Original Research Article

Seroprevalence of anti-SARS-CoV-2 antibodies in COVID-19 patients in Hyderabad, Sindh

ABSTRACT:

Aim: The world is affected by the severe acute respiratory syndrome coronavirus2 (SARS-CoV-2) pandemic. This virus has emerged as a human pathogen that can cause symptoms ranging from fever to Pneumonia, but it remains asymptomatic or mild. To better understand the virus's ongoing spread, identify those who have been infected, and track the immune response, accurate and robust immunological monitoring and SARS-CoV-2 detection assays are needed. **Method:** The estimate of serology tests to assess the presence of antibodies to SARS-CoV-2 in COVID- 19 patients at Asian Institute of Medical Sciences (AIMS) and Isra University & hospital. 1229 patients were selected including male and females with the age being 25 to 65 years living in the territories from 1st August to 30th November 2020. The anti-SARS-CoV-2 test was performed by electrochemiluminescence immunoassay analyzer. **Results:** Out of 1229 patients of COVID-19, 206 (17%) were positive and 1023 (83%) were negative. The results further revealed that higher percentage of positive COVID-19 were detected in males in all age groups as compared to females, and mostly are affected at age of 46-65 years male 40(24.69%) and female 14(17.5%). **Conclusion:** The seroprevalence of SARS-COV-2 antibodies has increased in old age population, which may aid in determining the true number of infected cases. Although the current study is based on a small sample of participants, the findings suggest a study with larger population to implement stronger and targeted interventions.

*Key words***:** COVID-19, Antibody, seroprevalence, SARS-COV-2 and ELISA

INTRODUCTION

SARS-CoV-2 antibody and antibodies detection tests are very important element of the adaptive immune response, provided that memory and specificity against future infection diseases. This is achieved through neutralisation by activation of complement to destroy cells by lysis, binding pathogens, opsonisation or presentation to immune cells to facilitate antibody dependent cell mediated cytotoxicity, degranulation and antibody-dependent cellular phagocytosis (ADCP) (Lu LL et al., 2018; Jacofsky, D et al., 2020; Adeniji, O.S et al., 2021). However, for many cases T cell immunity is predominant in intracellular infections (tuberculosis). Recently, the role of T cells to SARS-CoV-2 infection without seroconversion is widely discussed (Gallais et al., 2020). The reactivity of T cell responses to other coronavirus infections have been potentially explained, some of the variation in clinical severity in laboratory tests (Le Bert et al., 2020). It is also reported that a combination of T and B cell immunity is involved in generating protective memory and clearing covid-19 infection.

In Wuhan, China, on December 29, 2019, an outbreak of pneumonia linked to the novel 2019 novel coronavirus (2019-nCOV) was confirmed, affecting patients' lower respiratory tracts, and was linked to a local human South China Seafood Market (Li et al., 2020; WHO, 2020a; Zhu et al., 2019). The name 2019-nCOV has been changed to extreme acute respiratory syndrome coronavirus 2 at this time (SARS-CoV-2). The coronavirus belongs to a family *Coronaviridae* that cause various symptoms such as breathing difficulty, fever, lung infection and pneumonia (WMHC, 2020). These viruses are highly pathogenic which spread from animals worldwide and current SARS-CoV-2 is rapidly spreading from epicenter to the rest of the world (Wang et al., 2020). The available literature on the current epidemic features of COVID-19 largely focus on Wuhan, China and reported that COVID-19 infection is rapidly moving (Huang et al., 2020; Li et

al., 2020). However, data on reported cases and deaths can help with identifying the dynamics of disease transmission and estimating the percentage of the population infected in the COVID-19 pandemic, as well as providing a significant indicator for public health decision-making (Roda et al., 2020). Recently, mostly Asian countries, including Pakistan did not have adequate affordable nasopharyngeal swabs and RT-PCR screening of everyone suspected, and the risk of infection with SARS-CoV-2. In most cases, asymptomatic individuals or mildly affected are rarely screened. As a result, the numbers of confirmed cases are underestimated (Verity et al., 2020). In this context, sero-prevalence surveys are critical for determining the proportion of the population who may be protected against SARS-CoV-2 infection or who have developed antibodies against the virus (Lipsitch et al., 2020). WHO advises on monitoring changes in sero-prevalence over time is also critical at the outset of an outbreak to anticipate its dynamics and prepare an effective public health response (WHO, 2020b).

