SEROPREVALENCE OF SARS-CoV-2 SERUM IgG ANTIBODY IN ADULT POPULATION OF SIALKOT CITY, PAKISTAN: A CROSS-SECTIONAL STUDY

Shahid Mahmood,[1] Izza Jahangir,[2] Sidra Ghazanfar,[3] Ayesha Khalid,[4] Ghazia Qasmi,[5] Imrana Aslam[6]

Abstract

Background and Objectives: Seroprevalence studies provide necessary data on extent of SARS-CoV-2 infection in a community. Identifying immune response of individuals exposed to this virus is one way of assessing community disease transmission and herd immunity. The aim of this study was to measure SARS-CoV-2 IgG antibody among adult individuals of Sialkot city overall and stratified by age and sex. Additionally, to describe COVID-19 presenting symptoms and its duration in these participants.

Methods: This descriptive, cross-sectional study was undertaken in Sialkot city, where 453 participants completed in-person interview on COVID-19 symptoms, socio-demographic profile and provided 5ml blood sample for SARS-CoV-2 IgG antibody testing using electro-chemiluminescent immunoassay (ECLIA) technique. SPSS version 22 was utilized for data management and analysis. Sero-prevalence was calculated as percent of reactive for IgG among all tested. Chi-squared test was used for determining statistical significance with $p < 0.05$ was considered significant.

Results: Overall, sero-prevalence of SARS-CoV-2 IgG antibody among adults in Sialkot was 64%. Almost, 134(29.5%) of these positive for COVID-19 IgG antibody had antibody titres level 15 units/dl or above. Three out of every four individuals reported one or more symptoms of COVID-19 dry cough (17%), fever (30%) loss of taste (6%), sore throat (5%) and anosmia (4.6%).

Conclusion: Almost two-third of adult population in Sialkot city had SARS-CoV-2 IgG antibody during 2021 survey that implies that herd immunity level for COVID-19 is achieved and negligible number of COVID-19 cases reported in Sialkot city supports this evidence.

Key words: Sero-prevalence; COVID-19; SARs-CoV-2 IgG antibody; ECLIA; Sialkot, Herd immunity; Pandemic

How to Cite: *Mahmood S, Jahangir I, Ghazanfar S, Khalid A, Qasmi G, Aslam I. Seroprevalence of SARS-COV-2 Serum IGG Antibody in Adult Population of Sialkot City, Pakistan: A Cross-Sectional Study JAIMC 2022;20(2): 80-85*

SARS CoV-2 is a viral droplet infection and is a zoonotic disease. In late December 2019, number of unusual cases of pneumonia were reported in Wuhan, China. Considering SARS infection occurred in south-east Asia couple of year, these acute respiratory syndrome cases were called SAR-CoV-2.[1] Now known as Covid-19, these cases were traced back to a central animal market place of Wuhan, China.[2] In a time span of few weeks, these sporadic cases turned into an epidemic. This epidemic has now spread to 210 countries.[1,2] World health Organization declared Covid-19 a Public Health Emergency of International Concern (PHEIC).[3] Globally, to date, there has been more than 600 million confirmed cases in different waves of infection and six million deaths.[3]

First case of COVID-19 was reported in Pakistan on 26th February 2020, and most cases to date linked to importation of infection from Iran, China, Middle-

1. *Department of Community Medicine, Allama Iqbal Medical College, Lahore*
2. *Department of Community Medicine, Khawaja Safdar Medical College Sialkot*
3. *Department of Pathology, Khawaja Safdar Medical College Sialkot*
4,6. *Department of Community Medicine, Khawaja Safdar Medical College Sialkot*
5. *FMH College of Medicine and Surgery Lahore*

Correspondence:
Dr Shahid Mahmood, Associate Professor, Department of Community Medicine, Allama Iqbal Medical College, Lahore
Email: shahidsethi@hotmail.com

Submission Date: 06-04-2022
1st Revision Date: 15-05-2022
Acceptance Date: 09-06-2022

East and European countries. Loose borders and inadequate screening arrangements at port of entries resulted in spread of cases in all provinces of Pakistan. There has been five waves of infection in Punjab (largest province in Pakistan), with approximately 520,869 confirmed cases and 13,606 deaths.[4] The effective reproduction rate has come down from 1.56 to 1.2%, however there is impending threat of sixth wave as around 50% of individuals are fully vaccinated in major cities of Punjab.[4,5]

