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Research Article

CHIKUNGUNYA, SCRUB TYPHUS MONO, AND CO-INFECTION AMONG PATIENTS WITH UNDIFFERENTIATED FEBRILE ILLNESS: A HOSPITAL-BASED STUDY

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ABSTRACT

Objectives: Chikungunya virus is a common arthropod-related acute febrile disease and it is transmitted by *Aedes aegypti* or *Aedes albopictus* species. On the other hand, the bacterium *Orientia tsutsugamushi* causes scrub typhus, which is also an acute febrile illness with multiple organ involvement. Coinfection of chikungunya and scrub typhus may lead to severe manifestation including severe respiratory and central nervous system (CNS) complications. Coinfection of chikungunya and scrub typhus may lead to severe manifestation including severe respiratory and CNS complications. Therefore, the proper diagnosis can prevent the clinical complications. The aim and objective of our study is to find the seroprevalence of chikungunya and scrub typhus and coinfection of both through medical assessment and serological research of these patients presented with acute febrile infection at Diamond Harbour Government Medical College and Hospital.

Methods: A prospective study was conducted from August 2022 to January 2023 at VRDL, Department of Microbiology, Diamond Harbour Government Medical College and Hospital. Serum was collected for IgM antibody enzyme-linked immunosorbent assay (ELISA) for scrub typhus (In bios kit) and Chikungunya (NIV Chikungunya IgM Capture ELISA Kit) test. Four hundred and eighty-seven samples were tested for IgM antibody by chikungunya and scrub typhus ELISA kit.

Result: The present study demonstrated that, from the month of August 2022 to January 2023, 67% of chikungunya cases, 25% cases with only scrub typhus, and 8% cases with both chikungunya and scrub typhus presented positive. A present study shows that chikungunya is slightly more prevalent in males as compared to females, where scrub typhus is equally positive in both male and female patients.

Conclusion: Laboratory testing of both of the diseases can prevent the complication of other suspected disease in coinfected patients.

Keywords: Chikungunya, Scrub typhus, Coinfection, Enzyme-linked immunosorbent assay.

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INTRODUCTION

Chikungunya virus is a common arthropod-related acute febrile disease, and it is transmitted by *Aedes aegypti* or *Aedes albopictus* species [1]. In chikungunya fever, long-lasting arthralgia can develop. The most common symptoms of chikungunya are fever, headache, myalgia, backache, rashes, retro orbital pain, and severe arthralgia [2,3]. The bacterium *Orientia tsutsugamushi* causes scrub typhus, which is also an acute febrile illness with multiple organ involvement. The trombiculid mite bites humans and transmits the sickness. Scrub typhus is characterized by a high fever, rash, headache, lymphadenopathy, and myalgia [4].

Patients are tested for either chikungunya or scrub typhus. The coinfection goes unnoticed. Coinfection has been documented previously [5]. Coinfection of chikungunya and scrub typhus may lead to severe manifestation including severe respiratory and central nervous system complications. Therefore, the proper diagnosis can prevent the clinical complications [6]. It is important to know the coinfection status as the management of single and dual infection are different. One of the probable factor behind the occurrence of these co-infections can be attributed to the fact that most of these infections occur in the monsoon season which coincides with that of scrub typhus.

The aim of our study is to find the seroprevalence of chikungunya and scrub typhus and coinfection of both through medical assessment and serological research of these patients presented with acute febrile infection at Diamond Harbour Government medical college and hospital.

METHODS

Study site

A prospective study was conducted from August 2022–January 2023 at VRDL, Department of Microbiology, Diamond Harbour Government Medical College and Hospital.

Study design and inclusion and exclusion criteria

Patients suspected of scrub typhus infection and chikungunya infection with acute febrile illness (temperature >37.5°C) and onset of fever >7 days and arthralgia not explained by any other etiology were included in the recent study. Patients who were already positive for typhoid (confirmed by blood culture), Sarscov 2 (confirmed by real time Reverse transcription polymerase chain reaction [RT-PCR]), leptospirosis (IgM enzyme-linked immunosorbent assay [ELISA]), malaria (confirmed by thick and thin smear microscopy, Dual Antigen Test), dengue (confirmed by MAC IgM ELISA, NS1 antigen, IgG ELISA) influenza (confirmed by rRTPCR) were excluded from this study. All patients provided written informed consent.

