Am J Blood Res 2022;12(3):88-96
www.AJBlood.us /ISSN:2160-1992/AJBR0142423

*Original Article*

# Seroprevalence of Sars-Cov-2 antibodies among eligible blood donors of Peshawar, Pakistan

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Received February 18, 2022; Accepted June 16, 2022; Epub June 20, 2022; Published June 30, 2022

**Abstract:** Background and objectives: To determine the seroprevalence of SARS-CoV-2 antibodies and the associated risk factors among healthy blood donors from Peshawar Pakistan, during the second and third waves of the COVID-19 pandemic. Methods: The study was conducted on 4047 healthy (with no history or symptoms of COVID-19) blood donors attending regional blood center Peshawar between Nov 2020 and June 2021. Demographic data was collected and donors were screened for the presence of anti-SARS-CoV-2 antibodies using electrochemiluminescence immunoassay (ECLIA). Results: The mean age of the participants was 27.27±7.13 and the majority (99%) were males. Overall, 59% (2391/4047) of the blood donors were reactive for SARS-CoV-2 antibodies. An increasing trend in seropositivity was observed from 45.5% to 64.8% corresponding to the second and third wave of the pandemic in Pakistan. Logistic regression analysis revealed significantly higher odds of seropositivity among male donors compared to females. Similarly, in multivariable analysis, the odds ratio for seropositivity among blood types AB, A, and B were, 1.6, 1.4, and 1.3 (CI 95%) times higher compared to blood group O (*P*-value ≤0.0001). Conclusions: Seropositivity of SARS-CoV-2 antibodies among blood donors gradually increased during the second and third wave of the pandemic in Pakistan indicating a widespread prevalence of Covid-19 in the general population. Susceptibility to SARS-CoV-2 varies with ABO blood types, with blood group O associated with low risk of infection.

**Keywords:** Seropositivity, blood groups, Covid-19, general population

## Introduction

After almost two years into the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) pandemic [1], many questions yet remain unanswered related to the spread of the novel virus in different communities. As of September 2021, over 250 million confirmed cases, and around 5 million deaths due to COVID-19 have been reported across the globe [2].

Although epidemiological data points, based on laboratory-confirmed cases, provide important insight regarding epidemic dynamics, they do not accurately portray the extent of asymptomatic spread within a community. Evidence suggests that SARS-CoV-2 infections may have been underestimated, since a large proportion of infected individuals remain asymptomatic [3, 4], and may not have been tested. This is especially true in lower-middle-income countries (LMIC), such as Pakistan, where testing strategies rely heavily on symptomatic testing by real-time reverse transcription-polymerase chain reaction (RT-PCR), and contact tracing. Meanwhile, asymptomatic infection is rarely targeted, due to limitations in testing capacity, availability, accessibility to standard tests, and required diagnostic facilities [5, 6]. Population-based seroepidemiological data has more potential to accurately enumerate the magnitude of previously unknown, and asymptomatic infection at the community level [7]. Such studies may serve as powerful predictors of infection in non-vaccinated individuals, thus guiding future public health policies and pandemic control strategies [8, 9].

Data from ongoing seroprevalence surveys reflect the dynamics of the COVID-19 pandemic of SARS-CoV-2 spread. A landmark systematic review and meta-analysis by Bobrovitz et al, including 9.3 million participants from 74 countries, reported low seroprevalence (median 4.5%, IQR 2.4-8.4%) of SARS-CoV-2 antibodies in the general population [10]. However, there is notable heterogeneity in findings of available seroprevalence studies, with levels varying widely among different regions, ethnicities, and age groups [11-13]. After the first pandemic wave, studies from Pakistan showed a high seroprevalence of SARS-CoV-2, ranging between 33%-42% in various populations [14, 15], suggesting that the actual prevalence of infection has been underestimated, and is several folds higher than the number of RT-PCR-confirmed cases.

