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# Seroprevalence of IgG Antibodies Against Severe Acute Respiratory Syndrome Coronavirus 2 and Associated Risk Factors in Blood Donors

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## Abstract

**Objective:** To determine the seroprevalence of SARS-CoV-2 immunoglobulin G (IgG) antibody and identify risk factors among blood donors.

**Methodology:** This cross-sectional study was conducted at the Armed Forces Institute of transfusion (AFIT), from July to October, 2020. A total of 900 blood donors were enrolled according to the calculated sample size. Sociodemographic, behavioral and clinical data were collected through a preformed questionnaire. Seroprevalence of SARS-CoV-2 IgG antibody was detected using indirect enzyme linked immunosorbent assay (ELISA) of Vircell COVID 19.

**Results:** Out of a total 900 donors, 180 (20%) were found out to be positive for SARS- CoV-2 antibodies. Of these 180 SARS-CoV-2 IgG positive cases, asymptomatic donors were 140 (77.7%) whereas 60 (33.7%) were symptomatic. Respiratory symptoms were most common and gastrointestinal were least common among seropositive donors respectively. None of the ELISA IgG negative samples were found to be positive for SARS-CoV-2 RNA by RT-PCR.

**Conclusion**: A high seroprevalence of SARS-CoV-2 IgG antibody reflected a great extent of spread virus in blood donor population.

**Keywords:** Blood donors, COVID-19 IgG antibody, ELISA, SARS-CoV-2

## Introduction

Coronavirus disease 2019 (COVID-19) is caused by a novel beta-coronavirus known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which was first detected in Wuhan, China at the end of 2019. SARS-CoV-2 infection is manifested with mild or moderate symptoms (dry cough, fever, body aches, and diarrhea) but in a minority of cases, severe symptoms (respiratory distress, pneumonia and cytokine release syndrome) appear, which can be fatal. SARS-CoV-2, a positive sense-single stranded RNA virus, consists of four major structural proteins: Spike(S), nucleocapsid (N), membrane (M) and envelope (E). Rapid transmission of this virus across the globe lead to a global pandemic in 2020.[1,2,3] In Pakistan, SARS-CoV-2 cases were first reported in February, 2020 in individuals who had returned from Iran.4 A substantial burden on fragmented health care systems led to a limited COVID-19 testing mainly in symptomatic cases, thereby leaving asymptomatic cases, which was a source of spread of infection in the general population. [5]

It is significant to determine the seroprevalence of SARS-COV-2 antibody among blood donors to identify the extent of exposure to the disease and the development of possible herd immunity. Detection of antibodies among apparently healthy blood donors gives important information about the circulation of virus as blood donors represent a part of general population. This data can also be used to assess the effectiveness of potential mitigating strategies.[6] SARS-CoV-2 diagnosis is carried out by real time reverse transcription polymerase chain reaction (RT-PCR) but factors such as inadequate sample collection, fluctuating viral load and time between sample collection and symptoms onset influence its sensitivity by giving false negative results.[7] Validated serological assays can facilitate the prompt identification of cases that have been infected with SARS-CoV-2 thereby assisting in enhancing the strategies for blood safety.[8] A previous study conducted

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on blood donors in southern region of Pakistan from May to July, 2020 reported a 40% seroprevalence rate.

During that period, the number of confirmed cases were 0.2 million with 6000 deaths.[5] To date, Pakistan has reported more than 0.4 million cases with 9330 deaths.[9] Considering a surge in SARS-CoV-2 infected patients, this study was conducted to determine the seroprevalence of SARS-CoV-2 IgG antibody and associated risk factors among blood donors. Furthermore, nucleic acid testing was done to detect possible SARS-CoV-2 infection in blood donors.

## Methodology

A cross-sectional study based on seropositivity for SARS-CoV-2 was carried out on blood donors from July to October, 2020. All potential blood donors reporting to the Armed Forces Institute of Transfusion (AFIT) during the pandemic period were recruited using convenient sampling technique. The study protocol was approved by an Institutional Ethics Committee (AFIT-ERC-20-010) and written informed consent was obtained from all study participants. Sample size calculation was performed using single proportion formula with 95% confidence interval, margin of error 5% and a prevalence of 37.8% reported in a previous study.[5] The study enrolled 900 blood donors who met the blood donor selection criteria according to World Health Organization (WHO) guidelines. In addition, those donors who experienced any COVID-19 related symptoms[10] in the past 28 days of donation and had contact with confirmed or suspected SARS-CoV-2 patient were excluded.[11] Blood donors who have been previously symptomatic but were free of symptoms for more than 28 days were included. In the study a standardized questionnaire was used to collect sociodemographic data; age, gender and educational level, history of SARS-CoV-2 symptoms such as respiratory, gastrointestinal and behavioral factors like history of smoking. Serological detection of SARS-CoV-2 IgG antibody against recombinant antigen (a mixture of nucleocapsid N and spike protein S) was performed by indirect enzyme linked immunosorbent assay (ELISA) (Vircell Microbiologist, Spain) following manufacturer's instructions using serum used for routine transfusion transmissible infection markers. Internal positive, negative and cut off controls were run with each batch. A serum sample from recovered case with positive IgG antibody status was also included as an additional positive control. ELISA IgG negative samples were tested for SARS-CoV-2 ribonucleic acid (RNA) by real time PCR on Roche Cobas 6800 system. The primary outcome was to assess the seroprevalence of IgG antibody among blood donors while secondary outcome was to determine the association between IgG seropositivity and socio-demographics (age and educational level), behavioral characteristic including smoking and symptoms consistent with SARS-CoV-2 infection.

