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Original Research Article

Laboratory profile of scrub typhus in children admitted at Bankura Sammilani medical college, India: a cross-sectional study

Jadab Kumar Jana*, Anusree Krishna Mandal, M. Suhail Alam Mallick, Dipti Mahata

Department of Paediatrics, Bankura Sammilani Medical College and Hospital, Bankura, West Bengal, India

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***Correspondence:**
Dr. Jadab Kumar Jana,
E-mail: jadabjana69@gmail.com

ABSTRACT

Background: An in-depth evaluation of routine baseline investigations is required in clinically suspected cases of scrub typhus fever due to the unavailability and/or delayed arrival of serological testing in all remote health care facilities. This may assist medical professionals diagnose and promptly administer anti-scrub medication.
Methods: A cross-sectional research was conducted on 105 children who had scrub typhus fever admitted in a tertiary healthcare facility. The children were selected one after the other till desired sample size was achieved. Basic demographic information, clinical symptoms, and test results were all recorded. Software called EpiInfo 3.5.1 was used for analysing the collected information. For continuous variables, rate and ratio were used to express them, whereas standard deviation and mean were used for categorical variables. Once more, ANOVA was used for analysing association between categorical variables. P-values of <.05 were considered statistically significant.
Results: Of the 105 children, 90.48% were from country-side and 56.19% were male. The most common haematological abnormalities were anaemia (84.76%), leucocytosis (42.86%), and, thrombocytopenia (46.67%). The biochemical changes include hypoproteinaemia (28.57%), hypoalbuminemia (23.81%), hyponatremia (50.48%), and elevated serum levels of ALT (66.67%) and AST (84.76%). An extended duration of fever was shown to be related with a statistically significant (P value =< 0.05) association between mean haemoglobin concentration, leucocyte and thrombocyte count, and serum levels of sodium.
Conclusions: Early recognition and management of scrub typhus fever may be aided by the presence of anaemia, thrombocytopenia, leucocytosis, hypoproteinaemia, albuminemia, hyponatremia, and elevated serum levels of alanine and aspartate amino transferase.

Keywords: Children, Laboratory profile, Scrub typhus

INTRODUCTION

A re-emerging acute febrile illness that lacks differentiation is scrub typhus. *Orientia* (formerly Ricketsia) *tsutsugamushi*, an obligatory intracellular gram-negative coccobacillus that is a member of the Rickeetsae family, is the cause of it.[1,2] It is contracted by biting an infected "chigger," which is the 0.2-0.4 mm larval stage of trombiculid mites, which is only visible under a microscope or magnifying glass.[3,4] The bacteria multiply at the site of inoculation after the infected chigger bites, causing papules to ulcerate, turn necrotic, and eventually develop into an eschar. These lesions are typically found on the groin, genitalia, axilla, and neck where skin folds meet.[3,5] The bacteria cause endothelial cell invasion upon entering the bloodstream, which results in perivascular inflammatory lesions and disseminated vasculitis. The aforementioned conditions then cause microvascular leakage, tissue hypoperfusion, oedema, and end organ ischemic injury.[6] These pathological changes in the organ systems give rise to a wide range of clinical manifestations. The most frequent

clinical manifestations in children include fever, headache, vomiting, hepatosplenomegaly, abdominal pain, breathlessness, maculopapular rash, and lymphadenopathy.[6] Only 11-43% of patients have escarp, the pathognomic hallmark of scrub typhus and the untreated scrub typhus has a median fatality rate of 6% (0-70%).[7,8]

The current gold standard and reference test in serology is the indirect immunofluorescent antibody (IFA) test, which is not available in India.[9,10] It is based solely on the ELISA of *O. tsutsugamushi* to detect IgM, which is also not available in all remote health care facilities. So, in cases of undifferentiated fever, a high index of suspicion coupled with routine laboratory testing such as a complete blood count (CBC) and liver and renal function tests that are accessible in all healthcare facilities and careful interpretation of the results could let primary care physicians (PCPs) and paediatricians to prescribe early empirical antibiotics in order to reduce scrub typhus-related morbidity and mortality. The objective of this study was to analyze the laboratory profile of scrub typhus fever in children aged 1 month to 12 years old.

METHODS

This was hospital-based cross-sectional study conducted at Department of Pediatrics, Bankura Sammilani Medical College and Hospital (B.S.M.C.H.), West Bengal, India from September 2023 to November 2023. Children from one month to twelve years of age were included. This study was approved by the Institutional Ethics Committee.

