Cross-sectional Study

Clinical profile and biochemical abnormalities in Scrub Typhus: A cross-sectional study

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ABSTRACT

Introduction: Scrub Typhus (ST) is an acute febrile illness caused by obligate intracellular bacteria of the family Rickettsia. It is often unrecognized and neglected but prevalent in tropical regions of endemic areas. The tragedy behind this diagnostic dilemma is non-specific clinical signs and symptoms, limited awareness, unavailability of diagnostic facilities, and low index of suspicion among the physicians. To address the knowledge gap, we tried to find out a proper panel of laboratory investigations to diagnose the disease and predict its progression because of the uncertainty of the course of the disease in a tertiary care hospital in western Nepal.

Methods: This is a hospital laboratory-based prospective study conducted at Gandaki Medical College- Teaching Hospital (GMC-TH) for a period of two years. Among 988 cases of acute febrile illness, 40 seropositive cases of ST were enrolled in the study. We excluded those who did not give consent for the participation, those who were under 17 years of age, and those who had preexisting liver dysfunctions and other co-morbidities and dual seropositive with other infectious etiologies. We used descriptive statistics to analyze the data in terms of demography, clinical features, and laboratory parameters.

Results: Out of 988 febrile patients, we included 40 confirmed cases of ST aged between 17 and 70 years during the study-period. Maximum seropositive cases were from Tanahun district 14 (35%), with predominance among the women (70%). The cases were prevalent in the age group 30–60 years, 19 (47.5%), and in the month of October 15 (37.5%). The commonest complaints were fever in 40 (100%), headache in 20 (50%), eschar in 11 (27.5%). Laboratory parameters showed anemia in 22 (55%), hypoalbuminemia in 11 (27.5%), leukopenia in 5 (12.5%), leukocytosis in 9 (22.5%), thrombocytopenia in 13 (32.5%), raised transaminase levels, SGPT in 21 (52.5%) and SGOT in 14 (26%) ST patients.

Conclusion: We found clinical and laboratory profiles in patients with ST were varied and nonspecific. However, knowledge of these findings may evoke the recognition of ST and give a clue to the progression of the disease.

1. Introduction
1.1. Background: causation, pathogenesis, and transmission

Scrub typhus (ST) is caused by gram-negative, obligate intracellular organism Orientia tsutsugamushi. It is a mite-borne disease transmitted by the bite of a larva of trombiculid mites (chiggers). The organism is maintained by transovarian transmission in the vector [1]. After hatching the infected larva mites inoculate the organism into the skin. The bacteria multiply at the site of the bite forming eschar, a characteristic necrotic skin lesion. The organism proliferates in the endothelial layer of blood vessel, releases cytokines and damages the vessel integrity, causing fluid leakage, platelet activation, polymorph and monocyte proliferation leading to focal occlusive end-angiitis causing

Abbreviations: ALP, Alkaline phosphatase; ELISA, Enzyme Linked Immunosorbent Assay; SGOT, Serum Glutamate Oxaloacetate Transaminase; SGPT, Serum Glutamate Pyruvate Transaminase; WBC, White Blood Cell.

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micro-infarcts. This process affects various organs of the body. Illness varies from mild and self-limiting to fatal consequences [2]. After an incubation period of 6–21 days, onset is characterized by fever, chills, headache, myalgia, cough, skin rash, lymphadenopathy, and GI symptoms. The infection may lead to generalized vasculitis, leading to multi-organ dysfunction. Complications include encephalitis, interstitial pneumonia, acute renal failure, acute hepatic failure, acute hearing loss, and acute respiratory distress. These features resemble that of other infectious etiologies such as enteric fever, malaria, dengue, brucellosis, and leptospirosis [3]. ST is a neglected tropical disease and delay in diagnosis and treatment results in an increased case fatality rate. It is also a re-emerging health problem, and a burden to the socio-economic development of developing countries like Nepal [4]. This infection is common among those people associated with occupational and agricultural exposures. People acquire this disease by playing barefoot in the foothills and during open defecation [5]. A big outbreak of ST was also reported after the 2016 earthquake in Nepal [4]. Because of their outbreak potential, there is an urgent need for reliable diagnostic tests at all levels of health facilities to diagnose any patients with acute febrile illness for scrub typhus.

