Original Article

An Emerging Coinfection of Dengue and Scrub Typhus: A Hospital-based Study

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Abstract

Background: Dengue fever and scrub typhus are endemic diseases in India. The aim was to study and compare clinical, biochemical, and hematological parameters between the dengue and dengue-scrub study groups. **Materials and Methods:** A retrospective hospital-based, cross-sectional study conducted at a tertiary care rural center. Various clinical, biochemical, and hematological parameters of dengue patients with random selection as the control group were compared with the dengue-scrub coinfection group (46 cases) as cases. The statistical significance was determined using an independent *t*-test. $P < 0.05$ was considered statistically significant. The *t*-test and the Mann–Whitney *U*-test were used. **Results:** Among the 86 cases (2.05%), scrub and dengue contributed 46 cases, followed by 27 cases of COVID-19 and dengue, four cases of malaria and scrub, three cases of malaria and dengue, and a single case of dengue with typhoid and leptospirosis with dengue. Serum albumin ($P = 0.003$, moderate effect size), serum globulin ($P = 0.001$, large effect size), alkaline phosphatase ($P = 0.001$, moderate effect size), and serum urea ($P = 0.012$, moderate effect size) levels were significantly different between only dengue and dengue-scrub group. In addition, the difference in hemoglobin levels, red blood cell count, white blood cell count, platelet count, cell variables, and ratios of those with dengue and scrub versus those with only dengue was significant ($P < 0.05$) with moderate-to-large effect size. **Conclusion:** Certain biochemical and hematological parameters such as albumin, globulin, alkaline phosphatase, platelet count, neutrophil-to-lymphocyte ratio, and platelet lymphocyte ratio can be used to support the diagnosis of coinfection (dengue-scrub).

Keywords: Coinfection, dengue fever, neutrophils-to-lymphocytes ratio, platelet-to-lymphocyte ratio, scrub typhus

Introduction

Dengue fever and scrub typhus are two common causes of acute febrile illness of unknown origin in Asia.[1] Dengue fever, transmitted by the bite of the *Aedes aegypti* mosquito, is common in the Asian population and frequently manifests as an acute febrile illness of unknown origin.[1] Dengue fever can range from a mild illness characterized by fever, headache, myalgia, and rash to a severe illness characterized by shock, narrow pulse pressure, pleural effusion, ascites, and bleeding.[2] Scrub typhus is a nonspecific febrile disease caused by the organism *Orientia tsutsugamushi* that spreads to humans through chigger bites.[2] The majority of patients present with nonspecific symptoms. An eschar may develop at the site of a chigger bite, indicating scrub typhus. About one-third of scrub typhus patients show signs of organ dysfunction. Scrub typhus' clinical manifestations can be similar to tropical febrile illnesses such as malaria, dengue, typhoid, and leptospirosis.[3]

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Quick Response Code:

Website: http://www.mmjonweb.org

DOI: 10.4103/mj.mj_33_23

Concurrent scrub typhus infection in endemic areas can cause a diagnostic quandary during the seasonal increase in dengue cases. Rashes, thrombocytopenia, and hepatic dysfunction are all clinical and laboratory features of both diseases. Infection with both pathogens, on the other hand, is sporadic.[1] Because coinfection with other tropical diseases, such as dengue and malaria, is common, a panel of tests based on local endemicity is required.[2] During the 2021–2022 Acute undifferentiated febrile illness (AUFI) epidemic, while providing routine clinical care, it was observed by the team of researchers (of the present study) that a few cases had

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Submitted: 30-May-2023 **Revised:** 08-Aug-2023 **Accepted:** 17-Aug-2023 **Published:** 30-May-2024

How to cite this article: Rakhecha D, Patil BU, Joshi MN, Yelwatkar S. An emerging coinfection of dengue and scrub typhus: A hospital-based study. Mustansiriya Med J 2023;22:198-202.

positive serology findings for dengue and scrub typhus and a positive smear for malaria (designated here as coinfections).

The prevalence of acute febrile illness is highly variable ranging from 6.6% to 17.5% in parts of India.[4] There have been few studies on the effect of dengue and scrub coinfection on various blood parameters and clinical outcomes.[1] Thus, this study was conducted with the aim to compare clinical, biochemical, and hematological parameters between the dengue and dengue-scrub study groups.