Seroprevalence studies provide necessary data on extent of SARS-CoV-2 infection in a community. Identifying immune response of individuals exposed to this virus is one way of assessing community resistance against COVID-19 transmission.[6] In response to SARS-CoV-2, body produces IgM antibody initially and this remains for 10-21 days, followed by IgG antibody.[6,7] Enzyme-linked immunosorbent assay (ELISA) techniques are used for the antibodies detection using blood samples. Although, it is not an accurate method for the detection, but still preferred for the early detection.[7] We do not know the extent to which COVID-19 antibodies are present our population. There are very few reports available on seroprevalence of COVID-19 IgG antibodies in Punjab, especially in big cities like Lahore and Sialkot. Javed et al.[8] reported through their population sero-prevalence study in big cities of Pakistan including Lahore, Karachi, Quetta and Peshawar that overall seroprevalence of SARS-CoV-2 IgG antibody is 16% and antibody sero-positivity in males (OR= 1.22) was higher than females. Symptomatic subjects had 2.18 times higher odds of IgG sero-positivity.[8] On the other hand, Ahsan et al.[9] in their cross-sectional household survey found a lower seroprevalence (7.1% to 6.3%). No difference in seroprevalence was reported by the authors as regards the gender. However, in those aged 50 years and above, IgG sero-positivity was 10.1%.[9] Healthcare workers are predominantly exposed owing to their profession. Batool et al.[10] demonstrated that 33% of the health care workers were reactive for SARS-CoV-2 IgG antibody and an estimated 44% of the reactive cases were asymptomatic. Similarly, 17% of Combined Military Hospital Kharian Medical College staff (faculty, medical students, and administrative staff) were found positive for IgG antibodies using rapid antibody testing kit.[11]

According to Centers for Disease Control and Prevention (CDC-USA), "Serologic methods have public health value for monitoring and responding to the COVID-19 pandemic, and clinical utility in providing care for patients".[4] These antibody tests are essential tool to identify past infection and those people with asymptomatic infection.[8,9] The aim of this study was to measure SARS-CoV-2 IgG antibody levels among adult population of Sialkot city and describe sero-positivity in terms of age and sex. Additionally, we also described the IgG antibody tires among participants and salient symptoms related to COVID-19.

METHODS

This descriptive, cross-sectional study was undertaken in city of Sialkot (an industrial city in eastern Punjab Pakistan) between December 2020 to June 2021. A sample of 453 adult participants were recruited from community-based laboratories in Sialkot using convenient sampling technique. These individuals visited these laboratories to be tested for IgG antibody assays. After getting approval from Research Ethical Committee of Khawaja Safdar Medical College, Sialkot, a two-member team approached the said laboratories, explained the aims and objectives of the study and an informed consent was obtained from participants. A brief in-person interview was conducted regarding socio-demographic profile (age, sex, area of residence) and COVID-19 symptom (with duration of these symptoms). Five-milliliter (5ml) whole blood was drawn by a phlebotomist through venipuncture and then serum was separated for IgG antibody assay. We used Elecsys Anti SARS CoV2 which is an electrochemiluminescent immunoassay (ECLIA). This assay uses the nucleocapsid (N) antigen for the determination of high-affinity antibodies (including IgG).[12] A cut-off index (COI) of 1 or more was considered reactive or positive. A COI of less than 1 was reported as non-reactive or negative. The sensitivity and specificity of this assay is reported to be 97.2% and 99.8% res-

pectively.[12]

Laboratory reports were obtained from the laboratories and data were entered in Statistical Package for the Social Sciences (SPSS) version 22.0. Data were checked for inconsistencies and coded for age and sex, after assigning a unique identifier. Age was categorized into three groups (<25 years, 25-49 years and ≥ 50 years) and duration of COVID-19 symptoms was divided into three groups (≤7 days, 7-14 and >14 days). SARS CoV-2 IgG antibody reactivity or positivity status was described overall and stratified by age and sex (as percentage). Unadjusted seroprevalence of COVID-19 IgG antibody was reported in percent. Association of age and sex with sero-prevalence was determined using chi-square tests and p-value of less than 0.05 was considered statistically significant.

