

Seroprevalence of Anti-SARS-CoV-2 IgG Antibodies among HIV Infected Individuals Attending ART Centre at Pune: A Cross-Sectional Study

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Abstract

Background: We aimed to determine the anti-SARS-CoV-2 IgG antibodies among people living with HIV (PLHIV) in Pune, India. **Methods:** This cross-sectional study was conducted between March 2021 and June 2021. Demographic and clinical information related to coronavirus disease 2019 (COVID-19) were recorded on structured questionnaires. Blood samples were collected and tested for anti-SARS-CoV-2 IgG antibodies using commercial ELISA. **Results:** Of the 405 HIV infected individuals enrolled in the study, 223(55.1%) were females. Mean age and CD4 count of participants were 42 years (SD: 10) and 626 cells/mm³ (SD: 284) respectively. A total of 382 (95%) PLHIV were virologically suppressed. The seropositivity against SARS-CoV-2 was found in 221 PLHIV (54.6%, 95% CI: 49.7-59.4). No significant association was found between demographic or clinical factors and seropositivity. **Conclusion:** A high prevalence of anti-SARS-CoV-2 IgG antibodies was found among PLHIV attending ART centre indicating an exposure to the virus among them.

Keywords

HIV, SARS-CoV-2, COVID-19, antibodies, seroprevalence

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Introduction:

World Health Organization (WHO) declared novel Coronavirus Disease 2019 (COVID-19) outbreak as a pandemic and advised countries to take immediate actions on March 11, 2020.¹ India had a total 11,599,130 COVID-19 cases of which 2,479,682 were in Maharashtra state till March 21, 2021.²

The COVID-19 positivity rate of Pune city in Maharashtra state was 16.04% on July 1, 2020 which increased to 22.10% on July 27, 2020 and was highest in the country.³ A study conducted by scientists and epidemiologists from the Indian Institute of Science Education and Research (IISER), Pune, between 20th July and 5th August 2020 with random blood samples of 1664 persons taken by teams from five 'high-incidence' wards in Pune city had shown prevalence of 51.5%. The results mentioned an extensive spread of infection in the five wards with seroprevalence ranging from 36.1% to 65.4%.⁴ The Indian Council of Medical Research (ICMR) conducted the fourth round of national serosurvey in June-July

2021 and showed the prevalence of 67.6% among general population. It was 58% for the six districts in Maharashtra State.^{5,6}

This disease has impacted everyone across the globe especially individuals with comorbid conditions. World Health Organization mentioned that the risk of developing severe COVID-19 was 30% greater among people living with HIV (PLHIV) as compared to HIV uninfected individuals⁷ and those having lower CD4 count and not taking antiretroviral treatment (ART) were at increased risk of opportunistic infections.⁸ However, evidence whether PLHIV have an increased

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risk of acquisition of SARS-CoV-2 infection and/or COVID-19 clinical complications is conflicting.⁹

HIV is a major public health problem with 2.14 million estimated PLHIV in India. There are around 11,33,950 PLHIV on treatment under national program in 532 Antiretroviral Therapy (ART) centres by August 2017.¹⁰

The seroprevalence of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) antibodies greatly helps in understanding the disease transmission, estimated total infections, population susceptibility and the measures to be followed for mitigating the COVID-19 infection. The data on susceptibility to SARS-CoV-2 infections and the risk of mortality among PLHIV is evolving.

The purpose of this study was to determine the seroprevalence of anti-SARS-CoV-2 IgG antibodies among people living with HIV attending ART centre in Pune, India.

Material and Methods:

Study Design, Setting, and Participants

This observational cross-sectional study was conducted in a clinic at the Indian Council of Medical Research-National AIDS Research Institute (ICMR-NARI). This outpatient clinic is affiliated to the National AIDS Control Organization (NACO) and caters to a proportion of PLHIV in Pune city. HIV infected adults from March 2021 through June 2021 were included in the study.

Sample size was estimated to detect 40% probability of having COVID-19 antibodies among general population,⁴ with absolute precision of 5% at a 95% confidence level. A total of 405 HIV infected individuals attending ART centre regularly were selected consecutively for this observational study at the clinic.

Ethical Approval and Informed Consent

The study was approved by the ICMR-NARI Institutional Ethics Committee (Protocol number: NARI-EC/2020-30). Participants were recruited after obtaining written informed consent.