Materials and Methods

Study setting

The Mahatma Gandhi Institute of Medical Sciences is a medical school in the Central Indian village of Sevagram and linked to Kasturba Hospital. Each year, multiple patients from Maharashtra, Andhra Pradesh, Chhattisgarh, Madhya Pradesh, and Telangana visit the outpatient department and receive inpatient care.

Study design and ethical considerations

It was a retrospective hospital-based cross-sectional study. This study received approval from the Institutional Ethics Committee (IEC/MED/320/2022 dated October 01, 2022). The study involved the analysis of de-identified patient data. The data were stored in password-protected Microsoft Excel files to which only the authors had access to.

Sample size

Dengue and scrub typhus were found to be coinfected in 46 patients. Patients were admitted with complaints of fever lasting 3–7 days. There had been no previous history of fever or similar complaints for at least 3 months before the current episode. At the treating physician's request, all patients were tested for dengue, scrub typhus, leptospirosis, typhoid, malaria, and COVID-19, as well as mixed infections, due to the overlapping clinical features and high prevalence of all these diseases in the current area. The study included all patients who fulfilled the definition of AUF and tested positive for coinfections. Dengue and scrub co-infection samples were collected using hospital information system at the medicine department from January to December 2021. The data collected included 15–60-year-old hospitalized patients with acute febrile illness.

The structured pro forma entered the patient's demographic, clinical, biochemical, microbiological, and radiological data. Scrub typhus-specific IgM levels were determined using an enzyme-linked immunosorbent assay (ELISA). ELISA-based NS1 detection and IgM capture were used to diagnose dengue fever. Leptospirosis was diagnosed using an IgM-based immunoassay. In the department of pathology, a microscopic examination of thick and thin smears was performed to confirm malaria. COVID-19 was diagnosed using reverse transcription-polymerase chain reaction (RT-PCR) for viral RNA. Various hematological ratios, such as absolute neutrophils-to-lymphocytes ratio (NLR) and platelet-to-lymphocyte ratio (PLR), are known to have prognostic value and were investigated in this study. The hospital information system and the general medicine department obtained these patients' follow-up data. This research analyzed as much de-identified patient data as possible. Cases with insufficient clinical information were excluded from the study. The results were presented as mean, standard deviation, and range values for continuous measurements, while frequencies were presented as numbers and percentages.

Acute undifferentiated fever was defined as a fever lasting 2 weeks or less with no localizable or organ-specific clinical features in the patients.[5]

Scrub typhus is a probable case with consistent clinical features described in the Department of Health Research-Indian Council of Medical Research guidelines for diagnosing and managing rickettsia diseases and a positive IgM ELISA for scrub typhus.[6]

Dengue: clinical description consistent with dengue fever as defined by India's national vector-borne disease control program, with ELISA demonstrating positive NS1 antigen and IgM antibody in serum.[7]

Leptospirosis: a probable case has clinical features defined in the National Leptospirosis Prevention and Control Program guidelines and a positive result in an IgM-based immunoassay.[8]

Malaria: fever with parasitological diagnosis using light microscopy or rapid diagnostic tests.[9]

COVID-19: viral gene detection by RT-PCR has been found as the most reliable technique.[10]

Coinfection is the presence of multiple infectious etiology among the infections listed. A high degree of overlap between case definitions is more likely due to a high prevalence of prior infections, cross-reactivity, and subclinical infections than to a high prevalence of coinfections. When treating a patient with acute undifferentiated fever in India, the low accuracy of routine diagnostic tests should be considered.[11]

Microsoft Excel was used to enter and manage the data, and Jamovi software version 2.3.21 (https://www.jamovi.org) was used to analyze it. The prevalence of acute febrile illness was expressed as a percentage. The means of biochemical and hematological parameters were compared using the *t*-test (for normal distribution variables) and the Mann–Whitney *U*-test (for variables with nonnormal distribution). The effect size of the studied association was reported using Cohen's d and rank-biserial correlation values. When $P < 0.05$, differences were considered statistically significant.