1.2. Epidemiology

The prevalence of scrub typhus is unknown because of the imprecision of diagnosis and the inadequacy of reporting and surveillance systems. An estimated one billion people are at risk of ST and one million cases are reported annually [6]. The disease is endemic in eastern and southern Asia, northern Australia, islands of the western Pacific region, and the Indian ocean [4]. There were very few attempts to explore the disease in the Nepalese context. Only in 1981, Brown et al. found the index case of the disease in Eastern Nepal [7]. However, the possibility of the cases was not followed up for the next 25 years until in the year 2004, a study done in Patan hospital found 28/876 (3%) febrile patients were positive for scrub typhus serology [8]. Three months after the devastating earthquake in Nepal in August 2015, the eastern Nepal saw a big outbreak of ST. A total of 101 cases were reported from 16 districts in 2015. Out of them, eight cases died, accounting for a crude fatality rate of 8%. Further, in the year 2016, a disastrous outbreak of 831 cases was reported from 47 districts [9].

1.3. Scientific rationale of the study

Scrub Typhus is one of the most prevalent, under-diagnosed, neglected tropical diseases. It can be treated effectively if diagnosed timely with proper antibiotics. Despite the intensity and magnitude of the illness, neither a clear epidemiological picture has been discovered so far nor has the diagnostic dilemma among the physicians been sorted out [9]. This study is a descriptive cross-sectional study that aims to find out the clinical and laboratory profile of patients with ST in a tertiary care hospital in western Nepal.

2. Materials and methods

2.1. Registration

The manuscript is written in line with the STROCSS criteria [10]. It is registered in Research Registry at http://www.researchregistry.com on July 24, 2022 and the unique identifying number is researchregistry8128.

2.2. Ethical approval

The study protocol was reviewed and approved by the Institutional Review Committee (IRC) of Gandaki Medical College, Ref. 26/076/077.

2.3. Patient and public involvement

After taking informed consent, a preformed questionnaire was used to collect the demographic and health-related information.

2.4. Setting and time frame

This is a hospital-based descriptive cross-sectional study done in Gandaki Medical College Teaching Hospital and Research Center (GMC-TH), a 550-bed tertiary care hospital in Pokhara, Kaski district of Nepal from July 2019 to April 2022.

2.5. Study population

A total of 988 patients visiting GMC-TH with acute febrile illness were enrolled in this study. After taking informed consent, we collected the demographic and health-related information in a preformed questionnaire.

2.6. Inclusion and exclusion criteria

All patients with acute febrile illness from 17 years to 70 years with a definitive diagnosis of ST were included. Patients with acute febrile illness diagnosed with other infections such as dengue, leptospirosis, typhoid, brucella, and malaria were excluded. Additional testing for COVID-19 was done for samples received after February 2020. Patients with ST associated with other diseases were also excluded.

2.7. Sample size and sampling technique

A total of 988 patients with febrile illness were included by convenience sampling during the study period.

2.8. Sample processing

A total of 988 patients with acute febrile illness (axillary temperature >38 °C) were included in the study. After obtaining informed consent, 10 ml venous blood was collected by trained laboratory staff, aseptically with the standard operating procedure, and divided into three aliquots for serological, biochemical, and hematological tests in BD Vacutainer, yellow color-coded and lavender color-coded tubes. Collection and transportation of specimens were done according to the manufacturer’s instructions. Blood samples collected were received in the central laboratory.

2.8.1. Serological test

Dengue, leptospirosis, and kalazar were tested using rapid diagnostic test (RDT) kits as per the manufacturer’s instructions. Enteric fever was screened using Widal test. Brucellosis was tested by rapid chromato-graphic immunoassay [3,11]. Malaria was first screened by RDT and confirmed by thin and thick blood smear.