Data Collection

A structured questionnaire was used for collecting data on different explanatory variables including socio-demographic factors, clinical characteristics, and detailed clinical history of symptoms suggestive of COVID-19. Socio-demographic variables included age, gender, and employment status. Data regarding HIV infection, type of ART regimen, duration on ART, CD4 count and viral load were collected and entered into database. The data on replacement of antiretroviral drug(s) among PLHIV due to adverse effects of drug, drug-drug interactions or programme policy was entered under treatment substitution.¹¹

COVID-19 Antibodies Measurement

The blood samples were processed for plasma and peripheral blood mononuclear cells (PBMC) separation by using density gradient centrifugation. The plasma samples were tested for IgG antibodies against SARS-CoV-2 by using commercial COVID-19 IgG enzyme-linked immunosorbent assay (ELISA) kit, targeting Spike 2 and Nucleoprotein of SARS-CoV-2 protein (ErbaLisa COVID-19 IgG, Transasia Biomedicals Ltd, India) according to the manufacturer's instructions. Briefly, 1:10 diluted plasma sample (100 µL) and the known positive, negative and calibrator controls (provided in the kit) were added to each well and then incubated at 37 °C for 45 min. The wells were washed and the horseradish peroxidase conjugate was added (50 µl) to each well. The plate was incubated at 37 °C for 30 min. After washing, the substrate (50 µl) was added to each well and the plate incubated in the dark at ambient temperature for 15 min. The reaction was stopped and optical density (OD) was measured at 450 nm with 630 nm as a reference wavelength. The cut-off of the assay was calculated by multiplying mean Calibrator OD with Calibration factor (mentioned on calibrator control vial). The antibody index was calculated by dividing OD of each sample by cut-off value. The samples showing antibody index more than 1.1 were considered positive for IgG antibody to COVID-19. The samples with antibody index <0.9 were considered as negative for IgG antibody to COVID-19, and the samples with antibody index between 0.9–1.1 were considered borderline positive.

Outcome Measure

The presence of IgG antibodies against SARS-CoV-2 infection conducted by ELISA was considered as binary outcome variable. Age, gender, employment, type of ART regimen, duration on ART, treatment substitution, CD4 count and viral load were considered as explanatory variables.

Statistical Analysis

The demographic and clinical characteristics of study participants were summarized using mean (standard deviation; SD). Association of explanatory variables with the presence of SARS-CoV-2 antibodies was analyzed using Chi-square test. We reported 95% confidence intervals (CIs) for seroprevalence. IBM SPSS 20.0 (IBM Corp., Armonk, NY, USA) was used for the data analysis.

Results:

Demographic and Clinical Characteristics

Of the 405 HIV infected individuals enrolled in the study, 223(55.1%) were females. Mean age and CD4 count of participants were 42 years (SD: 10) and 626 cells/mm³ (SD: 284) respectively. More than half of the participants were aged more than 40 years (252, 62.2%). Mean duration of HIV

Table 1. Association of Anti-SARS-CoV-2 IgG Antibodies with Explanatory Variables.

Variables N = 405	Anti-SARS-CoV-2 IgG antibodies positive (n = 221, 54.6%)	Anti-SARS-CoV-2 IgG antibodies negative (n = 184, 45.4%)	Total	P-value
Gender				
Male	105 (57.7)	77 (42.3)	182 (44.9)	0.254
Female	116 (52.0)	107 (48.0)	223 (55.1)	
Age (in years)				
≤40	75 (49.0)	78 (51.0)	153 (37.8)	0.081
>40	146 (57.9)	106 (42.1)	252 (62.2)	
Employment				
Employed	155 (56.6)	119 (43.4)	274 (67.7)	0.242
Unemployed	66 (50.4)	65 (49.6)	131 (32.3)	
Latest CD4 (cells/mm³)				
≤500	86 (58.5)	61 (41.5)	147 (36.3)	0.230
>500	135 (52.3)	123 (47.7)	258 (63.7)	
HIV Viral load (copies/ml) (n = 402)				
≤ 1000	208 (54.5)	174 (45.5)	382 (95.0)	0.962
>1000	11 (55.0)	9 (45.0)	20 (5.0)	
Treatment Substitution				
Yes	194 (56.4)	150 (43.6)	344 (84.9)	0.079
No	27 (44.3)	34 (55.7)	61 (15.1)	
Duration on ART				
< 3 years	23 (48.9)	24 (51.1)	47 (11.6)	0.667
3.1- 5 years	24 (54.5)	20 (45.5)	44 (10.9)	
5.1 to 10 years	79 (52.7)	71 (47.3)	150 (37.0)	
>10 years	95 (57.9)	69 (42.1)	164 (40.5)	
Duration of HIV infection				
< 3 years	20 (48.8)	21 (51.2)	41 (10.1)	0.467
3.1- 5 years	15 (68.2)	7 (31.8)	22 (5.4)	
5.1 to 10 years	67 (52.3)	61 (47.7)	128 (31.6)	
>10 years	119 (55.6)	95 (44.4)	214 (52.8)	
ART regimen				
Zidovudine based	21 (51.2)	20 (48.8)	41 (10.1)	0.640
Tenofovir based	192 (54.5)	160 (45.5)	352 (86.9)	
Other	8 (66.7)	4 (33.3)	12 (3.0)	
Symptoms related to COVID-19 in last 30 days				
Yes	7 (58.3)	5 (41.7)	12 (3.0)	0.790
No	214 (54.5)	179 (45.5)	393 (97.0)	

infection was 10 years (SD: 4.8). The mean duration on antiretroviral treatment was 9 years (SD: 4.2) and 314 (77.5%) participants were on ART for more than five years. Majority of the participants (95%, 382/402) had suppressed viral load and 84.9% (344/405) had treatment substitution. Of the total, 63.7% (258/405) participants had CD4 count of more than 500 cells/cmm³ at the time of study enrollment (Table I).

