ORIGINAL RESEARCH

Cross Reactivity of Dengue, Scrub Typhus and Malaria among COVID-19 Positive Patients: A Tropical World Threat & A Sero-Diagnostic Challenge

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ABSTRACT

Purpose: Diagnosis of acute undifferentiated febrile illness (AUFI) has been a challenge and burden in clinical practice in the tropics. COVID-19 cases that present with fever alone may be difficult to distinguish from other AUFIs in the tropics. Malaria, Scrub Typhus and Dengue fever are among the most common endemic diseases in the tropics. With the availability of rapid sero-diagnostic tests for these infections, it has been observed that patient's samples frequently show seropositivity for two or more infections posing challenges in clinical diagnosis and treatment. This study was performed to determine the false-positive serological test (seropositivity) in COVID-19 patients for Scrub typhus, Dengue and Malaria.

Materials and Methods: The present study was a type of observational prospective study conducted from April 2020 to November 2020. A total of 574 febrile patients which were positive in Real time PCR for Covid-19, were included in the study.

Results: Dengue IgM antibody positive for 124, Scrub typhus IgM antibody positive in 107 and no positive in malarial test, were found.

Conclusion: Our experience suggests that false-positive in the serological test should be interpreted with caution and requires surveillance. There should be a continuous follow-up of these patients during COVID-19 pandemic and the importance of recognising false positive serological results in patients with COVID-19, especially in the resource-constrained tropical settings.

Key words: Acute Febrile Illness, Covid-19, Pandemic, Seropositivity, Undifferentiated.

INTRODUCTION

Diagnosis of acute undifferentiated febrile illness (AUFI) has been a challenge and burden in clinical practice in the tropics.[1]Most tropical infectious diseases including malaria, dengue, leptospirosis, and rickettsioses present with fever and nonspecific signs and symptoms. The outbreak of Coronavirus disease 2019 (COVID-19), due to infection with novel severe acute respiratory syndrome coronavirus 2(SARS CoV-2)has become pandemic with a wide range of symptoms and severities and fever being the most common sign and symptom.[2]Thus, COVID-19 cases that present with fever alone may be difficult to distinguish from other AUFIs in the tropics. Malaria, Scrub Typhus and Dengue fever are among the most common

endemic diseases in the tropics and they have similar clinical presentation. Etiological diagnosis is important in the management of these diseases which are associated with population density, urbanization, endemicity and mobility all favouring the disease spread.[3]With the availability of rapid sero-diagnostic tests for these infections, it has been observed that patient's samples frequently show seropositivity for two or more infections posing challenges in clinical diagnosis and treatment. The reasons could be endemicity of the disease leading to raised IgG antibody level and sharing of antigen and cross-reacting antibodies. Poor diagnosis continues to hinder Malaria, Scrub Typhus and Dengue control in the tropics. This is due to a combination of factors including nonspecific clinical presentation of the diseases, high prevalence of asymptomatic infection in many areas, lack of resources of insufficient access to train health care providers and health facilities, widespread practice of self-treatment for clinically suspected conditions.[4]Individuals with acute malaria infection generate high levels of antibodies that cross-react with the viral spike protein of the COVID-19 disease. Cross-reactive antibodies specifically recognized the sialic acid moiety on N-linked glycans of the spike protein and do not neutralize in vitro SARS CoV-2.[5] Scrub typhus is known to be prevalent in foothills of Himalayas, however in recent past, its outbreaks have also been reported from major parts of India so their infection may co-exist with COVID-19.[6] Laboratory diagnosis of dengue during the febrile phase is established directly by detection of virus expressed soluble non-structural protein 1 (NS1 antigen) and/or dengue-specific IgM antibodies by means of ELISA.[7]To overcome these difficulties, tests employing rapid antigen detection, detection of post infection IgM antibodies can serve as alternatives. During this COVID-19 pandemic, false-positive results in serological test should be interpreted with caution and requires surveillance and continuous follow-up of these patients. It must be borne in mind that malaria /dengue can coexist with other infections, and thus confirmation of these infections do not rule out the possibility of the patients not suffering from COVID-19. This study was performed to determine the false-positive serological test(seropositivity)in COVID-19 patients for Scrub typhus, Dengue and Malaria.

MATERIALS & METHODS

The present study was a type of observational prospective study conducted in the Department of Microbiology, Rajendra Institute of Medical Sciences, Ranchi from April 2020 to November 2020. All febrile patients with mild symptoms tested positive on nasopharyngeal and oropharyngeal swab for SARS- CoV-2 by Real time PCR, admitted at COVID Care Isolation Wards were included in the study. Covid -19 diagnosed positive patients with critical or severe infections and admitted in ICU- set up were excluded from the study. Using strict aseptic precautions, 3-5 mL of venous blood was collected in appropriate sterile vials for serological, molecular testing. Serum was used for testing Dengue NS1 Antigen (PAN BIO kit), Dengue IgM Antibody (NIV, Pune kit), Scrub Typhus IgM Antibody (INBIOS kit) by ELISA methods. Whole blood collected from positive patient was further tested for malaria antigen by rapid test (PARACHECK) and by direct microscopy after Giemsa Staining. The clot from blood was separated and used for performing Conventional PCR for scrub typhus of those samples which showed positive in Scrub Typhus IgM Antibody test. For PCR of Scrub Typhus16S rRNA gene was targeted using primers OT1-F (5-CGAATTAATGCTGAGTTTGCTTAG-3) and OT1-R (5-CTCTCAGACCAGCTACAGATCACA-3) and under following PCR cycling conditions: 95°C for 3 minutes (1 cycle), followed by 40 cycles of 90°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds.

