Original Article

Seroprevalence of SARS-CoV-2 IgG in blood donors in a teaching institute from Western part of Maharashtra

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Abstract

Introduction: COVID-19 is a disease caused by the severe acute respiratory syndrome coronavirus 2 that has appeared as a global pandemic in recent times. Currently, the transmission rate has slowed down significantly, but the definite pathological reason behind this is still unknown. Therefore, the prevalence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody must be studied to establish the relation between the rate of transmission and antibody presence. **Materials and Methods:** A clinical assessment was performed to evaluate the seroprevalence of SARS-CoV-2 Immunoglobulin G (IgG) antibodies among 299 healthy volunteers in the period of February to May 2021. Serum samples were analyzed using chemiluminescent microparticle immunoassay (CMIA) technology to detect the presence of IgG antibodies. **Result:** It was observed that 21% of the participants were seropositive, and 78% of the population was seronegative across the different genders. This confirmed that the generation of antibodies is independent of gender. Simultaneously, a *t*-test was performed that further suggested no statistical correlation between gender and seroprevalence. Moreover, a comprehensive analysis was performed to establish the relation between age and blood group with the seroprevalence. However, there was no statistical relationship found among these parameters. **Conclusion:** This study assisted in examining the underlying causes of high or low seroprevalence among healthy volunteers.

Keywords: Blood donors, IgG antibody, SARS-CoV-2, seroprevalence

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus that emerged in late 2019 in Wuhan, China, causing a global outbreak. Coronavirus is composed of a positive-sense single-stranded ribonucleic acid (RNA) genome surrounded by membranes containing crown-shaped, spike-like glycoproteins, and it belongs to the seventh member of the coronavirus family (*Coronaviridae*).[1] Coronavirus has infected numerous people and can cause moderate-to-severe respiratory problems.[2] Reverse transcription polymerase chain reaction (RT-PCR) has been used to identify and confirm the presence of SARS-CoV-2 in people; it can also detect prior or non-active viral infections.[3] Moreover, most of the infected individuals are asymptomatic and, therefore, sensitive and cost-effective diagnostic methods are necessary to know the pr precise frequency and seroprevalence among the population. Therefore, various research studies on SARS-CoV-2

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seroprevalence are being conducted to better comprehend the immunity developed in the immunity.[4] Blood donors are healthy individuals who are doing humanitarian work without any remuneration. This assessment can assist in creating subsets from the population that have been infected by SARS-CoV-2 without exhibiting symptoms, enabling authorities to roll out appropriate health guidelines, and gain insight into herd immunity.[5] Various studies used the prevalence of anti-SARS-CoV-2 (IgG and/or Immunoglobulin M (IgM) and/or Immunoglobulin A (IgA)) serum antibodies as the evaluating criteria. These serological tests may suggest past SARS infections by detecting the presence of SARS-CoV-2 antibodies.[6] The target for antibody detection in most serological research is either full-length or truncated forms of the nucleocapsid protein (NCP) or spike SARS-CoV-2 protein (SP).[7] These studies also investigated characteristics such as viral exposure, geographical factors, blood group, and age. Measuring the frequency and levels of antibodies in the population might assist in prioritizing immunization for the vulnerable set of populations.[8]

There are several laboratory tests available to detect the presence of SARS-CoV-2 antibodies. The serological diagnosis of SARS-CoV can be done by detection of specific antibodies (such as IgA, IgG, and IgM) or a combination of them. Additionally, they may employ different target antigens (spike, membrane, and nucleocapsid proteins) as complementary partners to bind and detect antibodies.[3,9]

The presence or absence of IgG antibodies in a blood sample from healthy individuals is essential to highlight the growth and spread of COVID-19. This study focuses on conducting a clinical assessment of the seroprevalence of SARS-CoV2 IgG antibodies among healthy blood donors. Later, the study is correlated with the ABO blood group to determine the association of antibody production with the blood group. The dependency on the age and gender of the donors was also determined in this study.

Materials and Methods

Type of the study

This is an observational cross-sectional study.

Place of the study

This serological survey was conducted at a blood center of a teaching institution in western Maharashtra, India.

Study duration

The study was conducted for 6 months from Feb to July 2021.

