

**How to Cite:**

Abbas, A., Wajahat, M., Sayan, M., Noureen, S., Shoaib, R., Khan, A. A., & Arikan, A. (2023). Seroprevalence of anti-SARS-CoV-2 total antibodies among COVID-19 patients in province of Punjab, Pakistan. *International Journal of Health Sciences*, 6(S8), 6667–6676. <https://doi.org/10.53730/ijhs.v6nS8.13945>

## **Seroprevalence of anti-SARS-CoV-2 total antibodies among COVID-19 patients in province of Punjab, Pakistan**

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**Abstract**---At the end of 2019, there was an outbreak of coronavirus in Wuhan, China which killed more than 1800 people and more than 70,000 people were infected during the first 5 days. Chinese researchers named it as a novel coronavirus disease 2019. Up to now, 231 million cases and more than 4 million deaths have been reported worldwide. The total number of cases of COVID cases in Pakistan was

1,236,888 so far and the total death toll was 27,500 and is increasing rapidly. OmniPATHM COVID-19 Total Antibody ELISA Test kit was used for serological analysis. We found that the highest number of patients with positive antibody test was Lahore with 48%. (n=442). Total antibodies against SARS-CoV-2 was found to be higher in men (43%) compared to women (41%). 13% of the children were found to be positive for SARS-CoV-2 IgG. In age wise distribution, patients in age group 41-50 year were recorded highest seroprevalance. We took the mean of antibody titer according to different age groups and we found the highest mean of antibody titer which was 171 in age group (21-30).

**Keywords**---COVID-19, SARS-CoV-2, ELISA, antibody testing.

## **Introduction**

At the end of year 2019, there was an outbreak of coronavirus in Wuhan, China which lead more than 1800 people to die and more than 70,000 people infected during the first 5 days. Chinese researchers named it as a novel coronavirus disease 2019. The international committee on Taxonomy of viruses (ICTV) has given the name of this virus as severe acute respiratory syndrome (SARS-COV-2) [1].

Novel SARS-CoV-2 creates pandemic as of December 3, 2021 and it has reached 219 countries and taken the lives of more than 5 million lives and the number of confirmed cases around the globe was above 265 million [2]. According to the Worldometer of December 3, 2021, total number of reported cases were 265,817,940 and number of deaths reported were 5,266,354 [2]. The country with the most confirmed COVID-19 cases is the United States with 49,934,791 almost half of which are in the state of New York. The United States is the most affected country in number of deaths as of December 3, 2021 with 808,608 deaths. In Europe, the Russia is at the top of the list with the highest death rate of 281,278 followed by UK which has 145,551 total deaths [2]. According to the last update about coronavirus in Pakistan on December 3, 2021, confirmed corona cases reported in Pakistan were 1,286,825 in which 28,767 deaths were reported. Of the 1,286,825 total cases, 1,245,606 patients were recovered from the virus [3].

Experience taken from other species of human coronavirus prescribes that immunity is developed partially after infection. However, this immunity is of short duration usually lasting for a year or two [4]. There is very little data found for SARS-CoV-1 which shows that neutralizing antibodies have capacity which can stop viral infection. These antibodies remain effective up to a duration of 17 years after infection [5]. Clinically done, some studies suggest that the onset of response generated by any antibody after acute infection of SARS-CoV-2 seems to be the same as in the case of various kinds of human coronaviruses. Antibody response is generated to counter the nucleocapsid (N) and spike (S) proteins in which the receptor binding domain is present on the spike protein of S1 subunit [6].

Numerous studies regarding seroconversion have given knowledge about seroconversion of IgG, IgA and IgM antibodies. The main results indicated here talk about isotopes of one kind of antibody. For IgG antibodies, after symptoms rise, 12-15 days was the mean and median time for antibodies [7]. For IgM antibody, mean or median time to seroconversion ranged from 4-14 days after symptoms arose [8]. Very few studies demonstrate IgA dynamics in comparison to IgG and IgM antibodies. Levels of IgA titer are highly described between 16-22 days after symptoms rise. However, no agreement on shift over time has been reached [9].