RESULTS

A total of 574 febrile patients with mild symptoms tested positive on nasopharyngeal and oropharyngeal swab for SARS CoV-2 by Real time PCR were included in the study. Among these patients,314(54.7%)were male and 260 (45.3%)were female with median age being 39 years and ranges from 2 years to 74 years (CI =37.5 -39.4; Chi-square = 4.39; df=1 ; p value =0.02). Male to female ratio was 6:5. The body temperature of all admitted patients ranged from 100-104 °F. Malaria parasites test for *Plasmodium falciparum* & *Plasmodium vivax* were negative on smear microscopy in all cases and in rapid test performed for malaria, none of them were positive for malarial antigens. Dengue virus NS1Agshowed negative results in all cases whereas 124 (21.6%; Chi-square = 3.84;df=1; p value < 0.0001)sera showed positive for Dengue IgM antibody test. (Figure 1) On the other hand, 107 (18.6% Chi-square = 224.53; df=1; p value < 0.0001) patients were tested positive for Scrub typhus IgM antibody (Figure 1) and on PCR for predicted amplification product of 220 bp for confirmation of Scrub Typhus co-infection, none showed result in gel Electrophoresis. (Figure 2)

Figure 1: Seropositivity of Malaria, Scrub typhus and Dengue in Serodiagnostic tests in SARS CoV-2 positive patients

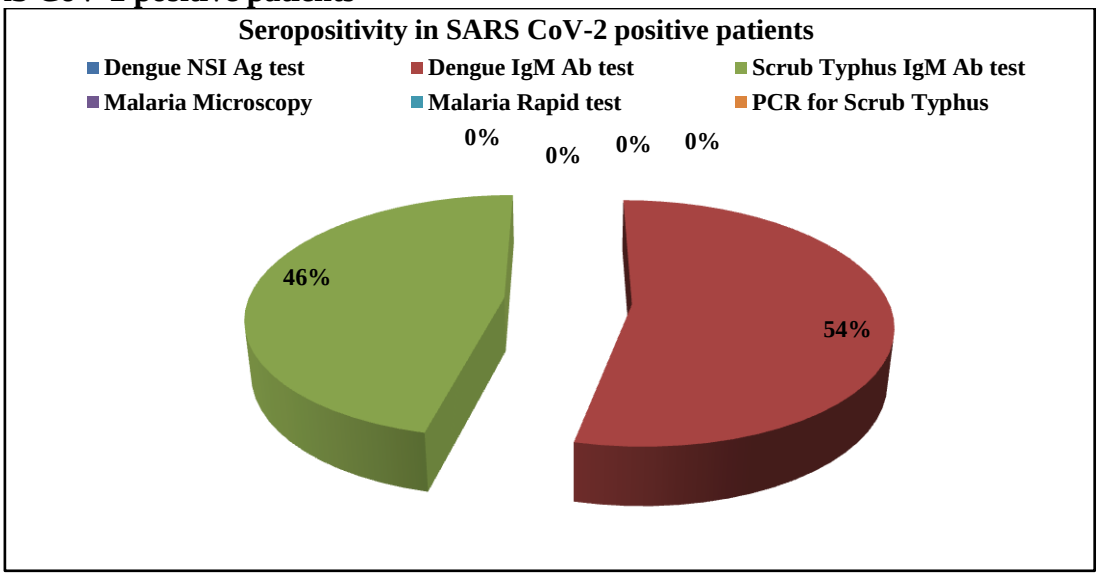
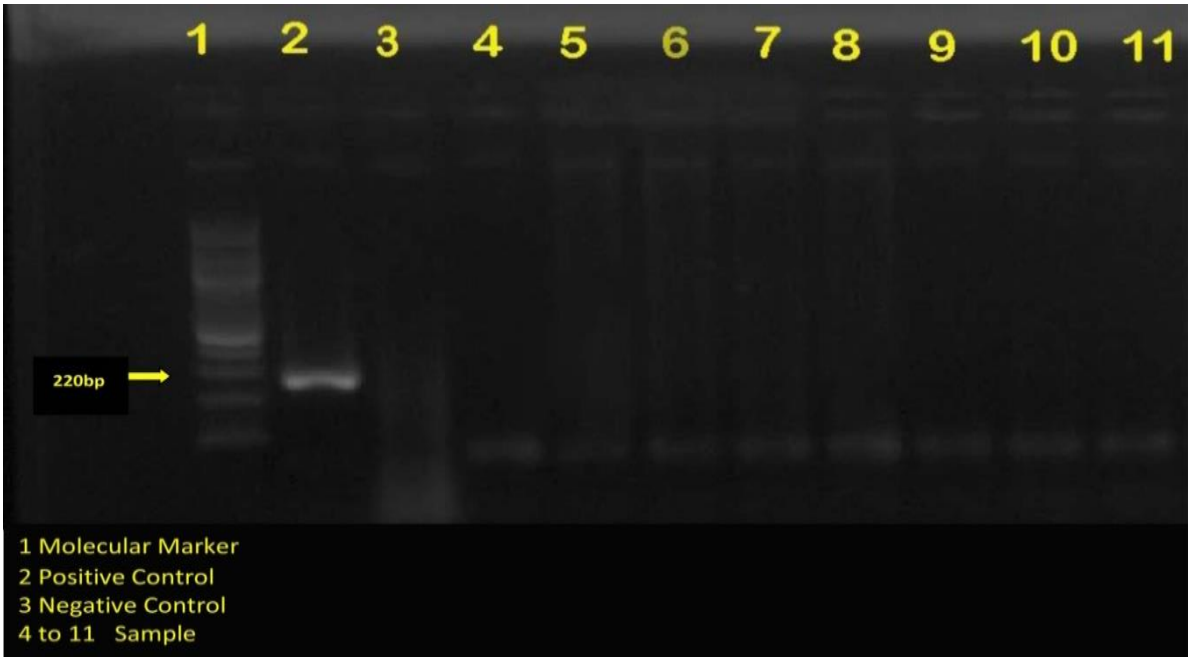