Statistical package for social sciences (SPSS) version 23.0 was used for data analysis. Categorical variables were presented as mean (SD) and continuous variables as frequency and percentages. Pearson Chi-square test was used to find the association between variables. A $P<0.05$ was considered as significant.

## Results

A total of 900 blood donors were recruited during the study period of four months. Males accounted for 99.7% (897/900) of the study population. The mean age of donors was 29.7(SD +7.9) years with the age group 18-29 years being the most prevalent and ≥50 years being the least prevalent. Various baseline characteristics of blood donors are shown in Table-I. Regarding history of symptoms, 24.2% blood donors had experienced SARS-CoV-2 related symptoms while 75.7% did not report any symptom.

An overall seroprevalence of SARS-CoV-2 IgG antibody was detected as 20% (n=180/900). Of these, seropositive donors who did not report any SARS-CoV-2 related symptom showed higher anti-SARS-COV-2 IgG seropositivity as compared to donors with history of COVID-19 related symptoms (14% versus 6%) (Table-II).

**Table I: Baseline Sociodemographic, Clinical and Behavioral Characteristics of Blood donors for SARS-CoV-2 IgG Testing.**

| Variables | Categories | Donors Number (%) |
|---|---|---|
| **Gender** | **Male** | **897(99.7)** |
| | **Female** | **3(0.3)** |
| Age groups (years) | 18-29 | 478 (53.1) |
| | 30-49 | 404 (44.9) |
| | ≥50 | 18 (2.0) |
| Educational Level | No higher education | 690 (76.8) |
| | Higher education | 210 (23.2) |
| COVID-19 related Symptoms | Yes | 223 (24.6) |
| | No | 677 (75.4) |
| Smoking | Yes | 113(12.3) |
| | No | 787(87.7) |

| Table II: Anti-SARS-CoV-2 IgG seroprevalence among blood donors | | |
|---|---|---|
| Donors | Number (%) | SARS-Cov-2 IgG Seroprevalence Number (%) |
| NO COVID related symptoms | 676 (75.4) | 140 (14) |
| COVID related symptoms | 224 (24.6) | 60 (6) |
| Total | 900 (100) | 180 (20) |

The correlation of SARS-COVID-2 IgG seropositivity with sociodemographic and behavioral characteristics of blood donors is shown in Table-III. Younger blood donors aged 18-29 years and lack of education in blood donors was found to be associated with increased seropositivity rate respectively (10.5 and 14.5%). However, age and educational background was not significantly associated with SARS-CoV-2 IgG seropositivity (P=0.4 and P=0.2). Non-smokers showed 19.3% SARS-CoV-2 IgG seroprevalence rate as compared to smokers (P=0.2). Majority of the donors experienced COVID-19 related respiratory symptoms whereas the least common were gastrointestinal. In seropositive cases, the donors with respiratory symptoms were 6.2% and gastrointestinal 2.5% while among seronegative cases, 11.2% experienced respiratory and 4.2% gastrointestinal symptoms. Overall, fever, shortness of breath and anosmia/ageusia showed significant association with SARS-CoV-2 IgG seropositivity.