A serology test, also known as an antibody test, is used to determine whether the human body produces antibodies in response to a variety of illnesses, as well as whether or not a person has developed immunity to a pathogen. Antibody experiments with no cross-reactivity to other related coronaviruses were used in the current COVID-19 pandemic to explicitly detect antibodies against SARS-CoV-2, which could result in a false positive and incorrectly suggest possible immunity. Serological tests include the enzyme-linked immunosorbent assay (ELISA), rapid diagnostic tests in the form of lateral flow assays, and the enzyme-linked fluorescent assay (ELFA) (VIDAS®, SARS-CoV-2 serology, Biomerieux, France). Though, such tests are not recommended for diagnostic purposes due to cross-reactivity with other coronaviruses, usually causing the type of flu in the population (Chan et al., 2009; WHO, 2020c). Only less than 40% of patients recorded that they had detectable antibodies, throughout the first $6 - 7$ days of COVID-19 infection (Zhao et

al., 2020). Few studies have already been published on the rapidity of antibody production (Chen et al., 2020), and clinical performance of immunoassays (Padoan et al., 2020). Currently there is no reported study that investigates whether antibodies to SARS-CoV-2 confer protection against subsequent COVID-19 infection by in Hyderabad, Sindh Pakistan. Therefore, this study intends to assess the presence of antibodies to SARS-CoV-2 and discusses immunity against consequent COVID-19 infection. In this report, we present data on the characteristics of patients with reported COVID-19 infection from various hospitals in Hyderabad, with the aim of determining asymptomatic cases.

MATERIAL AND METHODS

Specimen Collection and analysis

The present study was performed at the Asian Institute of Medical Sciences (AIMS) and Isra University & hospital, Pakistan. The study involved 1229 cases who were recruited from patients visiting hospitals from August 01, to November 30, 2020. The subjects included in the study were never diagnosed with COVID-19 and they do not have any COVID-19-related symptoms such as fever or cough and live in Hyderabad and adjoining areas. All the symptomatic and confirmed COVID-19 cases were excluded from the study. The 5ml blood sample was collected through an antecubital vein from the subjects in gel tubes. The serum was separated store at 6 to 8 ^{0}C until analyzed. The anti-SARS-CoV-2 test was performed by electrochemiluminescence immuno assay analyzer method on Cobas e-411 by Roche diagnostics International Ltd at Rotkreuz, Switzerland. It is a qualitative antibody assay against SARS-CoV-2 that use double-antigen sandwich technique utilizing recombinant protein and detected antibodies against nucleocapsid (N) proteins of coronavirus.

Statistical Analysis

The statistical analysis was performed using SPSS version 20.0. The variables were expressed as frequency and percentage of patients. The seroprevalence among groups stratified by characteristics of study subjects, including age (18-25, 26-35, 26-45, and 46-65 years) and gender. The P-value < 0.05 was considered statistically significant.

RESULTS

The random samples were collected from the representative Pakistani population in Hyderabad and the adjacent area. Patient distribution by age, sex (excluding criteria of patients having liver cirrhosis/cancer or other diseases). During the study period, 1229 cases were investigated for serology test and the pie chart shows the total number of serology positive cases were 206 and negative 1023 (Figure 1). However, the Fig: 2 show positive (17%) and negative (83%) cases respectively, in the whole population of Covid-19.

