Increasing IgG antibodies to SARS-CoV-2 in asymptomatic blood donors through the second COVID-19 wave in Karachi associated with exposure and immunity in the population

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Abstract

Introduction

One million cases of COVID-19 have been reported in Pakistan until August 1, 2021. However, SARS-CoV-2 PCR testing capacity is limited, and the true level of SARS-CoV-2 infections is unknown. Most individuals have asymptomatic or mild COVID-19 and remain undiagnosed. Volunteer healthy blood donors can be a control population for assessment of SARS-CoV-2 exposure. We determined COVID-19 seroprevalence during the second pandemic wave in Karachi.

Materials and Methods

We enrolled 558 healthy blood donors at the Aga Khan University Hospital blood bank between December 2020 and February 2021. Serum IgG reactivity were measured to spike and receptor binding domain (RBD) proteins. Clinical history was taken from individuals with a positive IgG result.

Results

Of the 558 study subjects, 553 (99.1%) were males and 5 (0.9%) were females with a mean ($\pm$ SD) age of 29.0 ± 7.4 years (range 17–53 years). Positive IgG responses to spike were detected in 298/558 blood donors (53.4%). Of the spike IgG positive cases, 93/298 individuals (31.2%) had IgG antibodies to RBD. Clinical information was available for 63.7% of spike IgG positive cases. Of these, 37% had flu-like symptoms, 25% had travelled, individuals had contact with COVID-19 suspected (18%) or confirmed (15%) cases. Within the year prior to blood donation, 29% of individuals had laboratory confirmed COVID-19.

Conclusions

The seroprevalence of 53.4% of IgG antibodies to spike and 31.2% of IgG to RBD of SARS-CoV-2 in healthy blood donors indicates high rates of exposure and gives insights into protective immunity in the population.

Introduction

COVID-19 is a highly contagious respiratory tract infection caused by SARS-CoV-2(1, 2). COVID-19 was declared a pandemic in March 2020 by the World Health Organization. The first case of COVID-19 in Pakistan was diagnosed on February 26, 2020. The first pandemic wave peaked in June, 2020 when approximately 5000–6000 cases were detected each day (3). The second wave started in November and December 2020 that lasted until January 2021 (4). A third wave of COVID-19 occurred between March and May 2021 (3) and soon after, in July a fourth wave was observed (5). The Government of Pakistan Covid database (<https://covid.gov.pk/>) shows that to date an estimated 1,039,695 covid cases have been reported in the country with death toll of 23,462 and case fatality rate (CFR) of 2% (Data available till 1st August 2021), <https://covid.gov.pk/stats/pakistan>. The magnitude of new daily COVID-19 cases identified has been lower than in many parts of the world such as, the USA where greater than 10,000 tests are conducted per 1 million individuals based on need (6). In Pakistan the testing capacity is limited and in January 2021, 40,000 SARS-CoV-2 PCR tests a day were conducted for approximately 200 million population (200/million) (7). The majority of individuals with COVID-19 tend to be asymptomatic or with minimal symptoms (8) and are

unlikely to be diagnosed. Given the limitation of PCR based diagnosis of COVID-19 there is likely under-reporting (9). In such a scenario, it becomes important to identify disease status and understand contextual transmission to devise strategies for prevention and control of this highly contagious disease. Seroprevalence assays can help estimate disease burden in the population. Previous seroprevalence data from Karachi has shown antibody positivity to range between 20 and 40% depending on study of community or hospital-based populations (10–12). A recent countrywide study has shown seroprevalence to be about 40% (13). These studies have tested for antibodies to nucleocapsid (N) protein of SARS-CoV-2. Meanwhile, it has been shown that antibodies to Spike (S) protein are associated with protective immunity against the virus (14). Here we measured IgG to both spike and the receptor binding domain (RBD) protein to investigate immunity against SARS-CoV-2 in the population. Blood donors represent healthy individuals in a community and therefore we studied in donors at a tertiary care facility in Karachi during the second COVID-19 wave from December 2020 until February 2021.

