Technical Note

Potential impact of SARS COV-2 infection on the performance of serological assays used to diagnose arboviral diseases

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ABSTRACT

Background: The COVID-19 pandemic caused by SARS-CoV-2 was first described in December 2019, in China. In addition, there has also been an increase in arboviral infections in recent years. As both infections have similar symptoms, misdiagnosis may occur if both outbreaks occur at the same time.
Objective: Our objective was to assess the potential impact of SARS-CoV-2 infection on diagnostic assays used for arboviral diseases.
Materials and methods: We conducted this study by testing samples obtained during the precovid phase (before November 2019) and during the covid period (after February 2020). Samples were further grouped as those with acute febrile illness (AFI) and those without. All samples were tested for anti SARS-CoV-2 Ab, Chikungunya and Dengue specific IgM antibodies to evaluate potential serological cross-reactions between COVID-19 and Arbovirus specific antibodies.
Results: One sample from the 62 cases of AFI during the pre-covid phase showed seropositivity for SARS-CoV-2 antibodies. Also, in asymptomatic individuals, arboviral seropositivity was significantly higher in the COVID period samples (22%) compared to pre-COVID samples (3%).
Conclusion: Due to similar clinical symptoms and cross reactions in both infections, relying solely on serological testing for arboviral diagnosis may be less sensitive; other clinical and laboratory parameters may be required.

1. Introduction

The Coronavirus disease (COVID-19) caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged from Wuhan, capital of Hubei Province, China in December 2019 and has spread worldwide. On March 11, 2020 COVID-19 was declared a pandemic by the World Health Organization. Upto December 2021, almost 281 million cases had been reported with 5 million deaths (World Health Organization, 2021).The COVID-19 pandemic has triggered a global economic crisis on a scale never seen before. The common symptoms of patients infected with SARS-CoV-2 includes fever, headache, loss of taste and smell, cough and sore throat.These symptoms vary from mild, moderate to severe in different groups of people which creates a diagnostic dilemma overloading the healthcare facilities (Baloch et al., 2020). The coexistence of COVID-19 and other viral infections has been a source of concern, especially for countries located in the tropical and subtropical regions where there is a higher prevalence of arboviral diseases (United Nations Department of Economic and Social Affairs (UN DESA), 2021; Liang et al., 2015).The co-circulation of arboviruses such as Dengue and Chikungunya,which are not completely understood, adds to the health-care burden. These arboviral infections usually presents as fever, headache, retro-orbital pain,musculoskeletal pain and transient rash (Lorenz et al., 2020; Saavedra-Velasco et al., 2020). Though there are some clinical signs and symptoms which may be indicative of COVID-19 and dengue infection, still there is a constant warning by the epidemiologists all over the world regarding the temporal coincidence relating to the COVID-19 and arboviruses (especially dengue) as both share similar clinical features and laboratory findings leading to diagnostic and management challenges (Lorenz et al., 2020; Saavedra-Velasco et al., 2020; Yan et al., 2020).Therefore in the present study we aimed to assess the potential impact of SARS-CoV-2 infection on diagnostic assay used for the diagnosis of arboviral diseases.

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2. Methodology

2.1. Patient cohort

In our study, we tested samples obtained during different time periods, during the precovid phase (August 2019–November 2019) and also during the covid pandemic (July 2020–March 2021). We investigated potential serological cross-reactions between COVID-19 and Arbovirus (Dengue and chikungunya virus) specific antibodies by performing commercial assays on sera obtained from well-characterized COVID-19 and arbovirus infected patients with demonstrated seropositivity. For our study convenience we have divided the samples into different groups. In the pre-covid phase, samples were categorized as those collected from patients without febrile illness (P1) and those with febrile illness patients (P2). During covid phase, samples from patients without febrile illness (C1) were grouped. From precovid phase, 50 samples were chosen in random from asymptomatic individuals (P1), 62 samples from patients with febrile illness (P2)-[seropositive for dengue ($n = 34$) and chikungunya ($n = 28$) IgM Ab]. And during the covid phase, 50 samples were taken from asymptomatic patients who had a past history of covid (C1).

2.2. Serological testing

All the above said samples collected during both pre-covid and covid phase were tested for the presence of anti SARS-CoV-2 total Ab (Wantai SARS CoV2 Ab ELISA kit), Chikungunya specific IgM antibodies(National Institute of Virology Chikungunya IgM capture ELISA kit) and Dengue specific IgM antibodies(National Institute of Virology Dengue IgM capture ELISA kit).

3. Results

In P1 group, all 50 samples tested were serologically negative for anti-SARS CoV-2 total antibodies. Three samples were seropositive and 10 were serologically equivocal for arbovirus antibodies, as listed in Table 1.

In P2, out of the 62 samples, only one sample was seropositive for both dengue and SARS CoV-2 as depicted in Table 2.