Inclusion criteria

Children of one month to 12 years of age who will be admitted to the pediatric ward and pediatric intensive care unit (PICU) with fever for ≥5 days and subsequently diagnosed as Scrub typhus fever on the basis of a positive IgM for O. tsutsugamushi done by ELISA methods with a normal cut-off value of ≤0.5 OD. Either the parents or carers of the children gave consent for this study.

Exclusion criteria

Age of the children: one month and > 12 years. Either parents or guardians of the children who didn't give consent. The children diagnosed scrub typhus with other co-infections such as dengue fever, enteric fever, leptospirosis, and malaria were excluded.

Sample size

Total 105 **[**Calculation of sample size: for a cross-sectional study, to estimate the sample size, n = $(Z\alpha)2*P*(1-P)/d^2$ has been used, where Z_α = the normal standard variate at a 95% confidence interval, P = the prevalence of interest in the event, i.e., thrombocytopenia, 47.36%, and d = the error of precision accepted, i.e., 10%.[11] Putting all these values into the aforementioned formula, the sample size is n = 95. Adding 10% non-responders to the final sample size n = 105].

Study technique

All children fulfilling the inclusion criteria were enrolled consecutively until the required sample size was achieved. A detailed physical examination of study participants was done and subjected to different laboratory investigations, such as a complete blood count (CBC), c-reactive protein (CRP), urine analysis, renal and liver function tests (RFT and LFT), and serological testing (IgM by ELISA) for *O. tsutsugamushi*. Malaria parasite dual antigen (MPDA), widal test, and serology for dengue and leptospirosis were done to rule out malaria, enteric fever, dengue, and leptospirosis, respectively. Other laboratory investigations, such as Trop-T, PT, INR, and aPTT, were ordered when required. Imaging studies such as chest X-rays, USGs, CTs, and MRIs were also advised on a demand basis. All study participants received a standard level of care as per IAP guidelines.[12]

A predesigned, pretested interviewer-administered questionnaire, which was prepared by pilot testing, was used to collect the data by interviewing either parents or carers after taking consent or the patient himself, in the case of older children, regarding socio-demography and the history of the present illness. A detailed clinical finding, laboratory reports, management received, any complications, and outcome were also meticulously recorded.

Statistical analysis

Collected data was put into the Microsoft Excel spread sheet for analysis with the help of EpiInfo software version 3.5.1 (Developer: Centre for Disease Control and Prevention, USA). Continuous variables were expressed in terms of mean and standard deviation, whereas rate and ratio were for categorical ones. Categorical variables were again analyzed by the analysis of variance (ANOVA) test to find an association among them. A P-value of <0.05 was set as statistically significant.

RESULTS

A total of 105 children with scrub typhus fever were enrolled over a three-month period between September 2023 and November 2023. The purpose of this cross-sectional study was to analyze the laboratory profiles that could assist the primary care physician (PCP) in starting anti-scrub medication beforehand in those areas where an ELISA test for *O. tsutsugamushi* is not available.

There were 105 children total, with a male to female ratio of 1.28:1. The number of male children outnumbered the number of female children. Children between the ages of

one and five were the majority affected, accounting up 36.19% of all study participants. The overwhelming majority of the children, 90.48%, were from the countryside. The basic demographic information about research participants is displayed in Table 1.

Table 1: Basic demographic characteristics.

Variables	Subgroups	Total	Percentages (%)
Gender	Male	59	56.19
	Female	46	43.81
Age	1 month to 1 yrs	16	07.80
	>1 yr-5 yrs	38	36.19
	>5 yrs-10 yrs	28	26.67
	>10 yrs	23	21.90
Residency	Rural	95	90.48
	Urban	10	09.52

Table 1 highlights that while overall there were more male than female children in this study, the female infant was more affected in the one-month to one-year age group represented in Figure 1.

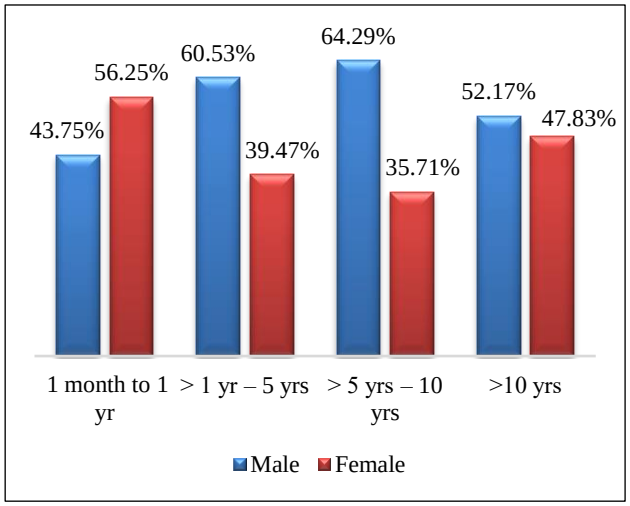

Figure 1: Gender and age-wise admission pattern.