All the ELISA IgG negative samples tested for SARS-CoV-2 RNA were found out to be negative by PCR.

| Table III: Seroprevalence of SARS-COV-2 IgG antibody with respect to various characteristics of blood donors | | | |
|---|---|---|---|
| **Characteristics** | **Tested N (%)** | **SARS-COV-2 IgG antibody N (%)** | |
| | | **Yes** | **No** |
| **Age Groups** | | | |
| 18-29 | 478 (53.1) | 94 (10.5) | 384 (42.6) |
| 30-49 | 404 (44.9) | 81 (9) | 323 (35.9) |
| ≥50 | 18 (2.0) | 5 (0.5) | 13 (1.5) |
| **Edu. Level** | | | |
| No education | 690 (76.8) | 131(14.5) | 559 (62.1) |
| Educated | 210 (23.2) | 49 (5.4) | 161 (17.8) |
| **Smoking** | | | |
| Yes | 113 (12.3) | 6 (0.6) | |
| No | 787 (87.7) | 107 (11.8) | |
| | | 174 (19.3) | |
| | | 613 (68.1) | |

# Discussion

Sero-epidemiological studies are needed because of the ongoing COVID-19 pandemic, which assists in evaluating the community spread of SARS-COV-2 infection. Natural infection may result in the development of herd immunity, which can only be established if a large proportion of the general population is immune to COVID-19 infection. This curtails the transmission of the virus from an infected person to non-infected or susceptible individuals. Serological surveys may assist in the estimation of extent to which herd immunity prevails.[1] The current study was conducted in the Northern region of Pakistan to illustrate the seroprevalence of SARS-CoV-2 IgG antibody by ELISA and associated risk factors among blood donors in the late phase of pandemic, because the later the period, the higher could be the prevalence.

Our study showed presence of IgG in 20% blood donors which is relatively lower than the 37.8% reported in a previous study from southern city of Pakistan. Despite exclusion of previously symptomatic cases, a high rate was observed because this was the most affected region of Pakistan and small numbers of individuals were enrolled in this study due to which variation in prevalence may be observed.[5] A study from Guangzhou, China reported very low 0.09% prevalence of IgG antibody in only 2 out of 2199 voluntary blood donors. Therefore, they characterized blood donors as low risk population as compared to high-risk group such as health care workers for COVID-19 infection and also considered sampling time an influencing factor for low prevalence of IgG.[12] Similarly, studies from Wuhan and Shenzhen, China reported 2.2% and 0.02% SARS-CoV-2 IgG antibody positivity, respectively.[13] These findings have shown the effect of strict social restrictions and better surveillance systems in China as compared to our country. Another survey-based study from Italy showed 0.9% SARS-CoV-2 IgG seropositivity which is far less than reported in our study. However, study participants were recruited from a region with a moderate incidence of infection which might be responsible for low rate whereas in the highly affected region of Italy, Lombardy documented 23% IgG positivity rate.[14,15] The prevalence rate may vary due to the variations in the epidemiology of study population, stage of epidemic and methodology.

The gold standard for COVID-19 diagnosis RT-PCR is limited and asymptomatic SARS-CoV-2 infection makes blood safety worthy of consideration.

Asymptomatic individuals have very low viral RNA, usually ranges from 2 to 4 log copies per milliliter; it could be detected in whole blood, serum or plasma. Therefore, the theoretical possibility of viral transmission by blood cannot be overlooked.[16] In view of this, ELISA IgG negative samples (n=597) were tested for SARS-CoV-2 RNA by RT-PCR. None of the sample was found to be positive which is consistent with the findings of a study conducted in Italy.[15] A multi-centered study from China also reported no evidence of SARS-CoV-2 RNA among voluntary blood donors which is in agreement with the current findings regarding only absence of SARS-CoV-2 RNA.[17] All ELISA IgG negative samples were tested by using sensitive real time PCR assay of Roche Cobas 6800 system with better limit of detection as compared to other NAT methods and the results suggest that this virus may not appear a direct threat to blood safety.

Regarding substantial risk factors, cigarette smoking increases the risk of acquiring viral infections in a multifactorial way including suppression of the pulmonary immune function and alteration of muco-ciliary defense mechanisms. In our study, IgG seropositivity was found to be higher in healthy non-smokers as compared to smokers. This may be consistent with the notion that smoking is a risk factor for progression of the COVID-19 disease in individuals with underlying comorbidities.[18] The youngest blood donors aged from 18-29 years had shown higher IgG seropositivity rate. This was expected as youngest blood donors constitute the core workforce, are willing to be engaged in strenuous physical activities and thus more likely to be exposed to infection despite adhering to social distancing restrictions. Better knowledge and awareness on the preventive measures associated with SARS-CoV-2 infection may curb exposure to infection. High seropositivity was observed among donors lacking higher education, and this was predictable.

Our study has several limitations. Firstly, enrolled blood donors were majority males so SARS-CoV-2 IgG prevalence is not widely applicable to general population. Secondly, NAT-testing for SARS-CoV-2 active infection was not performed on nasal swabs which might have high viral load as compared to serum samples tested so far. Thirdly, we could not identify the neutralization activity and half-life of detected antibodies thus effectiveness of herd immunity cannot be established.

## Conclusion

In conclusion, 20% of the blood donor population was found to be positive for SARS-CoV-2 IgG antibody which is a reflection of widespread asymptomatic virus circulation in our population.

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