Materials And Methods

Study setting

The study was conducted at Aga Khan University Hospital (AKUH) from December 2020 to February 2021. AKUH is a 700-bedded hospital with facilities of trauma, surgery, and bone marrow transplants. The blood bank at AKUH has an estimated annual collection of 25,000 to 30,000 units of whole blood from healthy non-remunerated blood donors. Approximately 90% of these blood donors are exchange donors donating blood for admitted patients. AKU laboratory is accredited by College of American Pathologists and follows the national and international standards for blood collection, manufacturing, storage, and transportation. Each blood donor is registered for blood donation following a donor interview to exclude the presence of transfusion transmitted infections and to ensure donor safety. Following pandemic, a question related to the risk of having COVID-19 infection in previous 4 weeks was added in the donor screening. Any donor with a history of COVID-19 related symptoms or confirmed infection was excluded from enrollment as a blood donor. Each donated blood unit is typed on automated gel platform (IH-1000, DiaMed GmbH, Cressier FR, Switzerland) and screened for malaria (ICT, BionaxNow, Abbott), syphilis (latex agglutination, Sfilide RPR, Milano, Italy), viral hepatitis B, C and HIV (Chemiluminescence assay, Vitros ECiQ Immunodiagnostic system, Orthoclinical diagnostics, Johnson & Johnson, United States).

Participants and research strategy

The study was approved by Ethical Review Committee of The Aga Khan University (#2020-5152-11688) and was conducted according to good clinical practices and the Declaration of Helsinki. Blood donors who presented to the blood bank for donations were informed about the study and if they consented, they were recruited. Recruitment started was between December 2020 and February 2021.

Serum was collected and tested for the presence of IgG antibodies to spike. Once IgG antibody responses were available, those with a positive IgG antibody were then contacted by phone for further information including a risk assessment of COVID-19 infection through an in-house questionnaire. A case was identified as someone with confirmed COVID-19 denoted by either a positive SARS-CoV-2 PCR or antibody against SARS-CoV-2. Exposure to a suspected or confirmed case of COVID-19 was self-reported by the blood donors, their laboratory reports were not available for review and confirmation.

Laboratory analysis

Sample collection

From each donor, 4 ml of blood sample was collected in gold-top serum separator tube (BD vacutainer® blood collection tube). The serum was separated, and aliquots were stored at -80°C till further analysis.

ELISA for IgG to spike and RBD

Recombinant spike and RBD protein were obtained from IBET, NOVA University, Portugal. All serum samples were tested in duplicate using in-house enzyme linked absorbent assay (ELISA)(15) and as per protocol described by Figueiredo-Campos et al (16). As a first step, all serum samples collected from study subjects were tested for IgG antibodies to spike protein and those having a reactive result for IgG to spike protein were further tested for IgG to RBD. A standard curve of comprising a titration of positive IgG antibody sera was run on each plate (Supplementary Fig. 1). For ELISA against SARS-CoV-2 Spike and/or RBD protein, the 96-well ELISA plate was coated with 50µl of Spike or RBD protein at a concentration of 2 µg/ml in PBS. Briefly, the wells were blocked with 200 µl of PBS + 0.1% Tween (PBS-T) + 3% non-fat milk. Subsequently, the wells were washed and 100 µl each of serum samples were diluted 1:100 in PBS-T + 1% non-fat milk powder were added to the plate and incubated for 2 hours at room temperature. Wells were washed and stained with goat anti-human IgG Fc (HRP). Finally, the plate was developed using TMB substrate solution which stopped with 0.5M sulfuric acid. The optical density was read at 450nm. A cut-off for IgG positive results was established on testing of 100 samples of pre-pandemic sera. These were found to be negative and an OD reading of 0.5 at 450 nm was used to identify positive IgG to both Spike and RBD.

Statistical analysis

Data was analyzed through SPSS version 24. Normality of data was checked through Shapiro walk test and mean $\pm$ SD was used for normally distributed and median (IQR) for skewed continuous data. Frequency or proportion was used to give estimates for categorical data. Chi-square test was used to compare the frequencies of various antibodies with respect to age groups (with 30 years as the cutoff) and blood types and the threshold of significance was a *p-value* < 0.05.

Results

Demographics of the study population

During the study period December 2020 until February 2021, the AKUH blood bank approached 1809 healthy non-remunerated blood donors regarding this study. Of them, 558 subjects (31%) consented to participate in the study. Study subjects included 553 (99.1%) males and 5 (0.9%) females with a mean ($\pm$ SD) age of 29.0 ± 7.4 years (range 17–53 years). Samples were collected in two sets during the peak of the second COVID-19 wave in Karachi, the first was of 322 blood donors in December 2020 and the second was of 236 donors collected in February 2021.

The ABO blood groups of the study subjects were A (n = 133; 23.8%), B (n = 200; 35.8%), AB (n = 39; 7.0%) and O (n = 186; 33.3%), Supplementary Table 2.

