Original Research Article

Seroprevalence of anti-SARS-cov-2 antibodies in covid-19 patients in Hyderabad, Pakistan

ABSTRACT

Pakistan is one of the Asian countries that are affected by the SARS-CoV-2 pandemic. This virus has emerged as a human pathogen that can cause symptoms ranging from fever to Pneumonia, but it is 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. We estimate serology tests to assess the presence of antibodies to SARS-CoV-2 in SARS-CoV-2 patients at Asian Institute of Medical Sciences (AIMS) and Isra University & hospital, Pakistan.We randomly selected 1229 patients including male and females with the age being 25 to 65 years those living in the territories on Aug 1, 2020. We used to test an anti-body detection strategy using Elecsys® Anti-SARS-CoV-2 and their patented SARS-CoV-2 complete antibody detection by ELISA. Out of 1229 patients, 206 were COVID positive and 1023 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. In Pakistan, the seroprevalence of SARS-COV-2 antibodies has increased, which may aid in determining the true number of infected cases.

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

INTRODUCTION

SARS-CoV-2 antibody and immunity antibodies 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 phagocytosis. 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 explains, 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 (CoV), belong 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 literatures 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. Few studies have already been published on the rapidity of antibody production (Chen et al., 2020), responses of mucosal antibody (Cervia et al., 2020), structural basis (Gavor 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, Pakistan. Therefore, this study intends to assess the presence of antibodies to SARS-CoV-2 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 the proportion of serious cases versus asymptomatic, moderate, or non-pneumonia cases.

MATERIALS AND METHODS

Study design and participants

In current study, a total of 1229 patients were randomly selected from Asian Institute of Medical Sciences (AIMS) and Isra University & hospital, Pakistan for seroprevalence testing on November-30-2020 from reported patient list on August-01- 2020 taking. We used implicit stratification by area, age, and sex, followed by systematic sampling with fractional polynomials, to determine at a cross-sectional study of adult (≥ 18 years) patients (Frankel, 2010). The collection of samples was in Gel tubes bottle with 5ml blood. All the samples were centrifuged at 6000 rpm for serum after clotting the blood and kept in fridge at 6 to 8 ^{0}C. The test was run according to the manufacturer's instructions. The anonymised data on patient age, sex, and MR codes was send to Isra Pathological Lab for review after sample collection and processing. All of the patients (n = 1229), were older than 18 years adults, and males patients comprises of 790 (73.38%) and females 439 (26.61%).

Procedure

Elecsys® Anti-SARS-CoV-2 was approved by the US Food and Drug Administration-(FDA) that reported to be 99.8% specificity and 100% sensitivity (≥14 days after a positive PCR test) was used. An immunoassay for in-vitro detection of antibodies (including IgG) to SARS-CoV-2 was obtained from human plasma and serum from a blood sample. The immunoassay is based on in-solution double-antigen sandwich format that can detect antibodies to the new coronavirus

infection and could be used to determine whether a person has been infected or has acquired immunity to the virus. The test is designed to help and recognize people who have developed an adaptive immune response to SARS-CoV-2, suggesting recent or previous infection (Elecsys, 2020).

Laboratory Diagnosis

Laboratory diagnosis involves complementary, direct or indirect methods, and plays a role in isolation, identification, prevention or treatment of mortality as well as morbidity, and the reduction of the transmission of SARS-CoV-2 infections. Direct and indirect tests are used to identify the disease-causing agent in patients and to determine the host's immune response to the disease. Pre-analytical procedures are an essential aspect of any infectious disease diagnosis before the test is selected (Tang et al., 2020).

Specimen Collection and Shipment

Prior to collection, adequate training must be provided to staff on biosafety measures, standard operating procedures for transport and collection. When samples are collected from both the upper and lower respiratory tract, the sensitivity to virus detection is high. In height viral loads in the respiratory tract were observed within 5–6 days of onset of symptoms. Upper respiratory samples are oropharyngeal (OP) and nasopharyngeal (NP) specimens. In this study, whole blood or serum was collected and the sensitivity of OP swabs to SARS-CoV-2 RNA was 32 percent compared to 63 percent of NP swabs (Mlcochova et al., 2020; Wang et al., 2020b).

Serology of COVID-19

Serological tests includes 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).