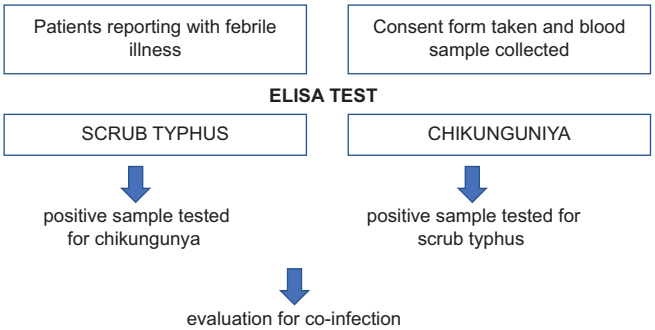
Sample collection and processing
Detailed clinical history was taken from all patients. 5 mL of venus blood was collected and serum and plasma were separated by centrifuge. The serum was kept at −20°C.

Diagnostic assays
Serum was collected for IgM antibody ELISA for scrub typhus (In bios kit) and Chikungunya (NIV CHIKUNGUNYA IgM Capture ELISA Kit) test. Four hundred eighty-seven samples were tested for IgM antibody by Chikungunya and Scrub Typhus ELISA kit. IgM antibody in the patient's serum were captured by anti-human IgM coated on to the solid surface(wells). In the next step, CHIK antigen is added which binds to captured human IgM in the sample. Unbound antigen is removed during the washing step, in the subsequent step biotinylated anti CHIK monoclonal antibodies are added, followed by Avidin–HRP. Subsequently, chromogenic substrate (TMB/H202) is added and the reaction is stopped by 1N H_2SO_4. The intensity of colour/optical density is measured at 450 nm. For scrub typhus test sera is diluted using provided sample dilution buffer for scrub typhus. Application of 100 µL in each well of 1/100 diluted test sera and controls are added. Incubation is done for 30 min at 37° temperature. After washing 100 ul per well ready to use enzyme HRP conjugate for scrub typhus IgM into all wells are added. After incubation and washing 150 ul per well of EnWash is added. After repetition of incubation and washing procedure 100 ul of liquid TMB substrate is added. After incubation 50 ul per well stop solution is added. The intensity of colour/optical density is measured at 450 nm.

Data management definition and analysis
Patient's demographic data were entered in printed form. Reports were uploaded to the NIE portal. Case definition for chikungunya diagnosis in patients with IgM positive ELISA were considered probable chikungunya cases. IgM positive value (OD>0.5) in acute febrile sickness with or without, is considered as a probable positive case of scrub typhus.

Patients reporting with febrile illness Consent form taken and blood sample collected.

ELISA TEST



Statistical analysis
Microsoft Excel was used for statistical analysis.

RESULTS

985 patients were reported with acute febrile illness from August 2022–January 2023. Four hundred and eighty–seven patients with acute unknown febrile illness reported to Diamond Harbour Government Medical College and Hospital, suspected of chikungunya and scrub typhus infection. Other patients were excluded from the study due to other illnesses. For all 487 cases, Chikungunya and scrub typhus IgM ELISA tests were performed.

Out of 487 samples, 26 samples were tested positive for only the Chikungunya IgM ELISA test and 10 cases were positive for only scrub typhus. 3 samples out of 487 were co-infected with Chikungunya and Scrub Typhus infection (Figs. 1 and 2).

Out of 26 positive case of chikungunya, female has slightly higher prevalence of positivity than male. In case of scrub typhus male and female has equal positivity rate.

In case of coinfection out of 3 case 2 were male and 1 was female (Fig. 3).

Out of all positive case of chikungunya, middle age people are more prevalent and paediatric patients are least positive with chikungunya IgM ELISA test.

In contrast scrub typhus is most prevalent in paediatric age group and least prevalent in young adult and middle age group (Figs. 4 and 5).