Sample size

A total of 299 volunteers were considered in this study and their blood samples were collected for assessment.

Study population

The population considered in this study was COVID-negative (never been COVID-positive before study participation) and non-vaccinated. The data collected in this study was during the second wave in India, where most of the causalities were reported.

Patient informed consent

An informed consent form for testing anti-SARS-CoV-2 was filled out by the participants.

Sample collection and processing

Serum/plasma samples were used to detect SARS-CoV-2 IgG antibodies by using chemiluminescent microparticle immunoassay (CMIA) technology (ARCHITECT *i*2000SR) technique. This assay is an automated, two-step immunoassay for the qualitative detection of IgG antibodies against SARS-CoV-2 NCP in human serum and plasma.[10] SARS-CoV-2 antibodies IgG Chemiluminescence immuno assay (CLIA) kits were obtained from Abott (Architect SARS-CoV-2 IgG) and biochemical tests were performed on the ARCHITECT *i*2000SR in accordance with the manufacturer's specifications. The index (sample/control) is calculated by comparing the relative light units in the sample to the calibrator's relative light units. Samples were interpreted as positive or negative according to the manufacturer's instructions.

Guidelines for reporting

The level of antibody determines the seropositivity of the sample. An antibody level >1.4 means the sample has significant antibodies, while an antibody level <1.4 is considered to be negative as per the kit insert provided with the kits and reagents by the manufacturer.

Ethical approval

Permission from the institutional ethics committee was obtained to conduct the study by letter number DYPV/EC/543/2022 dated September 4, 2020.

Statistical analysis

Descriptive analysis was performed to calculate the frequency, percentage, and mean of the observation. Data was presented in different frequency tables, cross-tabulations, and charts. All analysis reported in this study was completed on SPSS v23 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.)[11] and Microsoft Excel platforms.

Results

Gender dependency

Data used in this study was obtained from 299 healthy blood donors, whose blood samples were collected to detect the IgG antibody. Figure 1 shows the presence or absence of IgG antibodies in male and female healthy volunteers. It indicates comparable effects since both genders consist of about 21%

of the population as positive (presence of antibody) and about 78% of the population as negative (absence of antibody) in the serum sample. This further suggested that the presence or absence of antibodies did not depend on the gender of an individual. However, we observed a marginal lead for the female population. We conducted a *t*-test to evaluate the significance of the difference between the male and female population, as shown in Figure 1.

The *t*-test is a statistical testing method that is used to perform hypothesis testing to determine the effect of a process or treatment on the population, or whether these two populations are significantly the same or different. Here, these two populations were male and female, and they were evaluated as per the presence and absence of the antibodies. Table 1 shows the *t*-test statistics for males and females. Before the testing, the numbers were converted into percentages to represent the proportion of positive and negative samples in the male and female populations. Hypothesis testing is formulated based on the null hypothesis (H0) and an alternative hypothesis (HA).

H_0: $\mu 1 = \mu 2$, (i.e., there is no difference between the positive and negative sample among the male and female populations) (1)

H_A: $\mu 1 \neq \mu 2$ (i.e., there is a significant difference between the positive and negative sample among the male and female populations) (2)

The mean values of negative and positive in both gender populations were ~50. However, the variance among the male population is higher than the female population. Consequently, the difference between positive and negative antibody samples in the male population is relatively higher. Hypothesis testing depends on the *P*- value for one-tailed and two-tailed testing was performed. However, the two-tailed *P*- value was more relevant in this case, so we focused on two-tailed testing. Here, *P*- value = 1, which was higher than the standard critical significant value of 0.05 shows the 95% Confidence interval. Thus, if the *P*- value >0.05, then we failed to reject the null hypothesis that implies the difference between male and female populations is non-significant.

