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 estimation 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 males 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 an electrochemiluminescence immunoassay analyzer. **Results:** Out of 1229 participants 206 (17%) were positive with COVID-19, and 1023 (83%) were negative. The results further revealed that a higher percentage of positive COVID-19 were detected in males in all age groups as compared to females, and most of them 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 the 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 a larger population to implement stronger and targeted interventions.

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

INTRODUCTION

SARS-CoV-2 antibody detection tests are very important element of the adaptive immune response, provided that memory and specificity against future infection diseases. This is achieved through neutralization by activation of complement to destroy cells by lysis, binding pathogens, opsonization, 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 in SARS-CoV-2 infection without seroconversion is widely discussed (Gallais et al., 2020). The reactivity of T cell responses to other coronavirus infections has 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 the COVID-19 infection.

In Wuhan, China, on December 29, 2019, an outbreak of pneumonia linked to the 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 the epicenter to the rest of the world (Wang et al., 2020). The available literature on the current epidemic features of COVID-19 largely focuses 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 number of confirmed cases is underestimated (Verity et al., 2020). In this context, seroprevalence 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 seroprevalence 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 is used to determine the human body produces antibodies in response to a variety of illnesses, as well as not a person has developed immunity to a pathogen. Antibody experiments have been used that do not show the cross-reactivity to other coronaviruses 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 the clinical performance of immunoassays (Padoan et al., 2020). Currently no study has been reported to investigate the SARS-CoV-2 antibodies for conferring the protection against subsequent COVID-19 infection 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 from various hospitals in Hyderabad, aiming to determine 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 immunoassay 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 uses a 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, 17% positive and 83% negative cases in the whole population of COVID-19 were shown in Figure 2.

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 group I included 16.76 % positive and 84 % negative COVID-19 cases, while group II found 13.23 % positive and 86.76% negative, and group III suggest 18.41% positive whereas 81.58% were negative cases. Group IV observed that 22.31% positive and 77.68% negative, though a higher percentage of COVID-19 positive patients 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 revealed that in group I (18-25 years) confirm cases of males were 17.76 % of COVID-19 and 13.8 % for females while 83.50 % male, and 84.73 % females were negative. 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 figure of COVID-19 cases 239, among them 21.33% male and 13.48% females were positive although 78.66% male and 86.51% females were negative. Group IV (46-65 years) contained a total number 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 disclosed that a higher percentage of COVID-19 positivity was 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 specimens 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 65 years 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, older adults likely 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). However, few studies launched for medicinal plants for remedies and boost the immune response against SARS-CoV-2 infection. Nigella sativa plant has potent anti-SARS-CoV-2 activity, and it might be a useful source for developing novel antiviral therapies for coronavirus. (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.

CONCLUSION

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

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.

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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	

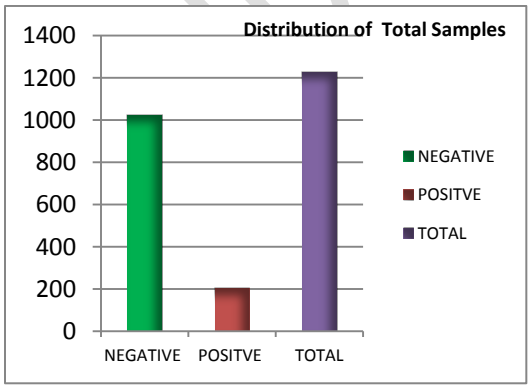

Fig.1: Seroprevalence of COVID-19 in Hyderabad and adjacent area, Pakistan

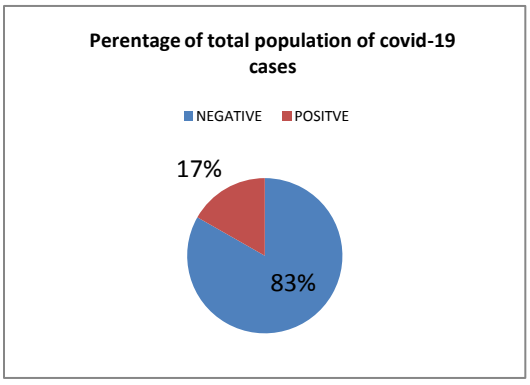

Fig. 2: Percentage of whole Population of Covid-19 Cases