Recent data showed that highest positivity rate of chikungunya was seen during the month of November as well as the positivity of scrub typhus was slowly increasing from October 2022 to January 2023 (Fig. 6).

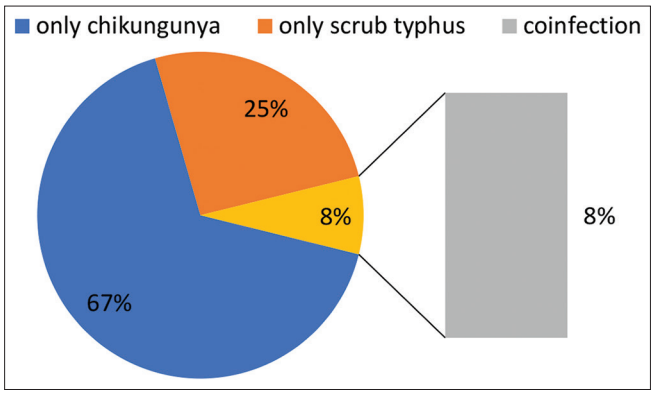
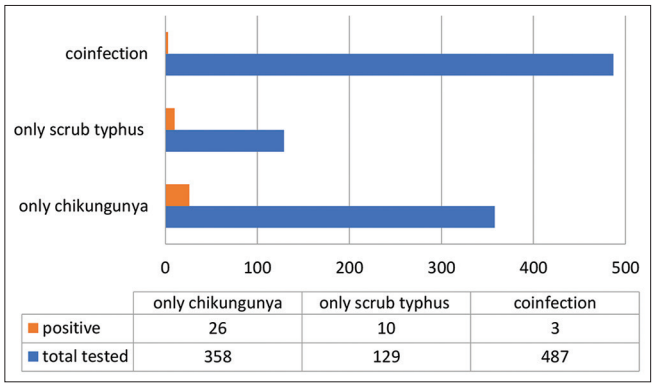
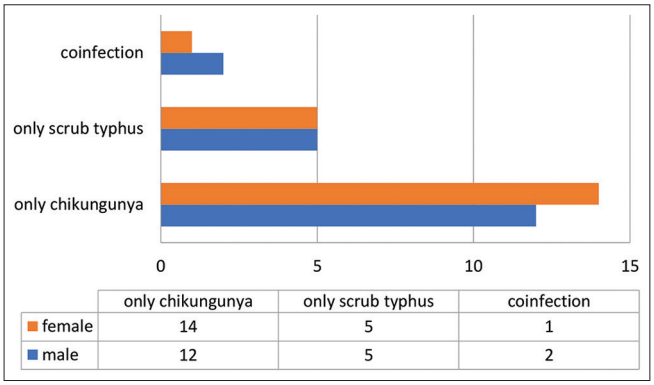


Fig. 1: Patients presenting with positive serology–chikungunya and scrub typhus



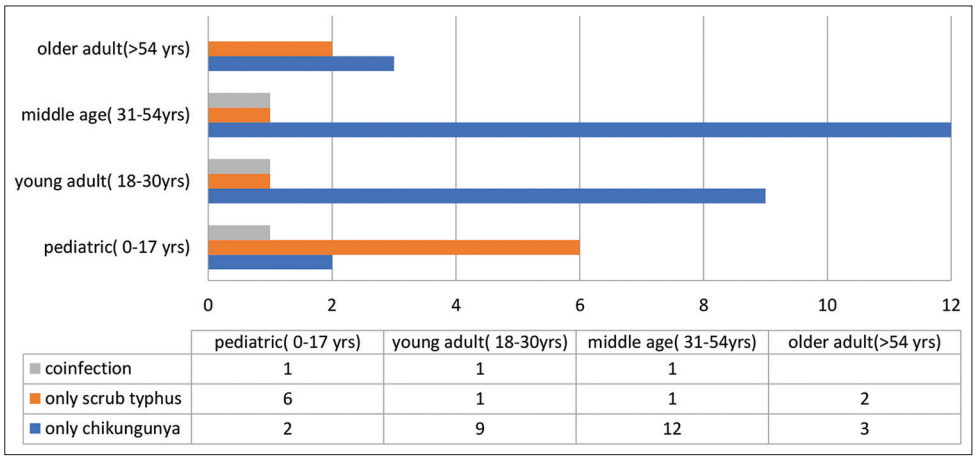
	only chikungunya	only scrub typhus	coinfection
positive	26	10	3
total tested	358	129	487

