leukocytosis, thrombocytopenia, hyponatraemia, hypoalbuminaemia, and SGOT elevation are significantly associated with scrub typhus. IgM ELISA is more sensitive than nested PCR in detecting positive cases of scrub. Scrub PCR is highly specific for scrub typhus but has very low sensitivity. The low sensitivity of PCR is because it was performed on blood samples and not on the eschar aspirate.

Limitations

The PCR test and IgM ELISA method have not been compared with the gold standard indirect immunofluorescence assay; hence, the sensitivity and specificity of these tests against the gold standard could not be established. The low sensitivity of PCR could have been improved by sending aspirates from the eschar, as it has a higher yield (99.1%) than that from blood (37.5%).

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