RESULTS

Of 514 individuals invited for this study, 471 completed the interview and provided blood sample for detecting SARS Cov-2 IgG antibodies. Non-response was 8%, where data of 18 participants were excluded due to inconclusive laboratory results and missing information of included variables. Finally, data of 453 individuals are presented here (Figure 1). Table 1 shows the socio-demographic characteristics of the participants. More than half of these participants were aged between 25 to 49 years (57%), whereas 122 (27%) were aged above 50 years. More men than women participated in our study (64% versus 36% respectively. Three out of every four individuals reported one or more symptoms of COVID-19 dry cough (17%), fever (30%) loss of taste (6%), sore throat (5%) and anosmia (4.6%) (Table 1). Duration of these symptoms were two weeks among 50% of these individuals, whereas one in four reported that they had the symptoms for less than 7 days (Table 1).

Figure 2 presents SARS-Cov-2 IgG antibody status using our laboratory analysis. We found that 289(63.7%) had varied levels of antibody and labelled as reactive using our cut-off (COI=1 or more). Only 134(29.5%) of these reactive for COVID-19 IgG antibody had antibody titres level 15 units/dl or above (Figure 3). When stratified by age and sex, it was found that 191(66.2%) aged 25-49 years were reactive to IgG antibody, whereas only 21% of those aged 50 years and above had this antibody. Difference in being reactive for COVID-19 IgG antibody in relation to age was statistically significant (p<0.001) (Table 2). We report that 181 (62.6%) men and 108 (37.4%) women were reactive to COVID-19 IgG antibodies, however, this difference based on gender was not statistically significant (p=0.34) (Table2).

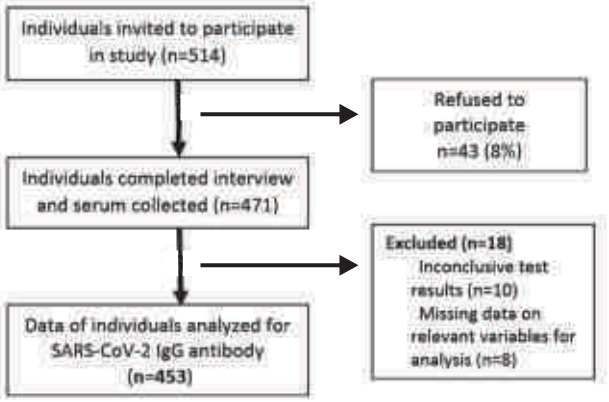

Figure 1: *Enrolment of participants and eligibility for analysis to measure seroprevalence of SARS-CoV-2 serum IgG antibody among adult urban population of Sialkot city, Pakistan*

Table 1: *Socio-demographic characteristics and presenting symptoms among adults tested for post-exposure SARS-CoV-2 IgG antibodies in city of Sialkot Pakistan (n=453)*

Characteristics/ presenting symptoms	Adults tested for SARS-CoV-2 IgG (n=453)	
	Numbers	Percentage
Age (in year)		
Less than 25	74	16.4 %
25-49	257	56.7 %
50 years and above	122	26.9 %
Sex		
Men	291	64.2 %
Women	162	35.8 %
Showed COVID-19 symptoms		
Yes	341	75.3 %
No	112	24.7 %
Had dry cough	78	17.2 %
Had fever	135	29.8 %
Had lethargy	37	8.2 %
Had Headache	05	1.1 %
Had Anosmia	21	4.6 %
Had loss of taste	26	5.7 %
Had sore throat	24	5.3 %
Had dyspnea	03	0.7 %
Duration of Symptoms (n=341)		
⩽ 7 days	94	27.5 %
7-14 days	168	49.2 %
>14 days	79	23.1%

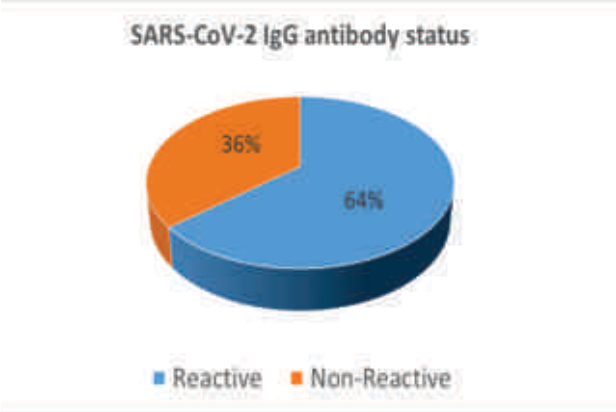

Figure 2. *Pie diagram showing the proportion of individuals labelled reactive for SARS-CoV-2 IgG antibody among adults in Sialkot city, Pakistan (n=453)*