The results were distributed into 4 groups (I-IV) which include 18-25, 26-35, 36-45, and 46-65 years respectively. The results revealed that 16.76 % positive and 84 % negative COVID-19 cases were detected in group I, whereas groups II found 13.23 % positive and 86.76% negative cases, Group III suggest 18.41% positive whereas 81.58% were negative. Group IV observed 22.31% positive and 77.68% negative. A higher percentage of COVID-19 positive cases were detected in the age 56-65 years, as illustrated in Table 1.

The age and gender-wise comparison of COVID-19 serological antibody test results in different groups were shown in Table 2. The findings showed that 17.76 % male positive cases of COVID-19 and 13.8 % for females whereas 83.50 % male and 84.73 % females were negative in Group I. The group II (26-35 years) confirmed 15.57 % male and 8.84% females' positive cases of COVID-19 whereas 86.23 % male and 87.78 % females were negative. Group III (36-45 years) had a total COVID-19 cases 239, among them 21.33% male and 13.48%, females were positive whereas 78.66% male and 86.51% females were negative. Group IV (46-65 years) contained a total of 242 specimens from which 24.69% were male and 17.5% were female's positive patients of COVID-19 however 77.21% were male and 78.52% females were negative. The results revealed that a

higher percentage COVID-19 positivity were detected in males among all age groups as compared to females, and the most affected age group was 46-65 years in both genders (Table 2).

DISCUSSION

The current study determined sero-antibody in 206 cases (16.74%) and the serological data was useful to identify the magnitude of this pandemic disease. The seroprevalence records were related to the concept of people immunity and the lowest level of population immunity was essential to cease the spread of infection in society (Randolph & Barreiro., 2020). The previous study demonstrated that almost half of the specimen analyzed before the COVID-19 epidemic was T cell-mediated immunity against SARS-CoV-2 (Grifoni et al., 2020). The cross-immunity could be a key cause to reduce the mortality of COVID-19 in numerous Asian countries, however highly COVID-19 related epidemics disclose severe respiratory syndrome (Song et al., 2020).

The present study indicates that more positivity of COVID-19 was observed in male cases than females which suggested that the higher frequency of critical illness due to COVID-19 in men. Lversen et al., reported in 2020 the seroconversion of SARS-CoV-2 were frequent in male healthcare workers in comparison to female. The gender-linked variation in seroprevalence could be due to an unidentified pattern of transmission because women may follow suggestions very carefully. The change may be due to biological origin if changes in immunological response and severity of disease between both genders.

The important finding of the existing study the incidence of a COVID-19 was higher in the age group 46-65 years in both genders. Acemoglu et al., in 2020 reported that an outbreak was mainly concentrated among younger people; it may be very difficult to prevent the virus from spreading

among older adults. The risks of severe complications and mortality in COVID-19 patients were higher age group 46 to 65years however which was a well-observed risk factor for severe outcomes.

Another study was published by Ferguson et al., in 2020 on the COVID-19 epidemic and described a similar arrangement of age-specific infection fatality rate by using statistical models to describe the dynamics of transmission and mortality using surveillance data. However, it's likely that older adults have a weakened immune response to infection, but further research is required. According to one study, disease prevention can be improved if about 60% of the population produces antibodies (Altmann et al., 2020).A few studies launched for medicinal plant for remedies and boost the immune response against SARS-CoV-2 infection (Idrees et al., 2020; Yang et al., 2020). However, it is important to examine and research whether these antibody responses are long-lasting or not. Since RNA viruses have a propensity to mutate, it is still uncertain if these antibodies would be effective against disease if the virus mutates.

This study has many limitations because it was carried out in a specific area and the findings could not be applied directly to the general population and large sample size will require confirming the results. However, the males were highly COVID-19 positive for this more research is required to verify the gender-related difference.