Fig. 2: Positivity status of chikungunya, scrub typhus and co-infected cases



	only chikungunya	only scrub typhus	coinfection
female	14	5	1
male	12	5	2

Fig. 3: Gender distribution of positive case of chikungunya, scrub typhus and co-infected cases



	pediatric(0-17 yrs)	young adult(18-30yrs)	middle age(31-54yrs)	older adult(>54 yrs)
coinfection	1	1	1	
only scrub typhus	6	1	1	2
only chikungunya	2	9	12	3

Fig. 4: Age ranges among positive patients

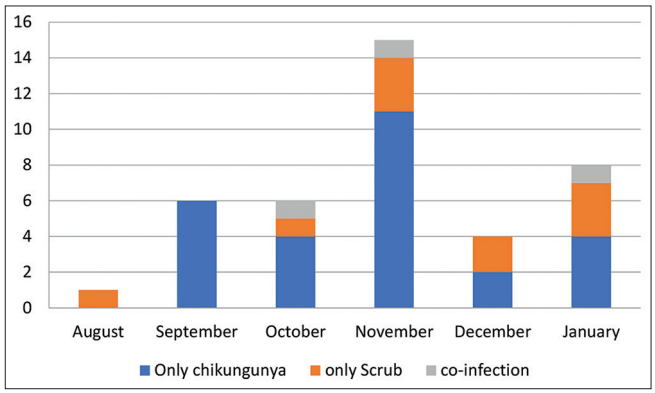


Fig. 5: Month wise distribution of positive cases

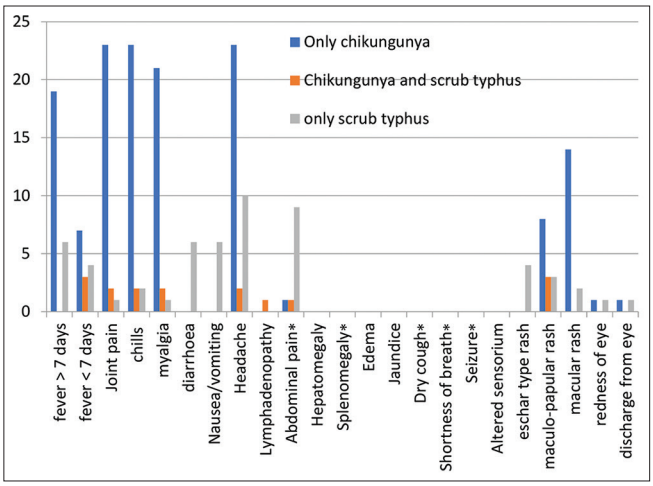


Fig. 6: Clinical features of positive cases

In our study, most of the chikungunya affected patients presented with fever for more than 7 days. Joint discomfort, myalgia, headaches, and macular rash are the most prevalent symptoms.

Fever, diarrhoea, vomiting, headache, abdominal discomfort, and an eschar-like rash are the most common symptoms of scrub typhus, while joint pain, chills, and myalgia are the least prevalent.

Among all positive patients for only chikungunya, 73% of patients presented with more than 7 days of fever and 26% presented with <7 days of fever. 88.4% patients complained of joint pain, chills, and a headache. 80.7% patient presented with myalgia. 53.84% patient presented with macular rash and 30.7% patient presented with maculopapular rash.

60% of patient with only scrub typhus presented with fever for more than 7 days and 40% presented with fever <7 days.10% patients presented with joint pain and myalgia, redness of eye and discharge from eye. Approximately 60% of the patients had diarrhoea, nausea, and vomiting. Every single patient complained of a headache. Thirty percent of the patients had a maculopapular rash, whereas 40% had an eschar-like rash.