The aim of study was to evaluate SARS-CoV-2 seropositivity of the population as an indicator of being affected by COVID-19 in cities in Punjab province, Pakistan.

## **Materials and Methods**

### **Ethical approval**

The ethical approval of the study was taken from the Near East University (NEU) Scientific Research Ethics Committee with the decision number 1370 NEU/2021/92 and from Reliance Hospital Lab, Islamabad with the registration number PHC Reg. No. R-18768. Informed consent was taken from each patient and from the guardian in case of a child under 18 years. Patients who were willing to take part in this study were enrolled in this study. Patients with no suspected symptoms of COVID and those who do not want to participate in this study were excluded from this study.

### **Study group**

This study was conducted at Reliance Hospital Lab in province of Punjab, Pakistan from November 10, 2020, to February 10, 2021. Across Punjab, serum samples were taken from three different cities which were Lahore, the capital of Punjab, Sialkot and Gujrat. In the study, a total of 919 SARS-CoV-2 suspected patients were enrolled based on their history, signs and symptoms from different areas of Punjab Pakistan. Samples were collected from patients with symptoms including fever, dry cough, tiredness and shortness of breath which are identified as COVID-19 symptoms by Center of Disease Control (CDC) guidelines [10].

### **Antibody measurement**

OmniPATH™ COVID-19 Total Antibody ELISA Test kit (Thermo Fisher Scientific, Waltham, USA) which detects IgA, IgM and IgG antibodies, was used in this study. OmniPATH™ measures antibodies that bind to the spike glycoprotein (S protein) which is the major surface protein that SARS-CoV-2 uses to bind to a receptor and invade cells. In the study, Dynex AGILITY automated ELISA device (Dynex Technologies, Sullyfield Circle Chantilly, VA, USA) was used as an ELISA reader.

ELISA test was performed according to the manufacturer test protocol. Microplate was read within a time duration of 10 minutes after the stop solution was added. Specimen results were determined using specimen index ratio. If the specimen

index ratio was  $<1.0$  the result was considered as negative and if it was  $>1.0$  then it was considered as a positive result.

### Statistical analysis

The statistical program used to evaluate the P value. A p-value measures the probability of an observed difference which could have occurred just by random chance. A small p value suggests the strong evidence in favor of alternate hypothesis.

### Results

In our study group 919 participants were enrolled in study. Among the study group; 337 (43%) and 313 (41 %) anti-SARS-CoV-2 positive patients were male and female, respectively. The mean age of the study group was 40 years. Among children category from 1 to 15 years, 121 (13 %) children were positive against SARS-CoV-2 antibody and are categorized in Table 1. The percentage of patients having different complaints including dry cough, fever, tiredness, body pain, sore throat, headaches and shortness of breath are categorized in Table 2.

Age distribution of the population had been done to evaluate which age group has high number of antibody titers in that time period. We found the highest mean of total antibody titer which was 171 in age group 21-30 and the lowest mean of total antibody titer i.e. 125 was found in age group 1-10. Highest antibody titer mean was recorded in males which was 158 which was higher than 137 recorded in females and it is listed in Table 3. In cities patients of Lahore had the highest mean of antibody titer which was 152 as compared to patients of Sialkot which was 122 and 115 among patients of Gujrat was recorded respectively listed in Table 4.