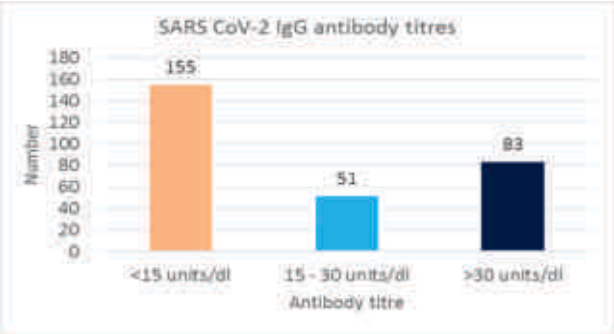

Figure 3. *Bar diagram showing IgG antibody titres among individuals labelled reactive for SARS-CoV-2 IgG antibody in Sialkot city, Pakistan (N=289)*

Table 2: *Comparison of age and sex of participants with SARS- CoV-2 IgG antibody reactive status in Sialkot city, Pakistan (n=453)*

Characteristics	*Status of SARS-CoV-2 IgG antibody status*		
	Reactive	Non-Reactive	*p*
Age (in years)			
Less than 25	36 (12.4%)	38 (23.2%)	**<0.001**
25-49	191 (66.2%)	66 (40.2%)	
50 years and above	62 (21.4%)	60 (36.6%)	
Sex			
Men	181 (62.6%)	110 (67.1%)	**0.34**
Women	108 (37.4%)	54 (32.9%)	

Footnotes: *p represents the probability of rejecting and null hypothesis when it is true. Calculated using Pearson's Chi-Squared test with relevant degree of Freedom; p<0.05 was considered for rejecting null hypothesis.*

DISCUSSION

The main objective of this study was to determine the SARS-CoV-2 IgG antibody among adults in Sialkot city and we found that two-third of these adults were reactive to SARS-CoV-2 IgG antibody; There was a significant association of this reactivity based on age, but no association by gender was found. Most predominant CVID-19 symptoms reported by the participant was fever followed by dry cough. Our study is one of few studies on seroprevalence of COVID-19 IgG antibody at population level in Punjab. We used standard laboratory method to detect IgG antibody with sensitivity and specificity of 97.2% and 99.8% respectively.[13]

Results of this study should be interpreted after considering few limitations. Cross-sectional design provides results at certain point in time and there are issues related to the limited generalization of results. Convenient sampling method was use due to financial and time constraints, which also might have affected the generalization of results. Additionally, since sero-positivity in population is a dynamic phenomenon, therefore measuring sero-positivity at one point in time may not provide information on duration of sero-positivity. Yet, our results are valid for similar settings using similar laboratory assay techniques.

We found higher seroprevalence (64%) compared to other studies undertaken in Pakistan. For instance, Ahsan et al.[9] reported sero-prevalence of 7.1% in a general population survey, whereas Batool et al.[10] showed sero-positivity of 33% among healthcare workers. Similarly, Naiyar et al.[11] reported that 17% of health care providers in CMH Kharian, Punjab were positive for COVID-19 IgG. Difference in these findings may be attributed to variation in using sampling methods, laboratory techniques. For example, Both Ahsan et al.[9] and Naiyar et al.[11] utilized rapid detection kits, where as in this study and Batool et al. used Chemi-luminescent assays.

Several studies around the globe have reported seroprevalence data on Covid-19 in recent times. The findings have wide variations, owing to sampling variations, sub-groups variation, geographical region,

ethnicity and presence of co-morbid/ immune modulating factors.[14] Those using rapid lateral-flow assay methods have shown to report inconsistent seroprevalence values than the Chemiluminescent assay techniques (some shown high and some lower values).[15,16] For instance, Shakiba et al.[17] showed in their population based cluster sample using Rapid testing kit that seroprevalence was 22% (95%CI: 19-26%) and proportion of seropositivity among those with no COVID-19 symptoms is higher comparatively. Similarly, Torres et al.[16] in brazil reported that old age, contact with COVID-19 patient and mixed ethnicity were the major risk factors and overall seroprevalence in studied population was 39%. Similar report was given by Haq et al.[18] where they used Roche® antibody detection kit in most populous cities of Pakistan by testing more than 15000 individuals. Seroprevalence in their study was 42.4%. It is strange that most affected countries have demonstrated lower IgG seroprevalence compared to low endemic countries (10-15% versus 30-40%).[19-21] There may be differences in population susceptibility for related flu viruses common in less developed countries and there may be an element of cross-immunity.[22] Cross-immunity may be one reason underlying the low mortality of COVID-19 in many Asian countries, where most coronavirus-related epidemics such as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) originated.[23,24]