Co-infected patients presented with fever, joint pain, chills, myalgia, headache, lymphadenopathy and maculopapular rash.

100% cases of co-infected patients presented with fever for <7 days and maculopapular type rash.66.6% sufferers suffered with joint pain, chills, myalgia, headache. 33.3% presented with lymphadenopathy and abdominal pain.

DISCUSSION

Scrub typhus is an infective zoonotic disease, caused by *O. tsutsugamushi.* In humans, it is transmitted by the bite of trombiculid mites which inhabit scrub vegetation. Present study demonstrated that from the month of August 2022 to January 2023, 67% of chikungunya cases, 25% cases with only scrub typhus and 8% cases with both chikungunya and scrub typhus presented positive.

Our study showed that among all positive cases of chikungunya, 7.6% were paediatrics, 34.6% are young aged people, 46.1% are middle aged people and 11.5% are older aged people.

In 2011–2012, Mohanty *et al.*, from Southern Odisha also reported 54.5% of Chikungunya cases are in the age group of 16–45 years [7]. Londhey *et al.*, have reported 50.3% cases of Chikungunya fever in the age group of 31–45 years from Mumbai in 2010–2015, which conforms with this study, in which the majority of cases (88.2%) of Chikungunya fever were in age group of 18–45 years [8]. Vijayakumar *et al.* found Chikungunya infection more commonly in the adult age group [9]. Among the scrub typhus positive infection, 60% are paediatric patients, 10% are young adults, 10% are middle aged adults and 20% are older aged.

A present study shows that chikungunya is slightly more prevalent in males as compared to females, where scrub typhus is equally positive in both male and female patients. The most expected cause can be due to occupation where men get exposed more in daytime. The proportion of males was more than females, both in the "chikungunya-only" and chikungunya-scrub typhus coinfection groups, possibly due

to higher involvement in male children's outdoor activities [10] in contrast to the above two studies, findings indicate that the proportion of chikungunya in 0-5-year-old children was higher than 6–14 years, as reported in another recent study with higher severe disease in infants and neonates [5].

Male preponderance was reported by Dinkar *et al.*, and Singh *et al.* [11].

In the study from North India by Mehta *et al.*, knee joint (92.5%) was the most commonly involved joint, followed by ankle joint (70%) and elbow joint (55%) in patients with Chikungunya fever [7]. The present study reported that fever, joint pain body rash are common in both chikungunya, scrub typhus and co-infected patient. Patients affected by scrub typhus presented with fever, joint pain, diarrhoea, nausea, headache, abdominal pain, eschar type rash or maculopapular rash. India belongs to the 'tsutsugamushi triangle', the postulated zone of highest prevalence of scrub typhus cases [12]. Present study shows that in only the case of chikungunya the most common symptoms are fever, joint pain (88.46%), chills (88.46%), myalgia (80.76%), headache (88%), macular rash (53.84%) and maculopapular rash (30.76%). The least common symptoms of only chikungunya cases are redness of the eye (3.8%), discharge from the eye (3.8%) and abdominal pain (3.8%).

CONCLUSION

Scrub typhus and chikungunya are two acute febrile illness which can affect simultaneously. Fever arthralgia are the common symptoms of both of the disease which can be confirmed by ELISA or PCR method. Laboratory testing of both of the disease can prevent the complication of other suspected disease in co-infected patients.

Application of molecular method for checking coinfection of scrub typhus and chikungunya infection is a need of the hour in view of serological cross reactivity.

Limitation

We could not carry the PCR method for our study of coinfection due to lack of funding. We propose the DNAPCR for Scrub Typhus and rRTPCR for chikungunya diagnosis in future.

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AUTHORS' CONTRIBUTIONS

PD conceived and designed the analysis, collected the data, performed the analysis, wrote the paper, SR, TKM, PH conceived and performed the analysis, UD conceived and designed the analysis, SS helped in clinical evaluation of symptomatic patients, SS helped in clinical evaluation of symptomatic patients. All authors reviewed the results and approved the final version of manuscript.

CONFLICT OF INTEREST

There was no conflict of interest.

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