Table 1. SARS –CoV-2 Prevalence among study participants

Characteristic	SARS-CoV-2 antibody positive patient, n (%)	Average of total antibody response, Null hypothesis $\mu$	P value
Participants			
Male	337 (43 %)	158 (43% of male corona positive population had antibody titer above 158, rejecting null hypothesis)	$<0.001$
Female	313 (41 %)	137 (41% of female corona positive population had antibody titer of 137, accepting null hypothesis)	0.877
Children	121 (13 %)	107 (13 % of children corona positive population had antibody titer more than 121, rejecting null hypothesis)	$<0.001$

Table 2. Calculation of frequency and p value of the symptoms in study patients

Symptoms Characteristic	SARS-CoV-2 antibody positive patient, n (%)	Average of total antibody response, Null hypothesis $\mu$	P value
Dry cough	192 (20.9%)	112 (almost 21% of corona positive population with dry cough had antibody titer more than 112, rejecting null hypothesis)	<0.001
Fever	296 (32%)	180 (32% of corona positive population with fever had antibody titer of 180, accepting null hypothesis)	0.352
Sore throat	79 (8.6%)	132 (almost 9% of corona positive population with sore throat had antibody titer more than 112, rejecting null hypothesis)	<0.001
Fatigue	204 (22%)	166 (22% of corona positive population with fatigue had antibody titer of 166, accepting null hypothesis)	0.284

Table 3. Percentage distribution and P value calculation of antibody titers among different age groups

Age Group Characteristic	SARS-CoV-2 antibody positive patient, n (%)	Average of total antibody response, Null hypothesis $\mu$	P value
1-10	80 (11%)	125 (11% corona positive population of this age group had antibody titer of more than 125, rejecting null hypothesis)	<0.001
11-20	76 (9.9%)	170 (almost 10% corona positive population of this age group had antibody titer of 170, accepting null hypothesis)	0.214
21-30	103 (13%)	171 (13% corona positive population of this age group had antibody titer of 171, accepting null hypothesis)	0.132
31-40	124 (16%)	155 (16% corona positive population of this age group had antibody titer of 155, accepting null hypothesis)	<0.001

		positive population of this age group had antibody titer of more than 155, rejecting null hypothesis)	
41-50	132 (17%)	121 (17% corona positive population of this age group had antibody titer of more than 121, rejecting null hypothesis)	<0.001
51-60	126 (16%)	126 (16% corona positive population of this age group had antibody titer of more than 126, rejecting null hypothesis)	<0.001
61-70	88 (11%)	128 (11% corona positive population of this age group had antibody titer of 128, accepting null hypothesis)	0.372
>70	42 (5%)	134 (5% corona positive population of this age group had antibody titer of more than 134, rejecting null hypothesis)	<0.001

Table 4. Prevalence of SARS-CoV-2 patients among different cities

Cities Characteristics	SARS-CoV-2 antibody positive patient, n (%)	Average of total antibody response, Null hypothesis $\mu$	P value
Lahore	442 (48%)	152 (48% corona positive population of Lahore had antibody titer more than 152, rejecting null hypothesis)	<0.001
Sialkot	194 (21%)	122 (21% corona positive population of Sialkot had antibody titer of 122, accepting null hypothesis)	0.998
Gujrat	135 (14.7%)	115 (14.7% corona positive population of Gujrat had antibody titer of 115, accepting null hypothesis)	0.321

P values were used in hypothesis testing to take decision whether to accept or reject the null hypothesis. The smaller the p value the more significant it is and null hypothesis was rejected.

If p value was  $< 0.00001$ , means it was significant and null hypothesis was rejected

If p value was  $> 0.00001$ , means was not significant and null hypothesis was accepted

## **Discussion**

According to recent studies performed in the United States (U.S) measured seroprevalence during month of April 2020 found was Idaho; 4.7%, 1.8% in Boise, in Los Angeles, California; and 14.0% in New York [11, 12, 13]. In comparison to our studies, we had estimated seroprevalence of 3 major big cities of Punjab, Pakistan. Lahore which is the capital of Punjab recorded a high prevalence of 57% which is very high as compared to New York, but we had collected samples at the peak time of the COVID-19 outbreak. Also, Lahore has not a healthy environment and it's a very highly populated city of Punjab. That's why people of Lahore were at great risk from infection with SARS-CoV-2.

