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# Estimate of anti-SARS-CoV-2 spike IgG antibodies prevalence among Iranian population based on blood donations: A serial cross-sectional study during the third wave of the pandemic

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## ARTICLE INFO

## ABSTRACT

*Objectives:* Iran is one of the countries that have been confronted with the SARS-CoV-2 epidemic since February 2020. This study aimed to determine the levels of specific IgG antibodies against SARS-CoV-2 among healthy blood donors to estimate the burden of the epidemic.

*Material and methods:* A serial cross-sectional study was conducted on blood donors who referred to 31 main blood donation centers in different provinces during the third weeks of September, October, and November 2020. A questionnaire was filled out to collect socio-demographic characteristics, history of contact with COVID-19 patients, and history of COVID-19. A blood sample was collected from each participant to assess the antibodies against SARS-CoV-2 using the ELISA method. The crude prevalence of anti-SARS-CoV-2 IgG was calculated. Then it was weighted based on the gender and age groups of the general population in each province and adjusted for test sensitivity and specificity.

*Results:* During three time points of the study, 3840, 3697, and 3152 participants enrolled. The seroprevalence of SARS-CoV-2 IgG antibodies was 19.59% (17.18–22.00), 22.67% (20.70–24.65), and 32.63% (29.93–35.33) over the three rounds of the study.

We found an association between the seropositivity and the highest educational level; AOR 0.76 (0.63–0.93), history of close contact with COVID-19 patients; AOR 1.69 (1.35–2.11), and history of confirmed SARS-CoV-2 infection; AOR 8.86 (5.38–14.60).

*Conclusion:* This study showed that about one-third of the population had been infected with COVID-19. Furthermore, a significant upward trend in seroprevalence was observed. The predisposing factors indicate the importance of public health.

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## 1. Introduction

Coronavirus disease 2019 (COVID-19) was reported in China in 2019 and spread around the world in a short time. World Health Organization (WHO) declared the coronavirus pandemic in March 2020. The emergence of the COVID-19 virus is one of the most challenging international health threats. The virus is highly transmissible [1]. As of 23 May 2021, 167,011,807 cases have been diagnosed with the infection, and 3,472,068 died globally [2].

Iran is one country that has been massively affected by the SARS-CoV-2 infection. The first confirmed COVID-19 case was reported in February 2020 in the city of Qom, and as of 25 May 2021, 2,843,523 people were infected, with 78,848 deaths [2]. The officially reported cases by the Ministry of Health of Iran are based on confirmed cases of molecular assays. Since many infected people experience mild-to-moderate disease without further medical investigation [3], the official reports might not have represented comprehensive information about the disease situation in the country.

*F. Ranjbar Kermani, A. Arabkhazaeli, P. Eshghi et al.*

Detection of specific IgG antibodies against SARS-CoV-2 S antigen helps estimate the number of people exposed to this virus in the past and assesses the burden of infection [4,5].

The World Health Organization (WHO) approved blood donors as a proper tool for seroepidemiological investigation of COVID-19 infection in the general population [6]. Seroprevalence of viral infection constructed by sampling from the people in the exact geographical locations at different and consecutive time points may help to draw a clearer picture of the epidemic.

A few studies have investigated the seroprevalence among blood donors in different countries and reported different seroprevalence rates of SARS-CoV-2 infection [7–9].

Iranian Blood Transfusion Organization (IBTO) is a national organization that conducts blood transfusion centers in all 31 provinces. The organization has access to donated blood. This study aimed to estimate SARS-CoV-2 IgG prevalence and antibody trend among the general population based on the results of testing SARS-CoV-2 IgG antibodies on blood donors in Iran before the start of vaccination programs during the third weeks in September 2020 through November 2020. This study provides essential baseline information on the epidemic progression of COVID-19 in the Iranian population. It can update the understanding and knowledge of the exposure and immune response to SARS-CoV-2 infection.