A positive test may indicate past infections with the virus. The Food and Drug Administration (FDA) has issued an Emergency Use Authorization (EUA) for its latest Elecsys® Anti-SARS-CoV-2 antibody test. The test is used to evaluate whether a patient has been exposed to SARS-CoV-2 and whether the patient has immune to SARS-CoV-2 antibodies. Roche has already begun to ship a new antibody test to leading laboratories worldwide and will increase production capacity by up to double-digit million per month to serve healthcare systems in countries that accept the CE mark (Li et al., 2020b), as well as the Roche's SARS-CoV2 antibody test in the U.S, which has a specificity of 99.8% and 100% sensitivity[3] (14 days post-PCR confirmation), it can help assess the immune response of patients to the virus. As the immunity to SARS-CoV-2 is more widely understood, the test may help to determine who has acquired virus immunity.

Diagnosis

A suspected case is defined as a case with fever, sore throat and cough that has a history of travel abroad or Karachi or Sukkur or contact with patients with a similar history of travel or other areas of persistent local transmission confirmed infection with COVID-19. However, cases may be symptomatic, asymptomatic or even fever-free. A confirmed case shall be referred to the

molecular test by PCR. Molecular testing of respiratory samples through sputum/ nasopharyngeal swab/ throat swab/ bronchoalveolar lavage and endotracheal aspirates. In a suspected case in Pakistan, the appropriate sample must be sent to designated reference laboratories in DOW University & hospital or Agha Khan University & hospital Karachi, Pakistan. Commercial tests will become available as the epidemic progresses.

In suspicious cases with negative molecular diagnosis, CT has been used to diagnose COVID-19; many of these patients had positive molecular tests on repeat testing (Huang et al., 2020). A random sample of patients was tested for seroprevalence of SARS-CoV-2 antibodies. Our goal was to deliver a nationwide estimation of SARS-CoV-2 exposure during the first wave of COVID-19 in the United States, with stratification by country, age, sex, race, and ethnicity, from July 2020 to July 2021.

Finally, the prevalence of Covid-19 cases in the local population of Hyderabad and the surrounding area will be determined. We investigated correlates for seropositivity cases in other population.

Statistical Analysis

We assumed a 5% prevalence of SARS-CoV-2 antibody across the region to measure the prevalence of patients. In order to prepare for the probability of a dropout, we randomly selected 1229 patients. In our study, we present prevalence estimates of 95% CIs, standardized to the Pakistani population, we used the distribution of all male females and age groups adults those who live in the territories, on Aug 1, 2020, identified through the Different hospitals. Finally, we calculated the sex-adjusted and age-adjusted correlates of seropositivity for patient MR race and

ratio of people living in poverty, ethnicity distribution, urban or rural classification, county mobility restriction and population density using Multilogistic Regression analysis. SPSS was used for all statistical analyses at $\alpha<0{\cdot}05$ significance (version 20.0).

RESULTS

The figure shows the number of total positive 206 cases and negative 1023 out of total 1229 number of cases (Figure 1).The sampling was representative of the Pakistani population especially in Hyderabad adjacent area. Patient distribution by age, sex (excluding criteria of patients having liver cirrhosis/cancer or other diseases), we selected the patients randomly basis and were analyzed by Roche e411 analyzer for antibody detection.

We first analyzed the results based on Age, which is divided into 4 groups. Group I-IV comprises of 18-25, 26-35, 26-45 and 46-65 years. The results revealed that percentages of positive (16.76 %) and negative (84 %) COVID-19 cases were detected at the group I, whereas groups II were found positive (13.23 %) and negative (86.76%), Group III were found positive (18.41%) and negative (81.58%), Group IV were found positive (22.31%) and negative (77.68%). Overall, this showed that higher positive COVID-19 was detected in the age 56-65 years, as illustrated in Table 1.

To further confirm our finding, regression analysis was carried out for comparing gender and age groups (Group I-IV) of Covid-19. The results showed that the male positive percentages of Covid 19 were 17.76 % and female positive percentages were 13.8 %,whereas in male negative percentages of 83.50 % and in female 84.73 % were detected in the Group I. The group II (age 26-35) showed the male positive percentages of Covid 19 were 15.57 % and female positive

percentages were 8.84%, whereas in male negative percentages of 86.23 %and in female 87.78 % were detected. The group III (age 36-45 years) having a total Covid 19 cases 239, showed that the male positive percentages of Covid 19 were 21.33% and female positive percentages were 13.48%, whereas in male negative percentages of 78.66% and in female 86.51% were recorded. Group IV (age between 46-65 years) contained 242 total samples in which the male positive percentages of Covid 19 were 24.69% and female positive percentages were 17.5%, whereas in male negative percentages of 77.21%, and in female 78.52% cases were detected. The results further revealed that higher percentage of positive COVID-19 were detected in males in all age groups as compared to females, and both male and female are affected most at age of 46-65 years. (Table 2).