Clinical symptoms and signs

The most frequently occurring clinical symptoms and signs in the present study were fever (100%), vomiting (26.67%), abdominal pain (20%), cough (27.62%), headache (32.38%), myalgia (25.71%), pallor (80.95%), hepatomegaly (60%), splenomegaly (54.28%), hepatosplenomegaly (43.81%), facial puffiness (27.62%), oedema (16.19%), lymphadenopathy (25.71%), conjunctival congestion (52.38%), and maculopapular skin rash (14.29%). A painless black crust at the site of the chigger bite, known as an eschar, is a pathognomonic sign of scrub typhus and was observed in 24.76% of the study participants. Clinical symptoms and signs are highlighted in Tables 2 and 3.

Table 2: Clinical symptoms at the time of presentation.

Variables	Subgroup	Total	Percentages
***Fever**	≥5-7 days	37	35.24
	8-14 days	52	49.52
	15-21 days	16	15.24
Gastroin-testinal symptoms	Vomiting	28	26.67
	Pain abdomen	21	20.00
	Loose stool	10	9.52
	Haematemesis	2	1.90
	Melaena	2	1.90
Respiratory symptoms	Cough	29	27.62
	Shortness of breath	11	10.48
CNS symptoms	Headache	34	32.38
	Altered sensorium	11	10.48
	Convulsion	8	7.62
Renal symptoms	Decrease urine output	3	2.86
	Haematuria	2	1.90
Musculoskele-tal symptoms	Myalgia	27	25.71
Ophthalmo-logical symptoms	Photophobia	5	4.76

*Mean ± SD = 9.71±4.22 days

Table 3: Clinical signs at the time of presentation.

Variables	Subgroup	Total no.	Percentage
Third spacing	Facial puffiness	29	27.62
	Oedema (pedal)	17	16.19
	Ascites	8	7.62
	Pleural effusion	3	2.86
	Pericardial effusion	1	0.95
Haemato-logical	Pallor	85	80.95
	Petechae	1	0.95
	Echymosis	1	0.95
	Lymphadenopathy	31	29.52
Gastro-intestinal	Hepatomegaly	63	60.00
	Splenomegaly	57	54.28
	Hepatosplenomegaly	46	43.81
Nervous system	Meningeal sign	10	9.52
Eye sign	Conjunctival congestion	55	52.38
Skin lesion	Eschar	26	24.76
	Maculopapular rash	15	14.29

Laboratory profiles

In order to identify any changes in organ function at the time of hospitalisation, various laboratory tests have been recommended in addition to ELISA for *O. tsutsugamushi*

IgM (cut-off value OD = <0.5). Thrombocytopenia, leucopoenia, anaemia, and leucocytosis were found in 46.67%, 3.81%, 42.86%, and 84.76% of the cases in the present study, respectively. In 86.67% and 91.43% of the study participants overall, acute-phase reactants like ESR and CRP were also elevated. Alanine and aspartate aminotransferase levels in the serum were elevated in 84.76% and 54.29% of the cases, respectively. In merely 3.81% of cases was there an increase in total serum bilirubin. Additionally, 28.57% and 23.81% of cases, respectively, had hypoproteinaemia and hypalbuminaemia. The present study also found hypokalaemia (22.86%), hyponatraemia (50.48%), uraemia (20%), and carotenemia (5.71%). Haematological and biochemical changes are highlighted in Table 4.

Table 4: Highlights the changes in haematological and biochemical parameter.

Variables	Subgroup	Total number	Percentages
Haemoglobin (gm/dl)	≥11	16	15.24
	<11	89	84.76
WBC (× 10^3 /mm^3)	>11	45	42.86
	4-11	56	53.33
	<4	4	3.81
Platelets (×10^5 /mm^3)	≥1.5	56	53.33
	<1.5	49	46.67
CRP (mg/L)	<6	9	8.57
	>6	96	91.43
ESR (mm in 1st hour)	≤20	14	13.33
	>20	91	86.67
ALT (U/L)	≤40	35	33.33
	>40	70	66.67
AST(U/L)	≤40	16	15.24
	>40	89	84.76
Bilirubin (mg/dl)	≤1	101	96.19
	>1	4	3.81
Total protein (gm/dl)	≥5.6	75	71.43
	<5.6	30	28.57
Albumin (gm/dl)	≥2.8	80	76.19
	<2.8	25	23.81
Sodium (mEq/L)	≥136	52	49.52
	<136	53	50.48
Potassium (mEq/L)	≥3.5	81	77.14
	<3.5	24	22.86
Urea (mg/dl)	≤28	84	80.00
	>28	21	20.00
Creatinine (mg/dl)	≤1.06	99	94.29
	>1.06	6	5.71
Urine analysis	≤5	101	96.19
(RBC/hpf)	>5	4	3.81