Although there is no concrete evidence that coronavirus disease 2019 (COVID-19) can be transmitted through blood transfusion, donor safety has been a general concern since the pandemic began [16]. Instead of routine screening of blood donors for SARS-CoV-2 by RT-PCR or immunoassay, WHO recommends that blood donors be deferred for four weeks if they experience any symptoms indicative of COVID-19, had contact with a known case, or travel history to an epidemic area [17]. Thus blood donors make ideal study subjects for serosurveys involving serial testing to monitor unknown viral circulation. This approach has been endorsed by the World Health Organization (WHO) and is currently implemented across the world to inform health policies, especially in LMIC and where serial testing and sampling are not feasible.

This current study was designed to determine the seroprevalence of SARS-CoV-2 antibodies among blood donors in Khyber Pakhtunkhwa (KP) province of Pakistan during the second and third epidemic waves and to establish potential risk factors for seropositivity. Amidst global vaccination drives, such studies can aid in assessing the immunological status of various populations, optimising vaccination strategies, and priortisation of vaccine recipients.

## Methodology

### *Study design and sampling*

This serial, cross-sectional study was conducted at the Regional Blood Centre (RBC) of Peshawar, the capital city of Khyber Pakhtunkhwa province of Pakistan from November 2020 to June 2021. RBC is a centralized blood bank, which collects blood from donors across KP, and is connected to the blood banks of all major hospitals within Peshawar. This study was approved by the Ethics Research Board of Khyber Medical University (Ref number: Dir/KMU-EB/SC/000838), while administrative approval was obtained from RBC. Potential donors were provided with information, translated into the national language (Urdu), regarding the background, and objectives of our study. Written informed consent was provided by all eligible participants before data and sample collection. Prior to blood collection, all the donors were assessed through a self-administered questionnaire, clinical assessment, and mini-health screening (triage) by the staff to confirm that the donors meet selection criteria following National Guidelines for Quality Control in Transfusion Medicine [18]. The process aims to ensure that the donors are healthy and free of transmissible infectious diseases. Donors who were found eligible according to the WHO and National Guidelines "Blood Donor Selection Criteria", were included in the study and those who were not eligible were excluded from the study [18]. Additionally, the donor was also deferred if they had experienced any COVID-like symptoms (cough, fever, flu, etc.) recently or have contact with a known case as per WHO guidelines [17]. Blood samples were collected by vein puncture method (antecubital vein) by a trained phlebotomist following standard procedure. The donors who consented to participate in the study also provide an additional 3 mL of blood in collection tubes containing EDTA. All the blood samples were then screened for routine Transfusion Transmissible Infections (TTI's). Screening for Hepatitis B, Hepatitis C, HIV, and Syphilis was done by Chemiluminescent Microparticle Immuno Assay (CMIA) assay Technique (Abbott ARCHITECT i2000, Abbott Park, Illinois, U.S.A.) while for malaria, screening by ICT.

*Study period*

To investigate a potential increasing trend, the sample collection period was broken down into four intervals: November 2020-December 2020, January 2021-February 2021, coinciding with the second wave, and March 2021-April 2021, and May 2021-June 2021, coinciding with the third wave in Pakistan's epidemic timeline. Following global pandemic trends, Pakistan experienced its second epidemic wave from November 2020 to January 2021 at a peak of which around 3,000 cases were reported daily for two weeks. This was followed by a period of sustained decline in cases until the third wave from March 2021 to May 2021, at a peak of which, around 5,000 cases were reported daily for a period of two weeks.

*SARS-CoV-2 antibody testing*

Plasma samples from TTI's seronegative blood donors were tested for Anti-SARS-COV-2 antibodies using Electro Chemiluminescence immunoassay (ELCIA) on Cobas e-411 Immunoassay analyzer (Roche Diagnostics, Rotkreuz, Switzerland) following manufacturer instructions. The assay measures total antibodies against the viral antigen (N-protein). A cut-off value of >1.0 was used to identify positive samples as per manufacturer recommendations.

*Statistical analysis*

Data were presented as mean ± standard deviation for numerical data, and frequencies and percentages [N (%)] for categorical variables. The outcome variable was SARS-CoV-2 seropositivity. Chi-square analysis and t-tests were applied to compare demographic characteristics between seropositive and seronegative donors, followed by logistics regression analysis to establish correlates of seroprevalence. A *P*-value of less than 0.05 was considered statistically significant. Data were recorded and analyzed using SPSS® version 24 (IBM carp, USA).