DISCUSSION

The clinical signs of 2019-nCoV infection are close to those of SARS-CoV, with fever, chest pain, dyspnea, dry cough, myalgia and exhaustion being the most common symptoms (Huang et al., 2020; Wang et al., 2020b, Zhu et al., 2020). However, diarrhea, dizziness, nausea, headache, vomiting, and stomach pain are some of the less common symptoms (Wang et al., 2020a). Some reports revealed that dry cough, fever, fatigue, and myalgia are the most common symptoms, according to the first 425 reported cases in Wuhan (China), with headache, diarrhea, sputum development, abdominal pain, and hemoptysis being less common (Dittadi et al., 2020).

Patients with COVID-19 are distributed among all age groups. The current study randomly 1229 patients were analyzed sero-antibody total protein observed 1023/1229(83.24%) were negative,

there were no covid-19 antibody produced while 206/1229(16.74%) were positive. this study investigating 1229 patients both male and female. The analyzed data showed more positive results as compared females, it was yet discovered why males were more positive than females. This study was cleared one point that was more males positive and also correlate china city Wuhan. While this potency provides new insights on the possibility and mode of reopening schools around the world, the immune response of children after infection is unknown. Our ELISA test was validated in an adult-only population; whether antibody feedback is delayed or qualitatively different in children needs to be examined further. The lower seroprevalence estimates among older adults suggest that targeted efforts to minimize social mixing of these individuals with others may have been successful.

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). Some studies reported the medicinal plant as a food to 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.

Antibody testing in the general population can help to determine the true number of asymptomatic carriers; however, it cannot be used for diagnosis, so PCR remains the preferred method of investigation. In terms of limitations, this is a single-site study, but it is the first of its kind to our knowledge, elucidating the seroprevalence of anti-SARS-CoV-2 antibodies, as well

as their relationship with age and gender. More local analysis is required to verify these findings. Furthermore, the number of pre-Covid samples available for research was limited.

CONCULSION

To determine prevalence of COVID-19 by total antibody testing in different age group and gender in population of Hyderabad and to conclude that over time, the seroprevalence of SARS-COV-2 antibodies has increased in Pakistan, which may aid in identifying the true number of COVID-19 cases. As a result, it is hoped that this study will be useful in resolving the continent's outbreak-related problems and will serve as a benchmark for future research. It is to be expected understanding the mechanisms of neutralizing antibodies performance will deliver appreciated implications for antibodies in treatment of SARS-CoV-2 in the nearby upcoming.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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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: Regression analysis comparison between gender and age group of Covid-19 total antibody tests

Age Group	Gender	Negative	Positive	Total	P value
		1023/1229	**206/1229**		
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	

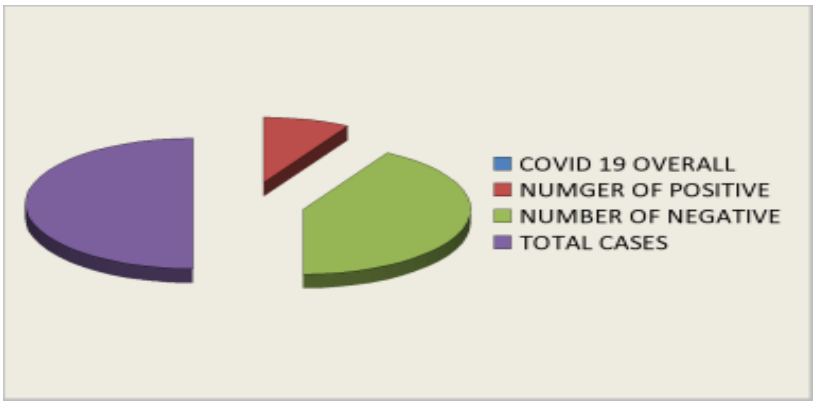

Fig.1: Detection cases in Hyderabad adjacent area, Pakistan