RBC- red blood corpuscles, WBC- white blood corpuscles, MCV- mean corpuscular volume, ESR- erythrocyte sedimentation rate, CRP- c-reactive protein, ALT – alanine aminotransferase and AST- aspartate aminotransferase

ANOVA was executed to analyse the association between haematological and biochemical features with the extended periods of fever. The results showed statistically significant (P value = <0.05) changes in mean leucocyte and platelet count, haemoglobin, and serum levels of sodium. The longer the fever went on, the lower the mean haemoglobin, platelets, and serum levels of sodium concentrations. Conversely, the longer the fever went on, the higher the mean leucocyte count. The length of fever did not affect any other laboratory parameter in a way that was statistically significant (P value = >0.05). Table 5 illustrates the association between haematological and biochemical parameters with the duration of fever in children who were enrolled.

Table 5: Association of haematological and biochemical parameters with prolonged fever in scrub typhus.

Laboratory parameter	Duration of fever			P value
	≤7 days	8-14 days	>14 days	
Hb% (gm/dl), mean±SD	9.85±1.14	9.08±1.49	8.94±0.85	0.011
RBC (×10^6/mm^3), mean±SD	4.03±0.41	4.03±0.46	3.93±0.42	0.702
WBC (×100^3/mm^3), mean±SD	8.87±4.33	11.13±5.37	11.3±4.37	0.03
Platelets(×10^5/mm^3), mean±SD	2.18±0.93	1.85±1.21	1.04±0.38	0.04
ALT (U/L), mean±SD	61.24±37.24	63.44 ±54.90	80.29±49.46	0.39
AST (U/L), mean±SD	61.24±37.28	79.24±72.98	89.14±81.31	0.26
Bilirubin (mg/dl), mean±SD	0.56±0.21	0.69±0.68	0.77±0.72	0.39
Total proten (gm/dl), mean±SD	6.29±1.07	5.99±1.15	5.79±0.91	0.246
Albumin (gm/dl), mean±SD	3.22±0.48	3.18±0.58	3.04±0.47	0.52
Sodium (mEq/L), mean±SD	136.55±4.38	135.91±4.91	133.21±2.99	0.046
Potassium (mEq/L), mean±SD	3.88±0.44	3.82±0.40	3.55±0.51	0.38
Urea (mg/dl), mean±SD	23.07±6.8	23.93±10.33	29.29±17.08	0.14
Creatinine (mg/dl), mean±SD	0.71±0.16	0.74±0.22	0.82±0.43	0.33

DISCUSSION

The objective of the present research was to carry out a thorough analysis of laboratory reports from 105 children, ranging in age from one month to twelve years, who had scrub typhus fever. This analysis would enable the PCP to diagnose the disease and start anti-scrub treatment early in areas where serological testing, such as IgM of scrub typhus, is not available.

Fifty-nine of the 105 children aged one month to twelve were male, and the remaining 46 were female. The male to female child ratio was 1.28:1. Boys may be more susceptible to chiggers because they engage in more outdoor play activities.[13-16] The current cohort's mean presentation age was 4.87 years, which is comparable to a study from Odisha, India.[11]

In all cases, there was a fever, which is consistent with the findings of the other studies.[17-19] Pallor (80.95), hepatomegaly (60%), splenomegaly (54.28%), hepatosplenomegaly (43.81%), conjunctival congestion (52.38%), vomiting (26.67%), abdominal pain (20%), cough (27.62%), headache (32.38), myalgia (25.71), facial puffiness (27.62%), oedema (16.19), maculopapular rash (14.29%), and lymphadenopathy (29.52%) were among the other more frequently observed clinical features in addition to fever. Although they varied in frequency, these observational findings were also reported in other studies conducted in various geographic locations.[20-24] "Eschar," the pathognomic sign of scrub typhus, was observed in 24.76% of cases in the present study, which is comparable to Das et al.'s finding.[11] Eschar's prevalence varies from 11% to 80%.[13,15,17,25] Some authors did not find it.[22,23,26] This discrepancy could be the result of an observer's error, the complexion of the patient, or a delay when arriving to the hospital after eschar falls off.