IgG to spike proteins in study subjects

Sera were tested for the presence of IgG antibodies to spike of SARS-CoV-2. Subsequently, all specimens with a positive IgG result to spike protein were tested for the presence of for IgG antibodies to RBD. Of the 558 individuals tested, IgG to spike protein was detected in 298 (53.4%) blood donors (Fig. 1A). Ninety-three of the 298 individuals (31.2%) who had IgG to spike also had IgG antibodies to RBD. A correlation analysis between IgG titers to spike and

RBD revealed a moderately significant positive correlation, (p value < 0.0001) and $r = 0.3787$) between the two data sets, Fig. 1B.

Demographics of individuals with a positive IgG response to spike

Individuals with positive IgG titer to spike protein had a mean ($\pm$ SD) age of 29.2 ± 7.2 years and a male predominance ($n = 297$; 99.7%). Individuals aged between 31–40 years had highest frequencies for spike antibodies (61.7%) and RBD (21%). The trend of positive IgG between spike and RBD for each age band was similar, with average 50% positive for spike (range 25–61.7%) and 17% positive for RBD (range 10.5–21%) across age groups (Table 1).

No statistically significant difference was observed in positivity rate in antibodies to spike protein (50.4 % vs. 58.4%, p -value 0.069) and RBD (15.5 vs. 18.7%, p -value 0.814) in the young donors who were < 30 years compared to the relatively older age group (Table 1).

Blood groups amongst study subjects with IgG to spike

Blood donors with blood group A, B, AB, and O showed IgG to spike in 52.6%, 56.5%, 43.5% and 52.6% respectively while IgG to RBD was observed in 37.1%, 32.7%, 17.6% and 27.6% in that order (Fig. 2). There was no statistically significant difference in the frequency of IgG to Spike (p -value 0.503) and that of IgG to RBD (p -value 0.342) with respect to various age groups.

Month-wise seroprevalence of SARS-CoV-2

Table 2 summarizes the seroprevalence of COVID-19 during peak and post peak seasons. No statistically significant difference ($p = 0.805$) was observed in the frequency of IgG to spike during December 2020 (54%) and February 2021 (52.5%). Similarly, though proportion of IgG to RBD (34.4 % vs. 26.6%) was higher in December 2020 as compared to February 2021 but the results were not statistically significant ($p = 0.059$).

Prior symptoms, risk factors and history of COVID-19

Of 298 study subjects with a positive IgG antibody to spike, only 190 (63.7%) could be contacted for additional information. The secondary questionnaire administered included information regarding any prior history (within the six months) of respiratory illness/flu-like symptoms, domestic/international travel history, or contact with individuals who were suspected or confirmed for COVID-19 PCR or prior SARS-CoV-2 antibody testing. Of the 190 study subjects for whom information was available (Table 3), 37.4% had a history of respiratory illness, 24.7% had a history of travel outside their home city, 17.9% had contact with suspected patient and 14.7% had contact with a PCR positive COVID-19 case, Table 2.

During donor interview, 56/190 (29%) individuals reported a confirmed SARS-CoV-2 laboratory test through either a positive PCR or antibody test.

Of 11/44 blood donors (25%) with a prior history of a positive SARS-CoV-2 PCR, 4 had (36.4%) positive IgG antibodies to RBD. Similarly, 4/12 (33.3%) blood donors with a prior laboratory confirmed antibody result to SARS-CoV-2, two had IgG to RBD (50%).

None of the study subjects had any co-morbidities. Those with confirmed COVID-19 had only mild disease without a history of hospital admission requiring intensive care treatment.

Discussion

Sero-epidemiological studies are helpful in estimating the true magnitude of the disease in a population by estimating the number of individuals with subclinical /asymptomatic infections. Further, they can provide insights into the immune protection present in the population. In this study, we found nearly 53% of healthy donors that visited our blood bank at the time of the second COVID-19 wave in Pakistan between December 2020 and February 2021, showed positivity for IgG against spike protein of SARS- CoV-2. This seroprevalence is significantly higher than the previously reported data for healthy blood donors from Europe such as; 0.9% in Italy, 1.9% in Denmark, 2.7% in France and 0.91% in Germany (17–19) (20). A study conducted in healthy blood donors in Lombardy, Italy (9), one of the first lock down region in Italy reported a frequency of 19.7% (378/1922) for SARS-CoV2 anti-S1 and anti-S2 IgG antibodies and 21.6% for neutralizing antibodies during March to June 2020 (21). A weighted SARS-CoV-2 seroprevalence of 4.0% was observed in 2857 blood donors from Brazil in April 2020 (22) while New York, United States observed a seroconversion of 1 in every 8 blood donors in June-July 2020 (23). A high level of exposure to the virus was observed in Saudi Arabia, where IgG to spike proteins was observed in 234/1212 blood donors (19.3%) during May to July 2020 (24). Moreover, an increase in seroprevalence was reported in blood donors in parallel with the rise of COVID-19 infection in Jordan (25). Previously, reports of healthy blood donors from Karachi tested in June 2020 (at the peak of the first COVID-19 wave) showed a seropositivity in 15/70 (21.4%) with an increase (to 37.7%) in July 2020 (12).