## Results

*Characteristics of eligible blood donors*

In total, 4,047 participants were enrolled between November 2020 and June 2021. The demographic characteristics of participating donors are shown in **Table 1**. Overall, males constituted 99.0% (4000/4047) of the study participants, meanwhile, 64.6% (2618/4047) of donors belonged to Peshawar city. The mean age of the total participants was 27.27±7.13 years. Our results shows a higher trend of blood donation in the younger population, with 82.5% (n=3339) donors ranging from 18 to 34 years old, while only 0.2% (n=7) donors were ≥55 years of age.

Among the male participants, 59.5% (2378/4000) were seropositive, while 27.7% (13/47) female donors were seropositive for SARS-CoV-2 antibodies. The mean age of seropositive blood donors was 27.3±7.2, whereas the mean age of seronegative donors was 27.1±6.9. Seroprevalence levels varied with age, ranging between 42.9% (3/7) in participants ≥55 years, to 64.9% (63/97) in participants 45-54 years old. Among various regions of KP, the highest seroprevalence levels were recorded in DI Khan (64.4%, n=116), Malakand (63%, n=243), Mardan (59.3%, n=112), followed by Peshawar (59.2%, n=1551), while lowest levels were found in Bannu (52.9%, n=185). The highest seroprevalence levels were among donors with blood group types AB, of 64.9% (276/425), and A, of 62.2% (681/1094) while the lowest seroprevalence levels, of 53.2% (649/1219) were among donors with blood group O (**Table 1**). Observed differences between mean age, or seroprevalence levels in different age categories, of seropositive and seronegative donors, were not statistically significant. Similarly, there was no statistical difference between seroprevalence levels in various regions of KP. In contrast, significantly higher seropositivity was observed in male blood donors compared to females, and among exchange blood donors, as compared to voluntary donors. Statistically significant differences were also detected in ABO blood types between seropositive, and seronegative blood donors, *p*-value: 0.00 (**Table 1**).

However, multivariate regression analysis revealed that male blood donors had significantly higher odds (*P*-value <0.001, OR 3.4; 1.8-6.6) of being seropositive, compared to female donors (**Table 2**). Bannu division had significantly lower odds of seropositivity, compared to the city of Peshawar (*P*-value 0.022, OR 0.8; 0.6-0.9). Compared to blood group O, groups A, B or

**Table 1.** Seroprevalence of anti-SARS-CoV-2 antibodies and associated risk factors among blood donors from Khyber Pakhtunkhwa, Pakistan

| Characteristics | | Antibody status (N) | | Total (N) | Seroprevalence (%) | *P*-value |
|---|---|---|---|---|---|---|
| | | Sero-negative | Sero-positive | | | |
| Gender | Female | 34 | 13 | 47 | 27.70 | 0.00 |
| | Male | 1622 | 2378 | 4000 | 59.50 | |
| Age (Years) | Mean ± SD | 27.1±6.9 | 27.3±7.2 | | | 0.20 |
| Age categories | 18-24 | 749 | 1047 | 1796 | 58.30 | 0.60 |
| | 25-34 | 625 | 918 | 1543 | 59.50 | |
| | 35-44 | 244 | 360 | 604 | 59.60 | |
| | 45-54 | 34 | 63 | 97 | 64.90 | |
| | ≥55 | 4 | 3 | 7 | 42.90 | |
| Region | Hazara | 36 | 45 | 81 | 55.60 | 0.09 |
| | Bannu | 165 | 185 | 350 | 52.90 | |
| | Peshawar | 1067 | 1551 | 2618 | 59.20 | |
| | Malakand | 143 | 243 | 386 | 63.00 | |
| | Kohat | 104 | 139 | 243 | 57.20 | |
| | DI Khan | 64 | 116 | 180 | 64.40 | |
| | Mardan | 77 | 112 | 189 | 59.30 | |
| Type of Donor | Exchange | 1023 | 1404 | 2427 | 57.80 | 0.03 |
| | Voluntary | 633 | 987 | 1620 | 60.90 | |
| ABO Blood Group | A | 413 | 681 | 1094 | 62.20 | <0.001 |
| | AB | 149 | 276 | 425 | 64.90 | |
| | B | 524 | 785 | 1309 | 60.00 | |
| | O | 570 | 649 | 1219 | 53.20 | |

AB were significantly associated with seropositivity, *P*-values ≤0.001 (**Table 2**).