In our study, ST was diagnosed by IgM ELISA Kit (InBios International, Seattle, Washington, USA) by detecting IgM antibodies in the serum sample [12]. The test is a flexible alternative to the indirect fluorescent assay (IFA) technique and has a sensitivity and specificity of more than 90.0%. The Wells of each plate are coated with a unique recombinant antigen mix. The sample is treated with the diluent at a 1:100 ratio and applied to each well. After incubation and washing, these wells are treated with HRP-conjugate. After a second incubation and washing step, TBM substrate is added. An acidic stop solution is then added and the degree of enzyme turnover of the substrate is determined by absorbance measurement at 450 nm. The result is recorded as either positive or negative. The cut-off value of optical density (OD) is fixed at 0.50 following recommendations for determining the endemic cut-off titer in the kit protocol.
2.8.2. Hematological test

Two milliliters of whole blood was taken in the lavender-top BD vacutainer containing EDTA anticoagulant. The tube was kept at room temperature. This test is done by Automated Hematology Analyzer, 5 parts, HEMA-D60S1 using a flow-cytometer principal (Bioeovapack Co., Ltd., Mingsheng Building, High-tech Zone, Jinan city, China) according to the manufacturer’s instruction.

2.8.3. Biochemical test

We collected 3 ml of blood in a yellow-top vacutainer, centrifuge the sample and feed the supernatant serum in the analyzer according to the manufacturer’s instructions, Bioelab AS-280, Auto chemistry analyzer. The reference range for different hematological and biochemical parameters was used as suggested by the hospital guidelines (Supplementary Materials).

2.9. Data management and analysis tool

Data obtained daily were entered daily in Microsoft excel 2016 and later exported to the Statistical Package of Social science (SPSS) for Windows version 24.0 for further statistical analysis. Descriptive statistics were used to analyze the data in terms of demography, clinical manifestations, and laboratory parameters. To check if the data were normally distributed, the Kolmogorov Smirnov test was used. For descriptive statistics-mean, SD or median, interquartile range, and frequency (percentage) were calculated.

3. Results

3.1. Demographics

3.1.1. Prevalence of ST among study participants

Out of 988 febrile cases, 98 tested positive for ST. 508 cases positive for ST were excluded because 26 were dual-positive and 32 had other comorbidities like pre-existing liver disease, blood disorder, diabetes, and hypertension under medications. Rest 890 patients negative for ST were diagnosed with COVID-19, leptospirosis, brucellosis, malaria, dengue, etc.

3.1.2. Gender-wise distribution

Table 1 shows that among 988 febrile cases, 508 were male and 488 were female. Out of 40 seropositive cases, 28 (70%) were female and 12 (30%) were male. This result showed a statistically significant association of ST cases with the gender of the patients (p < 0.001).

3.1.3. Age-wise distribution

Table 1 shows that the patients in the age group of 30–60 years suffered 47.5% from this disease followed by the age group below 30 years suffered 35% and the least proportion suffered was in the age group above 60 years which is 17.5%. The difference was statistically significant.

3.1.4. Geographical area-wise distribution

The majority of the cases were from kaski district 12 (30%) followed by Tanahun 14 (35%), syangja 10 (25%) and other districts 4 (10).

3.1.5. Month-wise distribution

Fig. 1 shows that the highest number of cases were found during the month of September to November, 25 (62.5%) followed by July to August, 10 (25%), and the least during December to June 5 (12.5%). The variation in month-wise distribution was statistically significant.

3.2. Clinical features

Fig. 2 shows that fever was a consistent symptom in all the patients. Twenty (50%) patients complained of headache and retro-orbital pain with or without redness of the eyes. GI symptoms including nausea, vomiting, and abdominal pain were complained by 11 (27.5%), and the pain was dull in nature and localized to the upper quadrant without guarding. However, eschar, a pathognomic feature was seen in only 11 (27.5%). Weakness, malaise, or prostration was complained by 14 (26%) patients. Some critically ill patients in ICU 4 (10%) complained of persistent cough and had diffuse alveolar infiltrate on chest X-ray.

3.3. Frequency of laboratory derangement

Table 2 shows the prevalence of abnormal laboratory parameters in our study participants. Biochemical parameters showed the raised level of SGPT, 21 (52.5%, N = 40), followed by SGOT, 14 (26%), ALP, 9 (22.5%). High bilirubin and hypoproteinemia were noted in 4 (10%) each. Hypoalbuminemia was found in 11 (27.5%). Hematological parameters showed anemia in 22 (55%), leucopenia in 5 (12.5%) patients, leukocytosis in 9 (22.5%) patients. Thrombocytopenia was found in 13 (32.5%), out of which 5 (12.5%) had severe thrombocytopenia and required transfusion.