Results

We had 4007 cases of AUFI in 2021, with more than half (59.57%) of the participants being males and 40.43% of females. The participants in the study were 32.6 (standard

deviation; S.D. =15.3) years old on average. The study participants' average hospital stay was 4.7 days (S.D. =1.9), with a median stay of 5 days. As shown in Figure 1, there were 1998 cases of COVID-19 disease out of 4007 patients with acute febrile illness (49.86%), 1687 cases of dengue (42.10%), 221 cases of scrub typhus (5.52%), and 86 cases of mixed infections (2.05%). Malaria, leptospirosis, and typhoid were responsible for less than 0.5% of the total prevalence of acute febrile illness. Among the 86 cases (2.05%), scrub and dengue contributed 46 cases (1.15%), followed by 27 cases of COVID-19 and dengue (0.67%), four cases of malaria and scrub (0.10%), three cases of malaria and dengue (0.07%), and a single case of dengue with typhoid (0.02%) and leptospirosis with dengue (0.02%) [Figure 2].

Fever was the most common clinical manifestation (100%). In addition, 15% of the dengue-scrub group had splenomegaly, 7% had breathlessness, 3.2% had bleeding, 2% had malaise and abdominal pain, and none had loose stools when comparing biochemical parameters between those who only had dengue and those who had dengue and scrub. Both groups were compared for biochemical parameters of serum protein, serum albumin, serum globulin, total and conjugated bilirubin, ALT, AST, ALP, blood urea, and serum creatinine levels. The mean value of serum albumin (P = 0.003, moderate effect size) was significantly low, whereas serum globulin (P = 0.001, large effect size), ALP (P = 0.001, moderate effect size), and blood urea (P = 0.012, moderate effect size) were significantly more in the cases [Table 1].

Table 2 shows the comparison between hematological parameters, including hemoglobin levels, red blood cell (RBC) count, white blood cell (WBC) count, platelet count, Packed cell volume (PCV), Plateletcrit (PCT), PLT distribution width (PDW), absolute GR count, N/L ratio, and P/L ratio, of those with dengue and scrub versus those with only dengue. Out of all the hematological variables studied in the two groups, values of MCH, MCHC, RDW, and LY# were not found to be significantly different in the two groups.

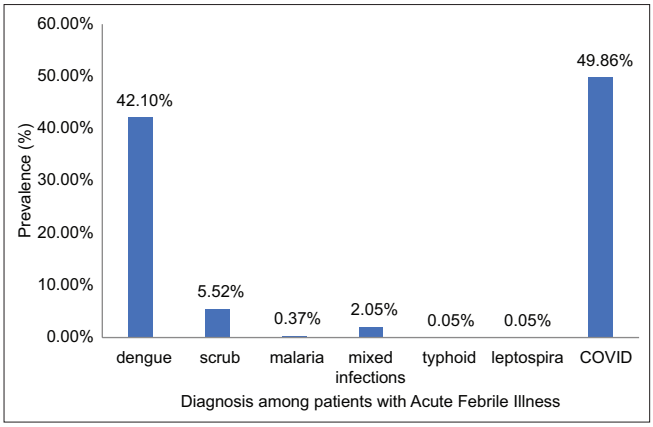

Figure 1: Prevalence of various diseases diagnosed among patients admitted for Acute febrile illness (AFI)

Discussion

Various tropical fevers with differing prevalence have been reported from different regions of India. Variation is determined by vector distribution, geography, season, and climate.[12] AUFI epidemics caused by malaria, dengue, scrub typhus, enteric fever, and leptospirosis occur during and immediately following the monsoon season.[13] Dengue fever has become endemic in most of South-east Asia, with outbreaks emerging in and around the rainy season.[14] Scrub typhus can cause fever and multi-organ dysfunction, resulting in septic shock; however, the disease does not respond to standard sepsis antibiotics. The empirical addition of doxycycline to the initial regimen will result in rapid clinical improvement.[2]

The endemicity of scrub typhus, dengue fever, and its life-threatening complications is well-known in central rural India. An illness with overlapping symptoms is caused by infection with multiple etiological agents. It also complicates the diagnosis and management of such patients. As a result of growing populations, frequent travel, improved laboratory services, and increased awareness, contagions are becoming

Table 1: Results from the comparison of biochemical profiles among those with dengue and those with dengue and scrub infection