An individual's various organ systems are invaded by *O. tsutsugamushi*, as shown by alterations in blood parameters, including biochemical and haematological ones. The three most notable haematological abnormalities in the current investigation were thrombocytopenia (46.67%), leukocytosis (42.86%), and anaemia (84.76%). The anemia found in this study is consistent with research conducted by Bairachary L.[5] According to researchers from various regions of India, the prevalence of anemia ranges from 8.3% to 74%.[19,27,28] Preexisting anemia in our study subjects may account for the higher percentages of anaemia in the present research. Leukocytosis was found in 42.86% of the cases in the present research, which is consistent with a study by Somashekar et al, which had the same finding.[21] As reported by several authors from various geographical locations, leukocytosis was found in 17.1-97.1% of cases.[11,19,24,29,30] In cases of scrub typhus fever, thrombocytopenia is the most frequent haematological abnormality reported. It can result from either immune or toxic marrow suppression.[31] Thrombocytopenia was found in 46.67% of the cases in this study. Das et al reported thrombocytopenia in 47.36% of cases, which is a comparable finding.[11] In contrast to dengue, which does not always correlate thrombocytopenia with elevated CRP, scrub typhus fever is characterized by both thrombocytopenia and raised CRP.[32-34]

Increased serum levels of bilirubin, ALT, and AST were found in 66.67%, 84.76%, and 3.81% of the cases in the present study, respectively. Similar to the results of this study, Shajahan et al reported that increased levels of ALT and AST were found in 66.7% and 93.3% of cases, respectively.[35]

Liver transaminase and bilirubin levels in the serum are disproportionately high in scrub typhus fever. Conversely, a proportionately higher increase in these parameters in serum levels is caused by leptospirosis.[36]

Hypoproteinemia and hypoalbuminemia, which were found in 28.57% and 23.81% of cases, respectively, in the present study, are two more biochemical abnormalities associated to scrub typhus fever.

The present study is consistent with Kumar et al observations from Chennai, Tamil Nadu, India, which showed that 23.5% of study participants had hypoalbuminemia.[37] Pathak et al from Nepal observed 71.1% hypoalbuminemia in the study participants, which is in contradiction to the present study.[38] Whatever the occurrence of hypoalbuminemia in scrub typhus fever, the cause could be inadequate nutrition during the illness, hepatic involvement and third spacing of fluid, and study participants' previous undernutrition, which calls for more investigation.

A total of 50.48% and 22.86% of the cases in the present study had hyponatremia and hypokalemia, respectively. In a manner comparable to the present study, Pathak et al and Bhandari et al revealed hyponatremia in respective cohorts of 48.17% and 50.9%.[38,39] While the incidence is lower than in the present study, Bhandari et al (12.7%) also documented hypokalemia in scrub typhus.[39] According to the present study, 5.71% of the patients had elevated serum creatinine levels, indicating acute kidney injury (AKI). This finding is consistent with a study done by Digra et al, which found 4.7% of cases to have this condition.[22] In contrast to the present study, Palanivel et al, Muthukrishnan et al, and Kumar et al reported AKI in 10%, 10.7%, and 20% of their cohorts, respectively.[17,34,40]

Evaluation of the relationship between the duration of fever in scrub typhus and haematological and biochemical parameters found the mean hemoglobin level, platelet count, and serum sodium level all decreased with a longer time fever duration. These relationships were statistically significant, with P values of 0.011, 0.04, and 0.046, respectively. With the duration of the fever, the total leukocyte counts increased, and the P value was 0.028, which is statistically significant. With the present study, Dash et al found that prolonged fever increases the total leukocyte count, and this increase is statistically significant (P value <0.0238).[11]

This study has some limitation. Since this was a single-centre, hospital-based, cross-sectional research study, selection bias existed in it. In addition, the sample size was tiny. As a result, it was not applicable to the general community. To generalise the results of this study, a larger sample size and community-based investigation at several locations are required.

CONCLUSION

In cases where a child has had a fever for five days or longer, abnormal laboratory parameters such as anaemia, thrombocytopenia, leucocytosis, hypoproteinaemia, albuminemia, elevated serum levels of alanine and aspartate amino transferase (AST>ALT), hyponatremia, hypokalaemia, uraemia, and creatinaemia may aid a PCP or paediatrician in diagnosing and treating scrub typhus fever earlier in children.

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