3.4. Laboratory abnormalities

Table 3 shows the derangement in laboratory parameters. Total bilirubin was slightly raised 1.33 (0.7–32.7), SGOT and SGPT were elevated, 54.50 (18–497) and 51.00 (15–457) respectively. Characteristic increment in transaminase level more than 1.5 SD was found in 4 out of 40 patients (table not shown). Anemia, albeit mild, was also found.

4. Discussion

4.1. Background

The transmission of the disease have been favored by several factors like the country’s ecological niche, epidemiologic behavior of the vector, and the altered environmental factors due to the earthquake.

Table 1

| Demographic variables | Frequency (%), N = 40 |
|-----------------------|----------------------|
| Age in years          |                      |
| Below 30              | 7 (17.5)             |
| 30–60                 | 19 (47.5)            |
| >60                   | 14 (35)              |
| Gender                |                      |
| Female                | 28 (70)              |
| Male                  | 12 (30)              |
| Address               |                      |
| Kaski                 | 12 (30)              |
| Syangja               | 10 (25)              |
| Tanahun               | 14 (35)              |
| Other                 | 4 (10)               |

Fig. 1. Months of Scrub Typhus infection.
4.2. Demographics

4.2.1. Prevalence

Sanitation have surged disease transmission [13]. Habitat after the collapse of houses. Moreover, overcrowding and lack of contact with rats that might have come out of the usual underground

monsoon predominant environment may be a crucial factor for typhus (30%) and Syangja 10 (25%). The presence of scrub bushes and reason for this disparity may be due to difference in the ecological niche [6]12.2% but was higher than the study done in Patan hospital [14]. The for ST, which was comparable to the study reported by Pokharel A et al.,

reason could be due to increased exposure of women to mites while working in scrub-prone agricultural fields during harvesting or cultivation of crops in the field. On the other hand, men were involved in office work, company, or factory.

4.2.2. Gender-wise distribution

Following the earthquake, people were obligated to remain in intimate contact with rats that might have come out of the usual underground habitat after the collapse of houses. Moreover, overcrowding and lack of sanitation have surged disease transmission [13].

4.2.3. Age-wise distribution

In the current study, majority of cases were found among 30–60 years age group, 19 (47.5%). This finding was similar to studies done by several authors [18,22–25]. The high prevalence among the middle-aged group may be due to exposure to mites while working in scrub vegetation and walking bare-foot in hills.

4.2.4. Month-wise distribution

We found a higher prevalence of ST from September to November, 25 (62.5%), followed by July to August, 10 (25%), and the least between December to June, 5 (12.5%). This finding was consistent with the study done by Zhang et al., [25] who found the epidemic period to be September to November. Kumar et al., [26] in South-India and Mishra A et al., [5] in Birgunj, Nepal reported a higher prevalence in July to August; Huang et al., [27] in June to July and Upadhya et al., [24] July to November. Monsoon season is favorable for mites to lay eggs, just following the post-monsoon period that coincides with the growth of scrub vegetation which is a habitat for mites. There is a high risk of mite-bites during these months because of increased activity in agriculture works.

4.3. Clinical features

In our study, fever is the most consistent symptom, 100%, similar to other studies [5,17,25,28,29]. However, Park et al., [16] reported 92.8% seropositive result among febrile cases in Changwon Hospital, Korea. Srivongaoan et al.,[30]found hypothermia <36 °C in severe cases as a consequence of septic shock. Though eschar is considered as a pathognomonic sign, we found eschar in 11 (27.5%) cases only. Kim et al., [31] recorded a variable occurrence from 7 to 80%. However, Tsay [32], reported 60%, Gurung et al., [29] from Gorkha, Nepal reported 30%, Mishra et al., [5] Birgunj, in 2019–2020, found Eschar in 11.5%, and Thapa S et al., [17] from Chitwan found eschar in 6.5% cases. Surprisingly, in a study from Andra Pradesh, India by Ramyashree et al., [33] did not report any eschar. Rather than the variable clinical presentations and skin manifestations, risk factors like habitat, travel to endemic regions, and occupation may give a clue to assist in diagnosis.