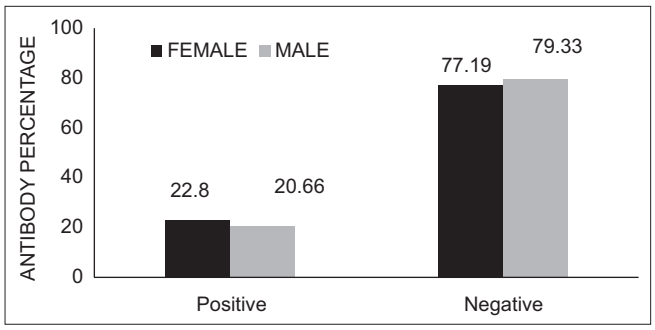


Figure 1: Percentage distribution of presence/absence of IgG antibody based on gender of an individuals

Age and antibody distribution

After determining the gender distribution of the positive and negative samples, we determined the distribution based on the age of healthy volunteers. The age of donors was grouped into 10-year bins, ranging from 10 years to 60 years, and we observed the presence (positive) or absence (negative) of the antibody accordingly. Table 2 shows the distribution of donors as per their age. Table 3 shows the same number as a percentage to make it independent of the total donors/samples in the specific age group.

The 10–20 years group showed the highest percentage of antibodies, that is 33%, and the 50–60 years group also had the same percentage of antibodies. However, in the 50–60 years

Table 1: *T*-test statistics of male and female categories based on the presence and absence of antibody

Parameters	Male	Female
Mean	49.99	49.99
Variance	1721.08	1479.13
Observations	2	2
Pearson Correlation	1	
Hypothesized Mean Difference	0	
df	1	
t Stat	0	
P (T≤t) one-tail	0.5	
t Critical one-tail	6.31	
P (T≤t) two-tail	1	
t Critical two-tail	12.70	

Table 2: Summary statistics of the antibody level by patient's age and blood groups

	Total	Mean	SD**	SE***	95%CI	*P**
Age	63	4.15	2.395241	0.301772	0.603234	0.84
18–25	25	4.47	2.438307	0.487661	1.006484	
26–30	12	4.18	2.892421	0.83497	1.837756	
31–35	13	4.06	2.416604	0.670245	1.460339	
36–40	6	4.25	2.338758	0.954794	2.454376	
41–45	3	2.83	1.450069	0.837198	3.602171	
≥46	3	3.3	1.55	0.9	4.647949	
Blood Group	63	4.15	2.39	0.3	0.6	0.56
A	16	3.74	2.347286	0.586821	1.25078	
AB	5	4.912	2.869986	1.283497	3.563558	
B	22	3.84	2.116049	0.451143	0.938203	
O	20	4.62	2.656009	0.593902	1.24305	

*One-Factor ANOVA, **SD=Standard Deviation, ***SE=Standard Error

Table 3: Antibody distribution based on the age of individuals. Number (Percentage %)

Age Range	Total	Positive	Negative
10–20	21 (7.02)	7 (33.33)	14 (66.66)
21–30	152 (80.83)	30 (19.73)	122 (80.26)
31–40	96 (32.1)	19 (19.79)	77 (80.2)
41–50	27 (9.03)	6 (22.22)	21 (77.77)
51–60	3 (1.0)	1 (33.33)	2 (66.66)

group, the total number of donors was only three so here data was weakly conclusive. Moreover, samples in the 21–30 years, 31–40 years, and 41–50 years age groups showed similar results, with approximately 20% positive and about 80% negative. These groups were highly populated in the sample and thus can be considered representative of the complete data, as shown in Table 3.

Location-based antibody distribution

The study was conducted in Maharashtra, India, during the second COVID-19 wave. Figure 2 shows the distribution of antibodies in different regions of Pune and neighboring cities. Conferring to the data, the highest number of samples was 21% of the total samples collected from the Pimpri area, indicating the highest number of antibody-containing samples present; 27.41% of positive cases were found in this region. The next regions were Chinchwad, Mulshi, Hinjewadi, and Alandi. These regions consist of about 5% to 8% of the total samples considered, while they showed approximately 22% of positive results and 78% of negative results. Other regions contributed non-significantly to the sample collection, which ranged from one to seven donors.

ABO blood group-antibody distribution

The majority of healthy volunteers in this study had A, B, and O blood groups, as shown in Figure 3, where the B blood group comprised 35.55% of the population but only had 20.56% positive antibody cases. Seventy-four patients had blood group O, accounting for approximately 25% of the population but had 27% positive antibody patients, the highest among all. The next blood group type, A, consisted of 90 individuals or approximately 30% of the total population; however, only 17.77% of them contained antibodies. The last blood type is AB, which only 30 individuals had, that is approximately 10% of all donors, but 16.66% of them had positive antibodies.