*Seroprevalence trends during the outbreak*

Overall, SARS-CoV-2 antibodies were detected in 59.0% (2386/4047) of our participating blood donors. As seen in **Figure 1**, an increasing trend in seroprevalence levels was observed during the sample collection period. Amidst the active second wave of the epidemic, seropositivity increased from 45.5% (174/382) in November 2020-December 2020 to 56% (985/1765) in January 2021-February 2021. This further jumped higher and 65% (1227/1900) seropositivity was detected in donation samples collected between March-June 2021, during the third epidemic wave.

## Discussion

This cross-sectional analysis presents the trend of seroprevalence in blood donors, without prior history of COVID-19, during the second and third SARS-CoV-2 epidemic waves in Khyber Pakhtunkhwa province, Pakistan. Overall, the seroprevalence rate was 59% among 4,047 eligible donors following three epidemic waves in KP. During the study period, a progressive increase in seroprevalence, from 45.5% to 64.8%, was observed as expected [19-21] concomitant with the COVID-19 incident rate in Pakistan at the time. In November 2020, at the start of the second epidemic wave in KP, 45.5% of blood donors were seropositive for SARS-CoV-2 antibodies, which jumped to 55.8% in February 2021, following the second wave, and up to 64.8% seropositivity between April-May 2021, during the third wave. Similar trends in seroprevalence have also been reported in different geographic regions across the world including Pakistan. According to a meta-analysis, SARS-COV-2 seroprevalence among the general populations was estimated to be 8% by end of 2020 [8]. However, higher levels were reported in serological studies in blood donors from around the globe [19, 20, 22]. Ongoing global sero-surveillance has revealed large heterogeneity in prevalence, which varies vastly across coun-

**Table 2.** Results of logistic regression analysis

| Parameter | Univariable analysis | | Multivariable analysis | |
|---|---|---|---|---|
| | OR (95% CI) | *P*-value | OR (95% CI) | *P*-value |
| Gender | | | | |
| Female | REF | | REF | |
| Male | 3.8 (2-7.3) | <0.001* | 3.4 (1.8-6.6) | <0.001* |
| Age (years) | 1.1 (0.9-1.1) | 0.242 | 1.0 (0.9-1.0) | 0.361 |
| Region | | | | |
| Peshawar | REF | | REF | |
| Hazara | 0.9 (0.6-1.3) | 0.506 | 0.9 (0.6-1.4) | 0.572 |
| Bannu | 0.7 (0.6-0.9) | 0.023** | 0.8 (0.6-0.9) | 0.023** |
| Mardan | 1.0 (0.7-1.3) | 0.997 | 0.9 (0.7-1.3) | 0.978 |
| Malakand | 1.2 (0.9-1.4) | 0.166 | 1.2 (0.9-1.4) | 0.191 |
| Kohat | 0.9 (0.7-1.2) | 0.536 | 0.9 (0.7-1.2) | 0.586 |
| DI Khan | 1.2 (0.9-1.7) | 0.170 | 1.2 (0.9-1.7) | 0.199 |
| Blood group type | | | | |
| O | REF | | REF | |
| A | 1.4 (1.2-1.7) | <0.001*** | 1.4 (1.2-1.7) | <0.001*** |
| AB | 1.6 (1.3-2.0) | <0.001*** | 1.5 (1.2-2.0) | <0.001*** |
| B | 1.3 (1.1-1.5) | 0.001*** | 1.3 (1.1-1.5) | 0.001*** |
| Donor type | | | | |
| Voluntary | REF | | REF | |
| Exchange | 0.9 (0.9-1.1) | 0.051 | 0.9 (0.8-1.0) | 0.059 |

*Compared to females, the male sex is significantly associated with antibodies seropositivity. **Donors from the Bannu region were significantly less seropositive compared to the Peshawar region. ***Compared to blood group O, blood groups A, B or AB were significantly associated with antibodies seroprevalence.