Twelve of the total participants (3%) were symptomatic (having influenza like illness symptoms) within the last 30 days of anti-SARS-CoV-2 IgG antibodies testing. COVID-19 testing was done prior to study recruitment, among 22% (89/405) participants and 20% (18/89) of them had infection.

On testing of anti-SARS-CoV-2 IgG antibodies by commercial ELISA, we found that more than half of the participants (54.6%, 221/405) were positive.

Gender, age, employment, CD4 count, viral load, and treatment substitution were not found to be associated with the

presence of antibodies against SARS-CoV-2 among study participants. Our study also revealed that the duration of HIV infection and duration on ART were not significantly associated with the study outcome.

Discussion:

The present study was conducted among PLHIV during the period of second wave of COVID-19 in India. The seroprevalence of IgG antibodies against SARS-CoV-2 in our study was 54.6%. Though the prevalence was higher among PLHIV indicating exposure to SARS-CoV-2, it was less than the national (67.6%) and Maharashtra state (58%) seroprevalence among general population as reported by the Indian Council of Medical Research in June-July 2021 after fourth round of serosurvey.^{5,6}

Our seroprevalence was much higher than that reported from North India among PLHIV (14%) which might be due to the

differences in the pandemic period and study design.¹² This seroprevalence among PLHIV varied from the general population in the same region that ranged from 24.08% to 50.76% around the study period.^{13,14}

A cross-sectional study from Rome on stored cryopreserved samples from 1st March 2020 to 30th November 2020 that included both the first and the second wave of the Italian COVID-19 pandemic has mentioned seroprevalence of 0.72% among PLHIV that was less as compared to data available from their general population.¹⁵ Another study from Umbria, Italy, on IgG antibodies against SARS-CoV-2 during the 4th to 31st May 2020 has mentioned seroprevalence of 4% and the authors have speculated that Umbrian PLHIV were careful as they perceived themselves to be at higher risk or that social isolation could have helped in the stigma related with HIV infection.¹⁶

In India, second wave started since the middle of March 2021 and on 9th April, the highest number of cases (144,829) was identified.¹⁷ Some of the reasons behind rise in cases were mentioned as COVID-19 appropriate protocol fatigue, mass gatherings, urban motility, increased testing and mutations in coronavirus. As per official records in June, India was approaching 3 million active cases of coronavirus and 200,000 people had died and it was widely acknowledged that the figure could be three to ten times higher.^{18,19}

A seroprevalence study among hospitalized patients at a tertiary referral center in North India has shown seroprevalence of 19.8% and has mentioned that majority patients were asymptomatic for COVID-19.²⁰ In our study, most of the HIV infected individuals did not experience any symptom of COVID-19 similar to study conducted in North India.¹⁴ We analyzed the factors associated with IgG antibodies against SARS-CoV-2 among PLHIV but none of the factors showed significant association. There are reports on whether HIV infected individuals have greater risk of acquiring SARS-CoV-2 and severe clinical outcomes of COVID-19. A systematic review during early phase of COVID-19 suggests that PLHIV with well-controlled disease are not at risk of poorer COVID-19 disease outcomes than the general population²¹ whereas a recent systematic review has mentioned that HIV is a significant risk factor for acquisition of SARS-CoV-2 infection as well as higher risk of mortality from COVID-19.²²

World Health Organization found that people with HIV were at higher risk of severe or critical illness at the time of admission and at higher risk of dying after admission to hospital with COVID-19 by adjusting for age, gender, and the presence of underlying conditions.²³ WHO has recommended prioritizing PLHIV for early COVID-19 vaccines considering that people living with HIV have a 30% increased risk of death if they are hospitalized and that vaccination for COVID-19 protects people from having severe disease, hospitalization and death. National AIDS Control Organization has recommended vaccination against COVID-19²⁴ and all ART centres across the country are advising and supporting PLHIV referrals to vaccination centres.

Limitations:

Our study estimated seroprevalence of anti-SARS-CoV-2 IgG antibodies and associated factors. However, it has few limitations. In this study, children living with HIV were not included for the detection of seroprevalence. Data on sociodemographic variable possibly associated with SARS-CoV-2 seropositivity like type of housing settlement and number of family members were not collected.

The cause-effect relationship could not be determined due to cross-sectional study design. We did not perform RT-PCR confirmatory test, therefore this study is an approximate representation of the COVID-19 distribution in HIV infected individuals attending ART centre. Being a single centre study, findings cannot be generalized to HIV infected population in the country. There was no control group in the study and the comparison with HIV uninfected population is done only on the basis of published seroprevalence study findings in Pune city and Maharashtra.

Conclusions:

Our study showed 54.6% SARS-CoV-2 seroprevalence among PLHIV in Pune during the period of March 2021 to June 2021. Further studies on prevalence and persistence of antibodies against SARS-CoV-2 in India will be important in understanding course of COVID-19 among vaccinated and unvaccinated PLHIV stratified by their immunological and virological suppression set up. This will also help in decision on requirement of booster doses of vaccines among them.

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