CONCULSION

The past year must explain that this pandemic has been affected by an unexpected pathogen that needs extraordinary procedures to fight against it. The seroprevalence of SARS-CoV-2 antibodies among asymptomatic peoples with different age groups and gender in Hyderabad was 16.76 %

which was smaller than expected. The present study will be useful in resolving the continent's outbreak-related problems and will serve as a benchmark for future research.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

The present study was approved by the ethical committee of Isra University, Hyderabad. All patients were well educated in their regional languages and with their complete information and understanding, a written agreement was signed, while uneducated patients' permission was taken with thumb impressions in the existence of their responsible eyewitnesses and registered in the investigation.

COMPETING INTERESTS

All authors have declared that no competing interests exist.

REFFERNECES

Lu LL, Suscovich TJ, Fortune SM, Alter G. 2018. Beyond binding: antibody effector functions in infectious diseases. Nat Rev Immunol 18:46–61.

Jacofsky, D., Jacofsky, E. M., & Jacofsky, M. (2020). Understanding Antibody Testing for COVID-19. The Journal of arthroplasty, 35(7S), S74–S81. https://doi.org/10.1016/j.arth.2020.04.055

Adeniji, O.S., et al., COVID-19 Severity Is Associated with Differential Antibody Fc- Mediated Innate Immune Functions. mBio, 2021. ***12****(2): p. e00281-21.*

Gallais F, A Velay, M.J. Wendling, C Nazon, M Partisani, J Sibilia, S Candon and S Fati Kremer, 2020. Intrafamilial exposure to SARS-CoV-2 induces cellular immune response without seroconversion. MedRxiv [Preprint]. doi: 10.1101/2020.06.21.20132449

Le Bert N, AT Tan, K Kunasegaran, CYL Tham, M Hafezi, A Chia, MHY ChnG, M Lin, N Tan, Linster, WN Chia, MI Chen, LF Wang, EE Ooi, S Kalimuddin, PA Tambyah, JG Low, YJ Tan and A Bertoletti, 2020. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and ARS, and uninfected controls. Nature, 584(7821):457-462. doi: 10.1038/s41586-020-2550-z.

Li, Q., Guan, X., Wu, P., Wang, X., Zhou, L., Tong, Y., & Feng, Z. (2020). Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia. *New England journal of medicine*. 382(13): 1199-1207, doi:10.1056/NEJMoa2001316.
World Health Organization, 2020a. Novel Coronavirus–China.

Zhu N, D Zhang, W Wang, X Li, B Yang, J Song, X Zhao, B Huang, W Shi, R Lu, P Niu, F Zhan, X Ma, D Wang, W Xu, G Wu, GF Gao and W Tan, 2020. China Novel Coronavirus Investigating and Research Team. A Novel Coronavirus from Patients with Pneumonia in China, 2019. The New England Journal of Medicine, 382(8): 727

733. doi: 10.1056/NEJMoa2001017.

WMHC, 2020. Wuhan Municipal Health and Health Commission's Briefing on the Current Pneumonia Epidemic Situation in Our City.

Wang C, PW Horby, FG Hayden and GF Gao George, 2020a. A novel coronavirus outbreak of global health concern. Lancet, 395(10223): 470-473. doi: 10.1016/S0140-6736(20)30185-9.

Huang C, Y Wang, X Li, L Ren, J Zhao, Y Hu, L Zhang, G Fan, J Xu, X Gu, Z Cheng, T Yu, J Xia, Y Wei, W Wu, X Xie, W Yin, H Li, M Liu, Y Xiao, H Gao, L Guo, J Xie, G Wang, R Jiang, Z Gao, Q Jin, J Wang and B Cao, 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet, 395(10223): 497 506. doi: 10.1016/S0140-6736(20)30183-5.