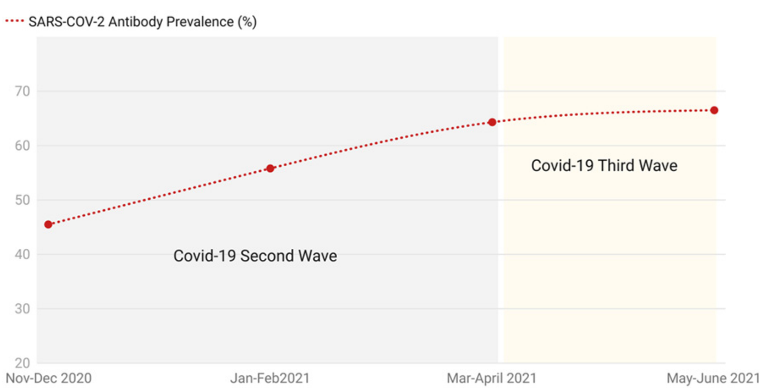


**Figure 1.** Dynamics of seroprevalence during the second and third waves of Covid-19 in Khyber Pakhtunkhwa, Pakistan. Percent seropositivity among eligible blood donors is plotted against various time points of sample collection. Each red circle represents the consolidated percentage of a time period. Connecting red lines show an increasing seroprevalence trend. Grey and pale yellow background reflect the second and third Covid-19 wave in Pakistan.

tries [13] and between different regions [23]. In this study, an overall seroprevalence of 59%, and seropositivity rate of 64.8% from April 2021 to May 2021, are on the higher spectrum of results recorded in serological studies conducted in blood donors globally. These findings are comparable only to a few other published data revealing exceptionally high rates of seropositivity, including a study from Malawi, which found a seroprevalence of 50% [24] and another study from South Africa showing 63% seroprevalence after three global waves in the pandemic [25].

In KP, which has a population of 35.53 million people, a cumulative of 132,627 COVID-19 cases were reported out of 1,459,291 tests conducted between March 2020 to May 2021. Our results, however, suggest that SARS-CoV-2 was in wider circulation in KP and the number of unknown cases may have been several folds higher than the number of officially confirmed cases. Although the study period mirrored the emergence of two waves in Pakistan's epidemic timeline, the resulting rates established are alarmingly high indicating that the actual number of infections has been underestimated based on laboratory-confirmed case counts. There could be a few potential explanations for the glaring gap between the number of people being tested in KP and the high prevalence observed in this study. It could be speculated that amongst its younger population demographics, the larger proportion of infection in Pakistan may be asymptomatic causing it to have remained undiagnosed. Testing strategy in Pakistan, similar to in many LMIC, does not actively opt to look for asymptomatic infections. Thus, the extent and prevalence of asymptomatic SARS-CoV-2 have widely remained undetected. Differences in health policies and level of adherence to preventative health measures may also have led to the high seropositivity in our cohort. In Pakistan, several non-pharmaceutical interventions were relied upon to mitigate viral spread. The government implemented a series of partial and smart lockdowns during each active wave, meanwhile, large social gatherings were prohibited, educational institutions were switched to online learning, and restrictions on economic and social activities were tightened. However, limited restrictions were placed on the individual mobility of citizens during these periods. Adherence to health control measures generally remained poor in most cities which may have contributed to wider viral circulation. Additionally, it can be speculated that the second and third waves were fuelled by the spread of beta and delta variants of SARS-CoV-2 [26, 27], although this did not significantly alter the course of Pakistan's epidemic trajectory as the number of severe cases, hospitalizations, and fatalities remained stable.