A study was done on seroprevalence in Faisalabad, Pakistan from April, 2020 to May, 2020 according to which overall prevalence was 17.18 and males were more effective than females [14]. Our study suggests prevalence of Gujrat which was 18% and this is in accordance with the study done in Faisalabad. Both cities are similar in population wise as well as environmental conditions. Also, our study had done after this study, so it means that people of Faisalabad had developed antibodies earlier due to infection as compared to Gujrat and the outbreak in Gujrat had been less severe than Faisalabad. Also, male patients (43%) are more affected than females (41%) in our study. This is because of the male patients are more associated with health care professionals than females and also males have high interaction with the public due to their jobs and business.

According to the study done by Xu et al (1) on 745 children and 3174 adults who had close contact with diagnosed patients found that rate of positive cases reported in adult population was 2.7 percent higher than that in children. Our results are in accordance with this finding as in our study the number of children with SARS-CoV-2 was 16% which was 4 times less than adults. In Pakistan adults are more involved in social relationships and social interaction. Also immune system of children is stronger than adults so they are at low risk of being infected. COVID-19 infected children have low level of neutralizing antibodies. So, high levels of neutralizing cross reactive HCoV antibodies might be the reason of increased susceptibility to SARS-CoV-2 in adults [16].

One research report pointed out that 63% of the Pakistani population is relatively younger, e.g., 15-33-year age group and population under this age group is highly affected [17, 18]. This study is in accordance with our results as we also have highest number of antibody titers recorded between age of 11 to 30 years. This is due to the fact that most of Pakistani young population is linked with those

professions which directly involves public dealing such as health care workers, laborers, transporters etc. so, they are at risk of developing anti-SARS-CoV-2.

According to one study conducted on 145 patients with SARS-CoV-2 disease suggests common symptoms in corona patients which included dry cough (81.4%), fever (75.2%), fatigue (40.7%) [19]. Similarly another study showed the clinical manifestations of coronavirus patients and that were fever in 87% of patients, dry cough in 65% of patients and fatigue in 42% of patients [20]. These results are in accordance with our findings as our study suggests 32% of patients with fever, 20.9% with dry cough, and 22% with fatigue because these symptoms are most commonly reported in SARS-CoV-2 patients in acute phase of infection.

According to one study, the highest death rate was found in the patients having the age of 60 years or more. Systematic analysis of 1.5 millions of SARS-CoV-2 patients from different countries reiterates the effect of age on death rate with more relevant on age greater than 50 years especially, the age greater than 60 years [21]. In our study, ratio of reactive SARS patients between the age of 40 to 60 years were more and are at high risk. This may be due to the immune system of older people because as the body ages the immune system becomes weak, and it develops low levels of inflammation and SARS- CoV-2 could be pushing the already weakened immune system over the edge. Also, old age people have higher comorbidities such as obesity, diabetes and heart failure. That's why old age people have a higher chance of being affected by SARS-CoV-2 as compared to other age groups.

## **Conclusion**

A high seroprevalence was recorded in the city of Lahore in Punjab province, Pakistan. There was not a big difference in SARS-CoV-2 cases among males and females due to same life conditions however, males are slightly higher in number because most of reported male patients were belong to health care profession. Children were found to have a smaller number of cases or less susceptibility to SARS-CoV-2 infection. Younger population of Punjab province had recorded a high number of antibody response which indicates their stronger immune response against SARS-CoV-2. Thus, SARS-CoV-2 attacks on highly populated cities and in younger population of Pakistan where the chance of transmission and social interaction is more as compared to low population cities.

## **Acknowledgments**

I acknowledge Dr. Murat and Dr. Ayşe, Department of Medical and Clinical Microbiology for their invaluable advice, assistance and comments on the manuscript, Near East University, Northern Cyprus. I also acknowledge Reliance Hospital Lab management who continuously supports me throughout the research in sample collection and management.

## **Conflict of Interest**

None declared



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