Accuracy and reliability of serology assay are disputed due to the possibility of false-positive cases, because of cross-reaction with another coronavirus such as common cold. Conversely, there can be false-negative cases as well. In a recent meta-analysis comparing several serological methods, the sensitivity of immune-chromatographic assays was lower than that of ELISA or CLIA, but all methods yielded high specificity closer to 99%.[25]

Non-pharmaceutical interventions such as increased testing rates, contact tracing, school closures, ban of mass gatherings, physical distancing, and restriction of movement were effective in reducing transmission rates of SARS-CoV-2. However, this type of intervention has tremendous societal and economic consequences potentially resulting in social disorganization and great recession. One approach to de-escalating public health measures and returning to a state of normalcy, while maintaining epidemiological vigilance and ability to respond fast to viral resurgence, is to identify people with immunity to SARS-CoV-2 and estimate their proportion in the entire population. Current study aimed to achieve this objective. Despite number of limitations, it provided a baseline data to build larger studies with representative samples.

Future studies should aim at series of cross-sectional surveys in different time-periods to demonstrate the trend of seroprevalence based on IgG antibody assays. Rural areas in most studies have been neglected and COVID-19 seroprevalence in rural population through randomized cluster sampling technique may be undertaken. Similarly, though incidence of COVID-19 in children is low but as the disease transmission slows down in adults, there is chance that disease may take refuge in children. Therefore, seroprevalence studies may also include a representative sample from children. Finally, follow-up study to evaluate the declining titres of IgG antibody should be considered to assess the level of herd immunity in population and predict future outbreaks.

CONCLUSIONS

Almost two-third of adult population in Sialkot city had SARS-CoV-2 IgG antibody during 2021 survey that implies that herd immunity level for COVID-19 is achieved and negligible number of COVID-19 cases reported in Sialkot city supports this evidence. Further seroprevalence studies are required at community settings to determine the sero-prevalence of COVID-19 involving wider population groups to draw epidemiological landscape of COVID-19 disease transmission for health managers to devise effective preventive strategies.