Seroprevalence of anti-SARS-CoV-2 antibodies are shown to persist for up to 6 months post-disease onset (16). In Portugal, it was shown that seroprevalence was 2.1% in September 2020 and 13.4% in March 2021 (9). In Pakistan, a multi-center study (during 15-30th July 2020) reported seropositivity ranging from 31.1 to 48.1% in 15,390 individuals from seven randomly selected cities from three most populous provinces of Pakistan (Khyber Pakhtunkhwa, Punjab, and Sindh) (13). Younas et al. reported seroprevalence of approximately 38% during peak time of July 2020 at Karachi, one of the largest cities of Pakistan (12). The current study performed December 2020 until February 2021 at the time of the second COVID-19 wave shows a further rise (to 53%) in seropositivity in Karachi. On January 1, 2021, 41,000 tests were conducted each day and approximately 2500 positive COVID-19 cases (6.1%) were confirmed within a 24 h period (7). Our data therefore alludes to much greater infection rates in Karachi than identified from PCR based testing alone. A higher seropositivity is suggestive of active disease transmission in the community and this may be related to over population, congested living conditions and low compliance with the SOPs like wearing mask in public and maintaining social distancing.

Our data showed an increasing frequency of seropositivity to spike and RBD in individuals who were between their second to fourth decades of life. Similar findings have been reported from Saudi Arabia and Pakistan (12, 24). Declining seropositivity in the fifth and later decades might be attributed to decreasing immunity with advancing age, but no conclusion can be made as the studied population comprised predominantly of young adults. Notably, we had very few individuals aged 50 years and over as it was younger individuals who comprised the blood donor cohort.

Though several population-based studies have demonstrated a high seroprevalence in males compared to females (19, 22, 26, 27), such a comparison could not be undertaken because of gender imbalance in the current study with predominantly more male donors. Another limitation of this study is that a detailed COVID-19 history was not taken prior to enrollment of study subjects and any individual who had no signs and symptoms of disease within the past four weeks was eligible for the study. The secondary questionnaire was administered later and only 64% of the study subjects could be followed up.

Previous studies have shown COVID-19 to be present more in individuals with blood group A (24). as compared to those having blood group O (28). However, here, we did not find any significant difference in seroprevalence among different ABO blood group types, corroborating an earlier report from Pakistan (12).

One or more symptoms that are associated with COVID-19 including fever, cough, and loss of smell/taste, fatigue, and malaise were observed in 71(37.4%) individuals. This suggests that almost 60% of the individuals who were infected by SARS-CoV-2 remained asymptomatic. This finding is congruent with a study in Quebec where half to two third seropositive donors were found to be asymptomatic (29). Almost 25% donors had national or international travelling and 15–18 % of them also had an exposure to a suspected or conformed patient with COVID-19. Another interesting finding is that 44/298 of the spike seropositive donors had also been previously tested by RT- PCR because of suggestive symptoms, but only 11 (25%) were positive. The reasons for this low PCR positivity are not known, but a low viral load at the time of PCR testing, inadequate sampling technique or variability in PCR-based diagnosis may be the possible explanations.

Interestingly, we observed a discrepancy between individuals who had positive IgG to spike and those who were positive to RBD. Overall, only 31.2% subjects having IgG to spike demonstrated IgG to RBD. This ratio of spike to RBD positivity was comparable across the age groups investigated. Previously, it has been shown that IgG to spike and RBD both demonstrate high specificity against SARS-CoV-2 (15, 30) and a good correlation with virus neutralization (VN) titers. However, Salazar et al. in 2020 reported anti IgG-RBD to have slightly better correlation with VN in his study on 68 patients with COVID-19 (31). In this data set, we were not able to directly test all samples here for virus neutralizing activity. However, we did test selected RBD antibody positive samples for neutralizing activity and found a correlation between IgG titers and SARS-CoV-2 neutralising effect (32). Therefore, we speculate that RBD antibodies found in our healthy blood donors reflected previous SARS-CoV-2 infection whilst a higher frequency of spike antibodies was secondary to cross-reactive antibodies. Cross-reactive antibodies against SARS-CoV-2 in pre-pandemic sera have been shown in studies from Africa (33, 34). Moreover, a high prevalence of cross-reactive antibodies has been identified in sub-Saharan Africa (35). While SARS-CoV-2 antibody positivity may protect against reinfection (36), the protective role of cross-reactive antibodies against SARS-CoV-2 needs further probing. No definitive conclusion can be drawn as we did not screen for antibodies against other coronaviruses in this study, due to lack of access to testing kits. Another limitation is that the study was conducted at a single institution and a relatively small sample size. Further, as with other seroprevalence studies, the rate of exposure may be under-estimated depending on the time of sampling after COVID-19 as antibody responses wane with time (37).