Biochemical parameter	Statistic value	df	*P*	Effect size
Protein[a]	1.068	90.0	0.288	0.2228
Albumin[b]	680	90.0	0.003*	0.3574
Globulin[a]	4.267	90.0	<0.001*	0.8899
Bilirubin (T)[a]	0.335	92.0	0.738	0.0692
Bilirubin (C)[a]	0.844	91.0	0.401	0.1751
ALT[b]	1090	92.0	0.913	0.0136
AST[b]	963	91.0	0.367	0.1092
ALP[b]	476	90.0	<0.001*	0.5499
Urea[b]	681	87.0	0.012*	0.3105
Creatinine[b]	1024	91.0	0.661	0.0532

*Values <0.05 were considered significant, [a]Student's *t*-test was applied, and effect size reported as Cohen's d, [b]Mann–Whitney *U*-test was applied, and effect size reported as rank-biserial correlation. ALT: Alkaline phosphatase, AST: Alanine transaminase, ALP: Alkaline phosphatase

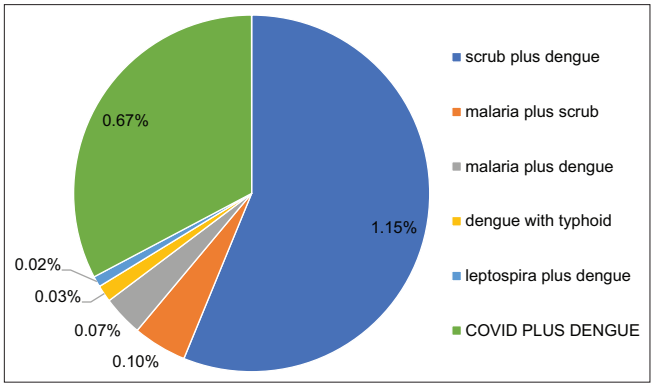

Figure 2: Distribution of Acute febrile illness (AFI) mixed infections

Table 2: Results from the test of association between tests and controls for hematological profile

Hematological parameter	Statistic value	df	*P*	Effect size
HB[a]	−3.162	92.0	0.002*	−0.652
RBC[a]	−2.702	92.0	0.008*	−0.557
WBC[b]	757	92.0	0.009*	0.3151
PLT[b]	312	92.0	<0.001*	0.7175
PCV[a]	−3.822	92.0	<0.001*	0.3961
MCH[b]	1025	92.0	0.550	0.0720
MCHC[b]	879	92.0	0.088	0.2046
RDW[b]	1090	92.0	0.916	0.0131
PCT[b]	335	92.0	<0.001*	0.6967
PDW[a]	599	92.0	<0.001*	0.4581
LY[#,b]	1086	92.0	0.892	0.0167
GR[#,b]	670	92.0	0.001*	0.3934
N/L ratio[b]	762	92.0	0.010*	0.3105
P/L ratio[b]	502	92.0	<0.001*	0.5455

[#]is used to denote absolute count.*Values <0.05 were considered significant, [a]Student's *t*-test was applied, and effect size reported as Cohen's d, [b]Mann–Whitney *U*-test was applied, and effect size reported as rank-biserial correlation. HB: Hemoglobin, RBC: Red blood cell, WBC: White blood cell, MCH: Mean corpuscular HB, RDW: Red cell distribution width, PDW: PLT distribution width, PLT: Platelet, PCT: Plateletcrit, PCV: Packed cell volume

a significant concern in tropical countries.[12] Clinicians have difficulty distinguishing scrub typhus from other causes of AUF in patients who lack eschar. As a result, performing a panel of tests based on local endemicity is exceptionally crucial.[2]