Figure 2: Gel image of PCR product by conventional polymerase chain reaction of Scrub Typhus



DISCUSSION

With the advent of rapid diagnostic serological tests for diagnosis of these diseases, there have been a lot of challenges in laboratory and clinical diagnosis of co-infection and cross-reactivity of tests. No significant data about such an association is available in India. In this study, neither smear microscopy nor rapid antigen test suggested malaria positivity in febrile COVID-19 diagnosed patients deferring the consideration of co-infection or cross reactivity of Malaria and COVID-19 as compared to study done by Laura C. Steinhardta et al in which Anti-Plasmodium IgG levels were significantly higher among SARS-CoV-2 serological assays showing false-positives but no association was found with active falciparum infection in malaria endemic settings.[8,9]

In dengue-endemic regions, 80% of COVID-19 cases are mild to moderate with nonspecific symptoms that mimics dengue.[10]The risk of dengue infection exists in 129 countries and seventy per cent of the actual burden of disease is in Asia.[11] In this study, Dengue NS1 Ag was not detected and Dengue IgM Ab was positive in 21.6% cases, as NS1 Ag is detected from first day till seventh day of fever, so the cause may be due to delayed testing of COVID-19 diagnosed febrile patients. Yan et al recently described two cases which were initially diagnosed as dengue but later confirmed to be COVID-19 from Singapore.[12]Marsha S. et al showed one patient tested positive for dengue IgM as well as NS1 in rapid diagnostic test among ninety five RT-PCR confirmed COVID-19 patient from Indonesia.[13]LokeshTiwari et al also reported the first paediatric case of serological cross-reactivity of dengue with SARS-CoV-2 infections from India.[14]Prasitsirikul et al,[15]Kembuan et al[16]and Sri Masyeni et al[17]also showed cross reactivity between these two infections from Thailand ,Indonesia and Asia respectively. A recent study found that a false-positive result could occur for both COVID-19 serology among dengue patients and reciprocally dengue serology among COVID-19 patients.[10]Antigenic cross-reactivity between dengue, JE andCOVID-19 could be due to similarity of specific SARS-CoV-2, JE and dengue protein structures regardless of genetic distance.[18] Decreased thrombopoietin production due to liver damage and dysfunctional bone marrow microenvironment, increased platelet clearance and consumption, and antiviral drugs may lead to the development of thrombocytopenia in patients with COVID-19 mimicking laboratory findings of dengue fever.[19]

Overlapping clinical pictures and co-epidemics of COVID-19 with other tropical diseases, such as Scrub Typhus has been a matter for concern in many tropical countries.[20] In this

study, 18.6% seropositivity was seen for Scrub Typhus IgMAb but as none showed positive results in Conventional PCR proving that the results are more coinciding toward cross reactions rather than co-infection. Hence, the false positive of COVID-19 serological testing kits is raising false alarms.[21]Also this is first study of its kind from this region and correlation between COVID-19 and Scrub Typhus has not been obtained in any study so far.
The Limitation of this study was that it was correlated only with Microbiological laboratory findings and was not correlated with clinical other than fever and Pathological or Biochemical test results.

CONCLUSIONS

During this pandemic, unless otherwise COVID-19 is ruled out as an aetiological infectious agent for the pyrexia, it will severely impact the public health and prognosis of the individual case. Our experience suggests that false-positive in the serological test should be interpreted with caution and requires surveillance. There should be a continuous follow-up of these patients during COVID-19 pandemic and the importance of recognising false positive serological results in patients with COVID-19.

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