Factors such as, bacilli Calmette-Guerin vaccination (38), high temperature and humidity (39), and exposure to other viral/bacterial pathogens (40) have all been suggested in contributing to increasing protective immunity against SARS-CoV-2 in the population. High seroprevalence rates suggest active virus transmission in the community and highlights the importance of use of protective measures such as use of mask and social distancing.

Overall, this study provides important insights into SARS-CoV-2 seroprevalence rates in the local population just prior to the introduction of COVID-19 vaccination in February 2021. Further, we propose that cross- reactive antibodies could enhance protective immunity against SARS-CoV-2 in this population.

Declarations

Funding Source:

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Conflict of interest:

None declared

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Tables

Table 1. Frequency of IgG to spike and RBD in various age bands.

Age group (years)	N (%)	IgG to Spike N (%)	IgG to RBD N (%)
17-20	74 (13.3)	35 (47.3)	10 (13.5)
21-30	275 (49.3)	141(51.3)	44 (16)
31-40	167 (29.9)	103 (61.7)	35 (21)
41-50	38 (6.8)	18 (47.4)	4(10.5)
51-60	4 (0.7)	1(25)	0
All	558 (100)	298 (53.4)	93 (16.7)
*P-value	-	0.069	0.814

Table 2. Comparison of IgG antibodies to SARS-CoV-2 spike and RBD proteins in December 2020 and February 2021.

	IgG-spike N (%)		IgG-RBD N (%)	
	December 2020	February 2021	December 2020	February 2021
Reactive	174 (54.0)	124(52.5)	60(34.4)	33(26.6)
Non-reactive	148 (46.0)	112(47.5)	114(65.5)	91(73.4)
All	322 (100)	236 (100)	174(100)	124(100)

The table depicts the number of healthy blood donors tested during December 2020 and February 2021 in Karachi Pakistan. Values given indicate the total number (N) of individuals tested in each case and the percentage of them who had a Positive IgG result based on the ELISA to either S (spike) or RBD (receptor binding protein). No statistically significant difference was found between December and February for IgG to spike *(p 0.805) and RBD ** (p= 0.059) positivity.

Table 3. Clinical characteristics of blood donors who had a positive IgG to Spike protein.

Clinical details of donors	Spike IgG	RBD IgG
	N/total donors (%)	N/spike IgG (%)
Flu- like symptoms	71 (37.3)	22 (31)
History of overseas travel	47 (25)	10 (21.3)
Contact with suspected COVID-19 case	34 (18)	9 (26.5)
Contact with confirmed COVID-19 case	28 (15)	6 (21.4)
Prior history of COVID-19 (PCR confirmed)	11 (5.8)	4 (36.4)
Prior history of COVID-19 (antibody confirmed)	4 (2.1)	2 (50)

One hundred and ninety individuals with a positive IgG to Spike could be contacted for information provided. The information depicts the number of individuals who had an affirmative result for each category

Figures

A.

B.

Figure 1

IgG antibody in 558 healthy blood donors. A, IgG positivity was determined in sera against Spike and RBD protein. IgG to Spike was measured in 558 individuals. IgG to RBD was determined in the 298 blood donors who had a positive IgG to Spike. B, A depicting results for IgG to spike/RBD, show p value < 0.0001) and $r^2 = 0.0678$. Spearman's rank correlation was run between IgG to Spike and RBD. $r = 0.3787$, 95% confidence interval: 0.2701 to 0.4777 and P value (two-tailed) < 0.0001 indicate a positive correlation between the two data sets. The dotted line in 'A and 'B' represents the cut-off for a positive IgG result in each case.

Figure 2

Seropositivity in blood donors in relation to their ABO blood groups. The graphs show the number of individuals tested (blue bars), those who had a positive IgG to spike (orange bar) and those who had a positive IgG to RBD (grey line) in each case. Data for individuals is shown as per their A, B, AB and O blood groups.

Supplementary Files

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- [SupplementaryInformation.docx](#)