Li C, C Zhao, J Bao, B Tang, Y Wang and B Gu, 2020b. Laboratory diagnosis of coronavirus isease-2019 (COVID 19). Clinica Chimica Acta, 510: 35-46. https://doi.org/10.1016/j.cca.2020.06.045

Roda WC, MB Varughese, D Han and MY Li, 2020. Why is it difficult to accurately predict the COVID-19 epidemic? Infectious Disease Modelling, 5: 271-81

Verity R, LC Okell, I Dorigatti, P Winskill, C Whittaker, N Imai, G Cuomo-Dannenburg, H Thompson, PGT Walker, H Fu, A Dighe, JT Griffin, M Baguelin, S Bhatia, A Boonyasiri, A Cori, Z Cucunubá, R FitzJohn, K Gaythorpe, W Green, A Hamlet, W Hinsley, D Laydon, G Nedjati-Gilani, R Riley, S van Elsland, E Volz, H Wang, Y Wang, X Xi, CA Donnelly, AC Ghani and NM Ferguson, 2020. Estimates of the severity of coronavirus disease 2019: a model-based analysis. Lancet Infectois Diseases, 20(6): 669-677. doi: 10.1016/S1473-3099(20)30243-7.

Lipsitch M, DL Swerdlow and L Finelli, 2020. Defining the epidemiology of Covid-19 - studies needed. The New England Journal of Medicine, 382: 1194-96

World Health Organization, 2020b. Population-based age-stratified seroepidemiological investigation protocol for COVID-19 virus infection. March 17.

Chan CM, H Tse, SS Wong, PC Woo, SK Lau, L Chen, BJ Zheng, JD Huang and KY Yuen, 2020. Examination of seroprevalence of coronavirus HKU1 infection with S protein-based ELISA and neutralization assay against viral spike pseudotyped virus. Journal of Clinical Virology 45(1): 54-60. https://doi.org/10.1016/j.jcv.2009.02.011

Zhao J, Q Yuan, H Wang, W Liu, X Liao, Y Su, X Wang, J Yuan, T Li, J Li, S Qian, C Hong, F Wang, Y Liu, Z Wang, Q He, Z Li, B He, T Zhang, Y Fu, S Ge, L Liu, J Zhang, N Xia and Z Zhang, 2020. Antibody Responses to SARS-CoV-2 in Patients With Novel Coronavirus Disease 2019. Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America, 71(16):2027-2034. doi: 10.1093/cid/ciaa344

Chen Y, A Zuiani, S Fischinger, J Mullur, C Atyeo, M Travers, FJN Lelis, KM Pullen, H Martin, P Tong, A Gautam, S Habibi, J Bensko, D Gakpo, J Feldman, BM Hauser, TM Caradonna, Y Cai, JS Burke, J Lin, JA Lederer, EC Lam, CL Lavine, MS Seaman, B Chen, AG Schmidt, AB Balazs, DA Lauffenburger, G Alte and DR Wesemann, 2020. Quick COVID-19 Healers Sustain Anti-SARS-CoV-2 Antibody Production. Cell, 183(6): 1496 1507.e16. doi: 10.1016/j.cell.2020.10.051.

Padoan A, F Bonfante, M Pagliari, A Bortolami, D Negrini, S Zuin, D Bozzato, C Cosma, L Sciacovelli and M Plebani, 2020. Analytical and clinical performances of five immunoassays for the detection of SARS-CoV-2 antibodies in comparison with neutralization activity. EBioMedicine, 62: 103101. doi:10.1016/j.ebiom.2020.103101.

Randolph HE, Barreiro LB, 2020. Herd immunity: understanding COVID-19 immunity. 52 (5): 737-41.

Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, Rawlings SA, Sutherland A, Premkumar L, Jadi RS, Marrama D, 2020. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. Cell. 25;181(7):1489-501.

Song SK, Lee DH, Nam JH, Kim KT, Do JS, Kang DW, Kim SG, Cho MR, 2020. IgG seroprevalence of COVID-19 among individuals without a history of the coronavirus disease infection in Daegu, Korea. Journal of Korean medical science. 2020 Jul 27;35(29).

Iversen K, Bundgaard H, Hasselbalch RB, Kristensen JH, Nielsen PB, Pries-Heje M, Knudsen AD, Christensen CE, Fogh K, Norsk JB, Andersen O, 2020. Risk of COVID-19 in health-care workers in Denmark: an observational cohort study. The Lancet Infectious Diseases. 1;20(12):1401-8.