Headache including retro-orbital pain and redness of the eyes was reported in 20 (50%) cases in our study. This complaint was similar to several studies [16,29,30]. Likewise, GI- upset including nausea, vomiting, and abdominal pain was found in 11 (27.5%) in our study. This finding is similar to that reported in other studies [25,30]. Weakness,
malaise, and prostration were found in 14 (26%) cases. This complaint was similar to the study done by other authors [25,30]

4.4. Deranged laboratory profile

In our study, biochemical parameters revealed raised levels of transaminase enzymes, SGPT and SGOT, in 21 (52.5%) and 14 (26%) respectively, and ALP in 9 (22.5%). Pokhrel et al., [6] reported an increase in transaminase by 70–90% and ALP by over 50%. Our finding is consistent with several studies [16,25,34–38] [16,39,40]. However, a study from North- East India done by Jamil Md et al., [41] found SGOT more than SGPT.

Our study showed hyperbilirubinemia in 4 (10%). This finding is similar to that reported by Cowan et al., [42]. Hypoproteinemia was found in 4 (10%) patients. Hypoalbuminemia was found in 11 (27.5%). This finding is consistent with several studies [16,30,31,36,39,43,44]. However, a previous study by Lee et al., [45] reported no difference in albumin level. This finding predicts a reversal of the A/G ratio suggesting acute hepatic injury. Hypoalbuminemia causes decreased plasma colloidal oncotic pressure, increases hydrostatic pressure causing endothelial permeability, and fluid leakage to the interstitial space resulting in peripheral edema and intravascular hypovolemia.

The dynamic pattern of laboratory indicators reveals that hemoglobin is decreased in 22 (55%) cases and not returning to normal in severe cases. This result is similar to other studies [39,46,47]. Leucopenia was found in 5 (12.5%) cases and leucocytosis in 9 (22.5%). These findings were consistent with many studies [25,35,38–40,48]. Thrombocytopenia was found in 13 (32.5%). Endothelial injury results in cascade reactions resulting in activation of platelets, generation of thrombin, and activation of fibrinolytic system due to hemostatic physiologic response [1]. Decreased platelet count also accompanies capillary permeability. This finding is similar to several studies [16,25,38,39,48].

5. Limitations

This is a single-center study and may not truly reflect the trend of ST. Because of the low sample size, the results may not be generalized across different clinical settings. We did not follow-up with the patients and have not recorded the clinical outcomes, which may be an important limitation. We suggest further studies to address these concerns and be carried out across multiple centers for a considerable time-period.

6. Conclusion

Although ST is endemic in Nepal, it is often neglected, and under-diagnosed due to non-specific clinical signs and symptoms, limited awareness, unavailability of diagnostic facilities, and low index of suspicion among the physicians. A proper panel of laboratory investigations is mandatory in resources-limited countries like Nepal to diagnose and assess the severity of this disease. We observed varied clinical features, a notable degree of liver dysfunction, and deranged hematological parameters which may predict the disease progression.

Provenance and peer review
Not commissioned, externally peer-reviewed.

Declaration of competing interest
No conflicts of interest.

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Ethical approval
Ethical clearance was given by the Institutional Review Committee of Gandaki Medical College (Ref. 26/076/077).

Consent
Written informed consent was obtained from each of the study participants.

Author contribution
Sanjeeb Shrestha (SS) and Mitesh Karn (MK) were involved in the conceptualization of the study. Sanjeeb Shrestha (SS), Rajesh Prajapati (RP), and Sanjib Mani Regmi (SR) were involved in the data analysis. All authors (SS, MK, RP, SR, AN, SP) were involved in the design of the study, data collection, literature review, writing and editing the manuscript, and approved the final version of the manuscript. SS and MK contributed equally.

Registration of research studies
1. Name of the registry: Research Registry (http://www.researchregistry.com).
2. Unique Identifying number or registration ID: researchregistry8128.
3. Hyperlink to your specific registration (must be publicly accessible and will be checked): https://www.researchregistry.com/browse-the-registry#home/registrationdetails/62dd5c36edfcf80024f16b20/

Guarantor
The Guarantor is the one or more people who accept full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.
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