In this study, scrub and dengue were mainly accountable for 46 cases of coinfection, followed by 27 cases of COVID-19 and dengue, four cases of malaria and scrub, three cases of malaria and dengue, and a single case of dengue with typhoid and leptospirosis. Compared to previous studies, we are reporting the maximum size of coinfection in a year [Figure 2]. In Himachal Pradesh, the prevalence of dual infections was reported to be 16 cases (1.3%). Scrub typhus and dengue coinfection were the most common, affecting 10 patients. Three patients had scrub typhus and leptospirosis, one had dengue fever, and another had chikungunya. One patient had scrub typhus, dengue fever, and vivax malaria simultaneously.[12] In a study conducted in a tertiary care hospital in Uttarakhand, coinfection was reported in 1.88% (46 cases) of AUF patients. The most common mixed infections were dengue with scrub typhus (15 cases), malaria with scrub typhus (11 cases), and malaria with dengue (eight cases).[15] Scrub typhus and dengue fever coinfections have also been reported from a tertiary care hospital on India's southern coast.[14] Malaria is a common coinfection with dengue and is becoming more of an issue in Asian countries.[16] Coinfections were found in 39% of 100 dengue patients, 12% were ICT positive for malaria, 9% for salmonella IgM, 8% for scrub typhus IgM, 8% for chikungunya IgM, and 2% for hepatitis B.[17] A study in the Puducherry pediatric population found 51 cases of dengue-scrub coinfection.[18] In 2017, a survey from Odisha reported four cases of coinfection with falciparum malaria and scrub typhus, three cases of dengue and scrub typhus, and three cases of enteric fever with scrub typhus.[4]

Fever was the most common clinical manifestation (100%). We discovered that 15% of the dengue-scrub group had splenomegaly, 7% had breathlessness, 3.2% had bleeding, 2% had malaise and abdominal pain, and none had loose stools. The current study found that dengue-scrub patients had significantly lower serum albumin ($P = 0.003$, moderate effect size) and serum globulin ($P = 0.001$, large effect size) than dengue patients. In addition, the dengue-scrub group had higher serum alkaline phosphatase (0.001, moderate effect size) and serum urea ($P = 0.012$, moderate effect size) than dengue patients. The study by Ahmad *et al*. found elevated transaminases (AST > ALT), bilirubin, and alkaline phosphatase, and Basheer *et al*. reported a case of dengue-scrub coinfection.[13,14] We found no significant difference between the dengue-scrub and dengue groups.

Thrombocytopenia is commonly associated with dengue fever and scrub typhus. Because thrombocytopenia in both infections is immune-mediated, coinfected patients likely experienced synergistic immune suppression of platelets, resulting in lower platelet counts.[14] In the current study, we discovered that the coinfection group had significantly lower platelet counts and hematocrit than dengue patients and a significant difference in hemoglobin levels, RBC count, WBC count, and absolute GR count.

This study showed a significant difference in PDW, N/L ratio, and P/L ratio between dengue-scrub coinfection and dengue Patients. There has yet to be any previous research into this parameter in the coinfection group – appropriate and timely laboratory investigations aid in resolving a mixed infection suspicion. High cutoff values for NS1 antigen and IgM ELISA for dengue and IgM for scrub typhus detected concurrently are strong indicators of concomitant disease. A combination of IgM and NS1 predicts current dengue infection better. They are highly sensitive and specific.[14]

The definition of fever duration in acute febrile illnesses ranges widely from 1 to 30 days. According to one study, coinfection causes a more extended hospital stay than dengue fever.[14] In our study, there was no significant difference in hospital stay duration between groups, possibly due to early detection of coinfection and treatment with doxycycline. The findings of this study would be helpful in alerting treating physicians in this region to include dengue fever, scrub typhus, malaria, and leptospirosis in the differential diagnosis of AUF in addition to COVID-19. When there is a high suspicion of coinfection and accurate serological tests are unavailable, hematological parameters such as PCV, PDW, N/L ratio, and P/L ratio can support the diagnosis. More research is needed to improve analyses and differentiation of signs and symptoms and biochemical and hematological parameters in patients with coinfections and individual infections. To rule out serological cross-reactivity, the study design should include molecular studies.

Conclusion

Various regions of India are endemic for dengue, scrub, typhoid fever, and malaria. During an epidemic, there is an increase in the prevalence of coinfection. It is challenging to differentiate mono-infection from coinfection on a clinical basis due to overlapping symptoms and signs. Therefore, a high suspicion index is required to identify coinfection. Early diagnosis and appropriate treatment can significantly alter disease outcomes; hence, a test panel should be performed in endemic areas to rule out coinfection. Specific biochemical and hematological parameters such as albumin, globulin, alkaline phosphatase, platelet count, NLR (neutrophil-to-lymphocyte ratio), and PLR (platelet–lymphocyte ratio) can be used to support the diagnosis of coinfection (dengue-scrub). Further studies are required for a better understanding. More accurate serological tests should be available to avoid false-positive results due to cross-reactivity.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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