Acemoglu D, Chernozhukov V, Werning I, Whinston MD, 2020. Optimal targeted lockdowns in a multi-group SIR model. National Bureau of Economic Research.

Ferguson N, Laydon D, Nedjati-Gilani G, et al. Report 9: Impact of non-pharmaceutical interventions (NPIs) to reduce COVID-19 mortality and healthcare demand. 2020.

Altmann DM, DC Douek and RJ Boyton, 2020. What policy makers need to know about COVID-19 protective immunity. Lancet 395(10236):1527-9.

Idrees M, S Khan, NH Memon and ZY Zhang, 2020. Effect of the Phytochemical Agents Against the SARS-CoV and Selected Some of them for Application to COVID-19: A Mini-Review. Current Pharmaceutical Biotechnology doi: 10.2174/1389201021666200703201458.

Yang F, Y Zhang, A Tariq, X Jiang, Z Ahmed, Z Zhihao, M Idrees, A Azizullah, M Adnan and RW Bussmann, 2020. Food as medicine: A possible preventive measure against coronavirus disease (COVID-19). Phytotherpy Research, 34(12):3124-3136. doi: 10.1002/ptr.6770.

Table 1: Summary of detection cases according to different groups of ages (18 - 65 years)

Age groups	Category	Detection cases		Total
		Negative	Positive	
18 to 25 years	Group I	273 (84%)	52 (16%)	325
26 to 35 years	Group II	367 (86.76%)	56 (13.23%)	423
36 to 45 years	Group II	195 (81.58%)	44 (18.41%)	239
46 to 65 years	Group IV	188 (77.68%)	54 (22.31%)	242

Table 2: Age and gender wise comparison of COVID-19 serological antibody tests

Age Group	Gender	Negative 1023/1229	Positive 206/1229	Total	P value
18-25 years	Male	162 (83.50 %)	35 (17.76%)	197	0.178
	Female	111(84.73%)	17(13.8%)	128	
26-35 Years	Male	238(86.23%)	43(15.57%)	276	0.051
	Female	129(87.78%)	13(8.84%)	147	
36-45 Years	Male	118(78.66%)	32(21.33%)	150	0.088
	Female	77(86.51%)	12(13.48%)	89	
46-65 Years	Male	122(77.21%)	40(24.69%)	162	0.135
	Female	66(78.52%)	14(17.5%)	80	
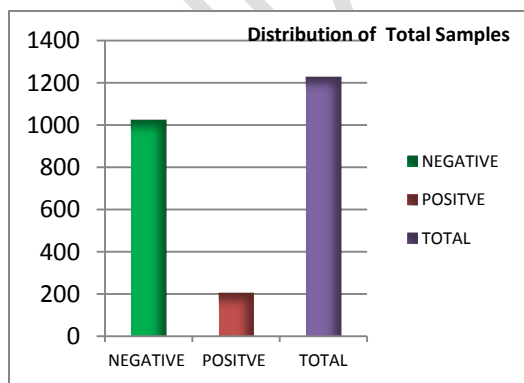
Fig.1: Seroprevalence of COVID-19 in Hyderabad and adjacent area, Pakistan
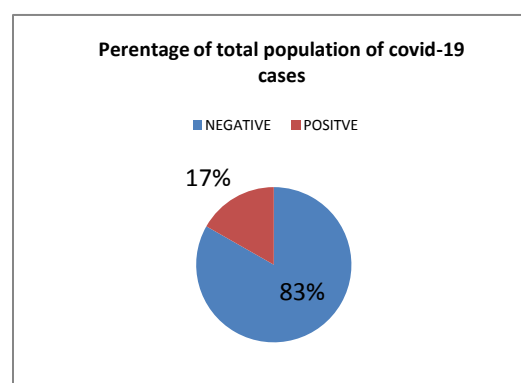
Fig. 2: Percentage of whole Population of Covid-19 Cases