Early in the pandemic, it was predicted that herd immunity against SARS-CoV-2 will be reached when 60%-80% of a population has developed antibody defense against the novel virus. However, among the scientific community, there is still no consensus about the strength and duration of immunity acquired through natural infection or vaccination with SARS-CoV-2. According to recent research, SARS-CoV-2 antibody titers begin to wane to undetectable levels, 3 to 5 months after infection [28]. Cases of reinfection with SARS-CoV-2 have been confirmed around the world, although the reported risk of recurring infection is low in Pakistan [29]. In Manaus, Brazil, an abrupt resurgence in hospitalizations related to COVID-19 was seen in January 2021; three months after an analysis of blood donors estimated that 76% of the population had already been infected [30]. This adds to a growing body of evidence suggesting that prior infection does not confer long-lasting immunological memory to SARS-CoV-2, making herd immunity through natural infection an unlikely exit strategy to the pandemic [31]. Nevertheless, based on our results, it could be stated that KP was gradually progressing towards reaching the theoretical herd immunity threshold prior to the vaccine becoming available and despite a low disease burden, compared to many countries. Amidst vaccination rollouts, sero-epidemiological surveys will be critical in assessing the cumulative immunological status of various populations and can provide rapid estimates to assist in prioritizing vaccine recipients especially if number of officially confirmed cases does not accurately reflect the true scale of infection in a community [32].

Participating donors in this study belonged to various regions from KP, but the majority were from Peshawar city (45.71%). By using Chi-square analysis, no disparity was observed between seroprevalence in various regions. This suggests the epidemic is geographically spread out across KP, providing evidence against the speculation that SARS-CoV-2 circulation is low in rural and remote settings, compared to densely populated urban centers. These observations were further confirmed when multivariate regression analysis revealed that compared to Peshawar, the Bannu division had significantly lower odds of seropositivity [OR 0.7 (95% CI 0.6-09) *P*-value 0.022]. Higher attack rates were expected in Peshawar, since it has been shown that residents in urban centers have greater risk of exposure to SARS-CoV-2 [33]. Males constituted 98.8% of the total participants, which mirrors the usual pattern of blood donations in KP. Statistical analysis revealed that compared to females donors, males were at significantly higher risk of infection (*P*-value <0.001), however it was difficult to draw further conclusions since such a large proportion of our study participants were males.

Our study reports the highest rates of seropositivity among blood donors having blood types AB (65%) and A (62%) while donors with blood group O (53%) recorded the lowest seropositivity (*P*-value <0.001). In multivariable regression analysis, the odds ratio for seropositivity among blood types AB, A, and B were, 1.6, 1.4, and 1.3, and all were significant (CI 95%, *P*-value ≤0.0001). These findings suggest that susceptibility to SARS-CoV-2 varies with ABO polymorphism, with blood group O associated with low risk, while individuals with groups AB and A are at higher risk, in concordance with the published evidence [34]. In individuals with group O, this could be attributable to anti-A antibodies which are absent in those with blood groups A and AB that could be responsible for binding corresponding antigens on the viral envelope and inhibiting attachment between virus and ACE-2 receptors on host cells [35]. This is further supported by the lower odds ratio in the regression analysis for blood group B, relative to groups A and AB. We found no statistical difference in seropositivity among different age groups despite evidence suggesting a high prevalence of COVID-19 in adults age 50 and above [23, 36]. This could be due to the fact that majority of the blood donors in our study were young (mean age =27.27 years).

There are limitations to this analysis. Donor populations constitute of healthy adults, having distinct demographics, that are not representative of general populations, as seen by predominant number of males (98.8%, n= 4000), and participants aged between 18 to 34 years (82.5%, n=3339). Similarly, only 0.2% of participating donors were aged ≥55, making it difficult to draw conclusions. Since age limit for blood donation in Pakistan is 18 to 65 years, we were unable to estimate seroprevalence in children, or the elderly. In addition, only 0.2% (n=7) of participating donors were aged ≥55, making it difficult to draw conclusions. Lastly, we were unable to assess relationship between behavioral aspects and risk of being infected with SARS-CoV-2. In the future, we aim to investigate risk of reinfection with SARS-CoV-2, and how this varies between gender, different age groups, and blood type of individuals.

### Acknowledgements

The research was conducted with Khyber Medical University student research support grant (approval letter no. KMU/ASRB/PG/IBMS/00234).

### Disclosure of conflict of interest

None.

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