Conflicts of interest *None*

Funding sources *None*

REFERENCES

1. Binti Hamzah FA, Lau C, Nazri H, Ligot DV, Lee G,

Tan CL, et al. CoronaTracker: Worldwide COVID-19 Outbreak Data Analysis and Prediction. [Submitted]. Bull World Health Organ. E-pub: 19 March 2020. doi: http://dx.doi.org/10.2471/BLT.20.255695
2. Bendavid E, Mulaney B, Sood N, Shah S, Bromley-Dulfano R, Lai C, et al. COVID-19 antibody seroprevalence in Santa Clara County, California. Int J Epidemiol, 2021, 410–419. doi: 10.1093/ije/dyab010
3. Bryant JE, Azman AS, Ferrari MJ, Arnold BF, Boni MF, Boum Y, et al. Serology for SARS-CoV-2: apprehensions, opportunities, and the path forward. Sci Immunol. 2020;5(47):eabc6347
4. Centers for disease control and prevention (CDC) Interim Guidelines for COVID-19 Antibody Testing in Clinical and Public Health Settings, available at: https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html,accessed on 01 November 2021.
5. Government of the Pakistan. Punjab Cases Details. Available at: www. https://covid.gov.pk/ stats/ punjab accessed on 30 August 2022.
6. Randolph HE, Barreiro LB. Herd immunity: understanding COVID-19. Immunity. 2020;52(5): 737–741.
7. Slot E, Hogema BM, Reusken CB, Reimerink JH, Molier M, Karregat JH, et al. Herd immunity is not a realistic exit strategy during a COVID-19 outbreak. [Updated 2020]. [Accessed June 5, 2020]
8. Javed W, Abidi, SHB, Baqar JB. Seroprevalence and characteristics of Coronavirus Disease (COVID-19) in workers with non-specific disease symptoms. BMC Infectious Diseases 2022; 22:481. https:// doi.org/ 10.1186/ s12879-022-07461-9
9. Ahsan AM, Shahzad K, Masood M, Umar M, Abbasi F, Hafeez A. COVID-19 seroprevalence in Pakistan: a cross-sectional study. BMJ Open 2022; 12:e055381. doi:10.1136/bmjopen-2021-055381
10. Batool H, Chughtai O, Khan MD, Chughtai AS, Ashraf S, Khan MJ. Seroprevalence of COVID-19 IgG antibodies among healthcare workers of Pakistan: a cross-sectional study assessing exposure to COVID-19 and identification of high-risk subgroups. BMJ Open 2021;11:e046276. doi:10.1136/bmjopen-2020-046276
11. Naiyar I, Anjum AF, Khalid AM, Noor I, Abdullah MS, Anwar MZ. Seroprevalence of COVID-19 and associated factors in a medical institution in Pakistan. Journal of Taibah University Medical Sciences 2021; 64(4):619-23. https://doi.org/10.1016/j.jtumed. 2021.04.004
12. Inbaraj LR, George CE, Chandrasingh S. Seroprevalence of COVID-19 infection in a rural district of South India: A population-based seroepidemiological study. PLoS ONE 2021; 16(3): e0249247. https://doi.org/ 10.1371/journal. pone.0249247
13. National SARS-CoV-2 Serology Assay Evaluation Group. Performance characteristics of five immunoassays for SARS-CoV-2: a head-to-head benchmark comparison. Lancet Infect Dis. 2020; 20 (12): 1390–400. https://doi.org/10.1016/S1473-3099(20)30634-4 PMID: 32979318
14. Kontou PI, Braliou GG, Dimou NL, Nikolopoulos G, Bagos PG. Antibody tests in detecting SARS-CoV-2 infection: a meta-analysis. Diagnostics (Basel) 2020; 10(5):319.
15. Vogel G. First antibody surveys draw fire for quality, bias. Science. 2020;368 (6489):350–351
16. Torres MKS, Lopes FT, de Lima1 ACR, Lima CNC, Brito WRDS, Santos BCD, et al. Seroprevalence and risk factors for COVID-19 in the metropolis of the Brazilian Amazon. Scientific Reports 2022; 12:8571. https://doi.org/10.1038/s41598-022-12629-z
17. Shakiba M, Hashemi Nazari SS, Mehrabian F, Rezvani SM, Ghasempour Z, Heidarzadeh A. Seroprevalence of COVID-19 virus infection in Guilan province, Iran. [Updated 2020]. [Accessed June 5, 2020].
18. Haq M, Rehman A, Ahmad J, Zafar U, Ahmed S, Khan MA, et al. SARS-CoV-2: big seroprevalence data from Pakistan—is herd immunity at hand? Infection 2021; 49:983–988. https://doi.org/10.1007/s15010-021-01629-2
19. Wang C , Hornby PW , Hayden FG , Gao GF.A novel coronavirus outbreak of global health concern. Lancet [published online January 24, 2020].DOI: 10.1016 / S0140-6736 (20) 30185-9
20. Song SK, Lee DH, Nam JH, Kim KT, Do JS, Kang DW, Kim SG, Cho MR. IgG Seroprevalence of COVID-19 among Individuals without a History of the Coronavirus Disease Infection in Daegu, Korea. J Korean Med Sci. 2020 Jul 27;35(29):e269. doi: 10.3346/ jkms. 2020.35.e269. PMID: 32715672; PMCID: PMC7384903.
21. Doi A, Iwata K, Kuroda H, Hasuike T, Nasu S, Kanda A, et al. Estimation of seroprevalence of novel coronavirus disease (COVID-19) using preserved serum at an outpatient setting in Kobe, Japan: a cross-sectional study. [Updated 2020]. [Accessed June 5, 2021].
22. Breedon AME, Saldanha RJ, Salisbury RL, Metzger DE, Werry MP, McPherson CJ, et al. COVID-19 Seroprevalence and Active Infection in an Asymptomatic Population. Front. Med 2021; 8:749732. doi: 10.3389/fmed.2021.749732
23. Alsuwaidi AR, Hosani AIA, Al –Memari S, Narchi H, Wareth AL, Kamal H, et al. Seroprevalence of COVID-19 infection in the Emirate of Abu Dhabi, United Arab Emirates: a population-based cross-sectional study. International Journal of Epidemiology 2021, 1077–1090 doi: 10.1093/ije/dyab077
24. Bawa MS, Desale AV, Pandve HT, Nimbalkar AV, Patil TV, Gophane LP, et al. Population based repeat cross-sectional seroprevalence survey of SARS-COV-2 IgG antibodies in Pimpri Chinchwad municipal corporation area, Maharashtra, India. J Family Med Prim Care 2022;11:2789-93.
25. Leslie M. T cells found in coronavirus patients 'bode well' for long-term immunity. Science. 2020; 368(6493): 809–810