Further, the impact of age and blood group on the antibody level (as a continuous variable) in all seropositive cases was investigated. The mean antibody value among positive cases was 4.15 units/ml [95% confidence interval (CI)], ranging from 2.83 units/ml in participants aged 41–45 years to 4.47 units/mL in participants aged 18–25 years [Table 2]. The *P*- value was calculated to establish the statistical relation and was calculated as 0.84 (>0.05 significance level) indicating failure to reject the null hypothesis, which further suggested no significant differences between the level of antibody across all age groups. Once we determined that there is no dependency of the antibody level on the age group, the next similar calculation was performed on the blood group and antibody level. The *P*- value for blood group and antibody was 0.56, which was thus >0.05. This again indicated that there is no significant relationship between antibody level and the blood group.

Discussions

To design and implement appropriate COVID-19 control measures, we need to comprehend the seroprevalence data of the SARS-CoV-2 antibody in the asymptomatic population. This study collected the seroprevalence data of healthy blood donors from February to May 2021 for 299 individuals. The

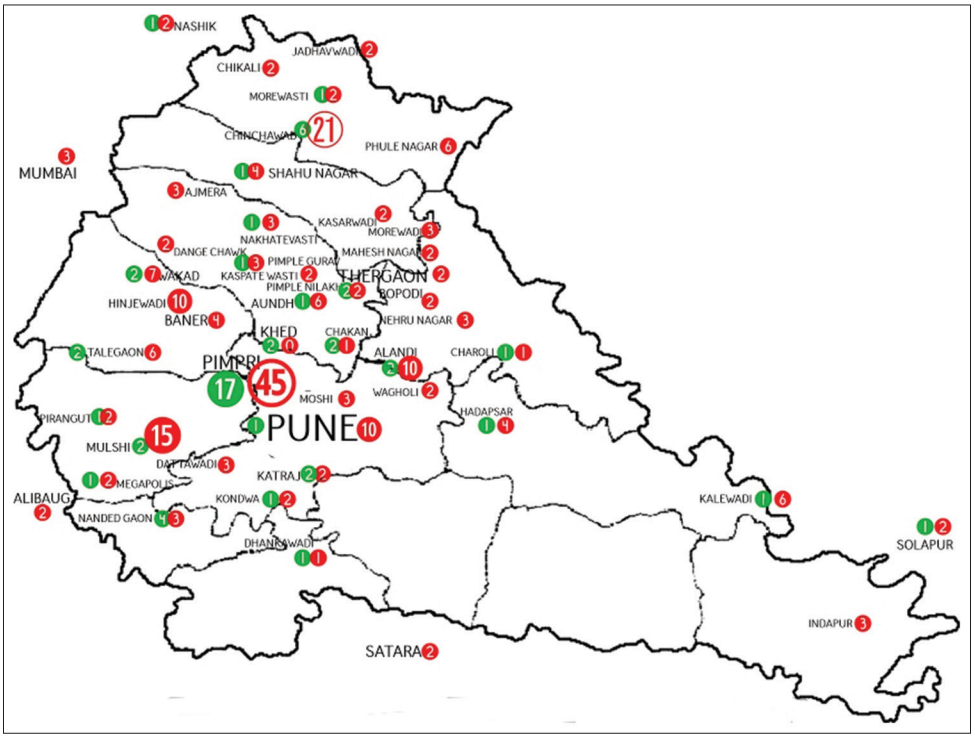
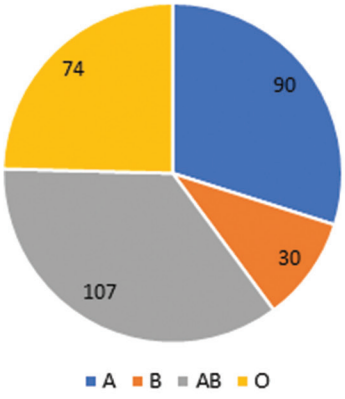


Figure 2: Pictorial representation of location wise distribution of presence and absence of antibody on Pune city map. **Green circle = Positive, Red circle = Negative