In C1, 50 of the anti SARS-CoV-2 antibody seropositive samples were tested for arbovirus antibodies, of which 11 were seropositive and 14 were serologically equivocal (see Table 3). There is significant increase in Dengue rates (p value −0.027) in C1 group as compared with rates detected in P1 group by chi square test.

4. Discussion

An additional strain has been put on health care systems by the ongoing SARS CoV-2 pandemic and the situation becomes more complicated in arboviruses endemic areas. At present, in addition to the SARS CoV-2 cases observed, arboviral infections continue to spread mainly in the tropical areas (Cattarino et al., 2020). Arboviral infections, dengue and Chikungunya, share similar clinical manifestations as are commonly observed with the SARS CoV-2 infected patients,which poses a diagnostic dilemma (Di Gennaro et al., 2020; Nacher et al., 2020). The primary diagnostic modality for SARS-CoV-2 infection is RT-PCR or antigen detection test from throat/nasal swab, whereas arboviral disease

Table 1

P1- samples from asymptomatic patients during pre-COVID phase.

P1 ($n = 50$)	Dengue IgM	Chikungunya IgM	SARS-CoV2 Total Ab
Positive	0	3 (6%)	0
Equivocal	2 (4%)	8 (16%)	0
Negative	48 (96%)	39 (78%)	50 (100%)

Table 2

P2- samples from patients having febrile illness during pre-COVID phase.

P2 ($n = 62$)	SARS-CoV2 Total Ab (Positive)
Tested positive for dengue IgM ($n = 34$) (54.8%)	1 (1.6%)
Tested positive for Chikungunya IgM ($n = 28$) (45.1%)	0

Table 3

C1- samples from asymptomatic patients with past history of COVID.

C1 Arboviruses serology		SARS CoV-2 Total Ab positive samples($n = 50$)
Dengue IgM	Positive	6 (12%)[a]
	Equivocal	5 (10%)
	Negative	39 (78%)
Chikungunya IgM	Positive	5 (10%)[b]
	Equivocal	9 (18%)
	Negative	36 (72%)

[a] Of the 6 samples which were seropositive for Dengue,3 were found to be seropositive for Chikungunya also,and 1 was found to be equivocal for Chikungunya.
[b] Of the 5 samples which were seropositive for Chikungunya,3 were found to be seropositive for dengue and 1 was found to be equivocal for dengue.

diagnosis primarily depends on serologic detection of antigen or antibodies in serum.

In our study, when we examined the pre-COVID samples, the set of samples from asymptomatic individuals (P1) did not show any seropositivity for SARS-CoV-2. However, from the pre-COVID samples drawn from cases of acute febrile illness (P2), one sample (1/62) showed seropositivity for SARS-CoV-2. In the asymptomatic pre-COVID samples (P1), 3 samples showed seropositivity to arbovirus; whereas during the COVID period, from samples of asymptomatic individuals who had serologic evidence of SARS-CoV-2 infection (C1), arboviral seropositivity was observed in 22% of samples. The seropositivity in P1 can be explained by prolonged IgM positivity or asymptomatic arboviral presentation, however in C1 this proportion is higher than expected (Chelluboina et al., 2019).

Lustig Y et al. in their study(March 17,2019-April 13,2019) observed simultaneous seropositivity for SARS CoV2 and Dengue, they attributed it to structural similarity, however in the current study our set of samples which were positive for arboviral serology from pre-covid period (P2), we observed SARS CoV2 seropositivity in only one case ($n = 62$) (Lustig et al., 2021).

In a study conducted by Stringari LL et al., it was observed that from a total of 7730 patients who were suspected with dengue and chikungunya, 210 were seropositive for anti-SARS-CoV-2 IgG (Stringari et al., 2021).

Although IgM/IgG-detecting serological assays are used to diagnose arboviral infections, they cannot be used to diagnose SARS-Cov-2.

SARS-CoV-2 can cause immune system hyper-stimulation. It is linked to changes in circulating leukocytes and a significant increase in pro-inflammatory cytokines such as interleukins (IL-6, IL-1), and Tumor necrosis factor (TNF-a), which can in some instances cause a "cytokine storm" in patients (Dotan et al., 2021).In addition to the virus's propensity to cause immune system hyper-stimulation, current research has shown a main sequence homology between humans and SARS-CoV-2 components.Antibodies produced by this acquired immune system cross-react with common molecules present in pathogens and self-components. Increased seropositivity for arboviral infections may be due to this altered immune response seen in SARS-CoV-2 infection (Dotan et al., 2021).

As a result, complete reliance on serological tests for arboviral diagnosis may be misleading due to similar clinical manifestations and cross reaction in both infections. It may be advisable to use other clinical and laboratory parameters to arrive at clinical diagnosis.

There are two main limitations of this study- first, diagnosis of dengue and chikungunya was based on IgM whereas SARS-Cov-2 sero-positivity was based on total Ig.

Second, PRNT (plaque reduction neutralisation test) could not be performed to verify arboviral diagnosis.

Data availability

No data was used for the research described in the article.

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