	Total	Positive	Negative
A	90	16	74
AB	30	5	25
B	107	22	85
O	74	20	54

Figure 3: Patient distribution based on blood type

presence/absence of IgG antibodies in the given population would indicate the fraction of the population that developed the SARS-CoV-2 antibody, which would further assist in providing valuable insight regarding the development of herd immunity.[12] Moreover, the study was conducted before the vaccination drive, so the prevalence of antibodies in healthy blood donors would indicate COVID-19 infection in the past. The study was conducted during the second wave of COVID-19 in India when a large population was infected by COVID-19.[13] In this study, we observed 21% of positive seroprevalence cases and 78% of negative seroprevalence cases in a population of 299 individuals. In addition, statistical analysis demonstrated that the seroprevalence of IgG antibodies is not influenced by gender, age, and blood group.

There were various similar studies conducted in different geographical regions of the world to understand the seroprevalence of IgG among healthy individuals. A study indicated that 17.9% of the Diamond Prince cruise population had developed SARS-CoV-2 antibodies when 3063 individuals were screened.[14] This number was 2.8% when a similar study was performed in the county of Santa Clara in California on a healthy population.[15] Moreover, a study conducted in Germany showed 14% seropositivity in April 2020.[16] Another study was conducted in Germany from March to June 2020, where 3186 regular blood donors were selected for screening to estimate the presence of SARS-CoV-2 IgG antibodies. Here, 0.91% of the population showed seropositivity. The study further deduced no statistical correlation with the gender of the individuals.[17] Compared to these other studies, this study's seroprevalence percentage was higher, which could mean that COVID-19 was more likely to have spread during data collection. Earlier, a study collected data from August 18 to September 20, 2020, for 29,082 individuals and it was observed that 6–7% of the population was seropositive.[18]

Recently, a post-vaccination seroprevalence study was performed where the overall prevalence of IgG/IgM antibodies was reported at 62.7%.[19] The scaled seroprevalence data indicated the effect of vaccination.[20] Moreover, in our study, we also examined the relationship of the blood group with seroprevalence. A, B, and O blood groups were studied, where the AB group was relatively lower in number, while other blood groups were equally evident. This study showed that there is no direct relation between the presence of antibodies and the type of blood group. Similarly, we also examined the relationship of age with the seroprevalence. This study showed that there is no statistical relationship between age and seroprevalence.

In a similar context, a study conducted in the initial period of COVID-19 at Sir Ganga Ram Hospital, Delhi, on 2586 patients showed that the A and B blood groups has more susceptibility to COVID-19.[21] Another study on the Saudi Arabian population also showed that there was no evident dependency of the blood group on the antibody level.[22] Another study conducted in South India from September to March 2021 on 1034 blood donors, showed no statistical significance of blood group and age with seroprevalence.[23] These studies aligned with our findings regarding the relationship of age and blood groups with seroprevalence. However, the study conducted in the Netherlands on 7361 donors showed antibodies were often present in the younger group of 18–30 years.[24] The study conducted in Quebec on 7691 donors found a positive relation between age with seroprevalence.[25]

Conclusions

In this study, it has been observed that during the second wave of COVID-19, the seroprevalence was very high (21%) in non-vaccinated individuals. This indicated the Serious impact of COVID-19 in individuals with no or mild symptoms. The seroprevalence ratio observed is relatively higher than the other studies reported earlier. We also concluded that there is no evident statistical relation found between gender and seroprevalence. Moreover, we also investigated the relationship of age and blood group with seroprevalence. However, no statistical correlation was found between these parameters. Later, it was discovered that all blood types were susceptible to the formation of IgG antibodies.

Institutional review board statement

The project was approved by the institutional review board for research.

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Data availability statement

All datasets generated or analyzed during the study are included in the manuscript.

Acknowledgement

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Abbreviation

SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2; CMIA – chemiluminescent microparticle immunoassay; RNA – ribonucleic acid; RT-PCR – reverse transcription polymerase chain reaction; Ig – immunoglobulin; NCP – nucleocapsid protein; SP – spike protein; CI – confidence interval.

Financial support and sponsorship

The institute handled the cost of the study.

Conflicts of interest

There are no conflicts of interest.

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