## 2. Methods

### 2.1. Study design and participants

This population-based serial cross-sectional study was conducted on voluntary blood donors. Of note, participants referred to all 31 main provincial blood donation centers and some satellite blood donation sites from the third week in September 2020 through November 2020.

According to Standard Operation Procedures (SOPs) of IBTO, all participants adhered to the particular criteria because of the COVID-19 outbreak, including a 28-day deferral after complete resolution of symptoms for blood donors diagnosed with COVID-19 or suspected respiratory infections and a 28-day deferral for donors who have had close contact with COVID-19 patients [10], have been added for blood donor eligibility. Eligible donors deemed suitable for whole blood donation could voluntarily enroll in the study.

### 2.2. Population size, sampling method

Considering a 14.5% prevalence of anti-SARS-CoV-2 IgG [11], a 5% alpha level (95% confidence level), and a precision of 1%, a sample size of 4761 was calculated for each time point.

A Quota sampling method was used for selecting study participants. The number of female donors was significantly less than male donors among Iranian blood donors, with the age group of 30 to 40 having the highest proportion [12]. Quotas were equally defined based on provinces with 31 levels, gender, and age group with three levels of 18–30, 31–45, and over 45 years. Therefore, convenient sampling was used to select equal samples in six quotas in 31 provinces at each time point.

### 2.3. Data and sample collection

Eligible donors who were accepted for whole blood donation were informed about this research, and blood donors who agreed to donate and answer the questionnaire were included in the study. A consent form was signed by donors who agreed to participate in the study.

Trained physicians filled out a questionnaire to gather the donors' information. The first part of the questionnaire covered the participants' socio-demographic characteristics, including gender, age, educational level, family size, blood group, and history of blood donation (first-time/ repeated/ regular).

According to the IBTO definition, a first-time donor is a donor who donates blood for the first time in his life; a repeated donor is one who donated blood in the past but not in the preceding 12 months, and a regular donor is a donor who has donated twice or more within the past 12 months [13].

In the second part of the questionnaire, the participants were asked two questions about the history of contact with COVID-19 patients or being tested for COVID-19. Individuals with positive results in diagnostics tests of RT-PCR and/or chest CT scans of the lungs were allocated to the positive group. Individuals referred by a physician for RT-PCR testing and/or chest CT scan but had negative results were assigned to the negative group. Participants who were not referred by a physician for RT-PCR testing or chest CT scan were in the no history group.

At the time of blood donation, an extra blood sample was collected for the study. The blood samples were centrifuged at 3000 rpm for 10 min by 2 h after collection, and their plasma was stored in the freezer at −20 °C or lower. The collected plasma samples were transported to the Blood Transfusion Research Center in Tehran regarding the cold chain condition.

### 2.4. Serological assay

The SARS-CoV-2 specific IgG testing was performed using EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG), Germany, according to the manufacturer's instructions. The kit is intended to identify individuals with an immune response compatible with SARS-CoV-2, indicating recent or previous infection.

The reagent wells of the ELISA were coated with an S1 domain of the spike protein of SARS-CoV-2 expressed recombinantly in the human cell line HEK 293. According to the instructions of the kit manufacturer, the interpretations of test results are as follows: the ratio of (patient sample)/Calibrator > 0.8: negative, the percentage of (patient representative)/Calibrator $\leq 0.8 > 1.1$: borderline, and the ratio of (patient sample)/Calibrator $\leq 1.1$: Positive.

According to the manufacturer test performance characteristics, the sensitivity and specificity of the EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG) test were 94.4% and 99.6%, respectively. In this study, to adjust seropositive results according to test performance in our laboratory facilities, the sensitivity and specificity of the EUROIMMUN test were re-calculated by the Quality Control Laboratory (QCL) of IBTO. To determine sensitivity, 300 samples were collected from SARS-CoV-2 positive RT-PCR patients (all of them were positive with an Iranian EIA Test kit) (Anti-SARS-CoV-2 ELISA (IgG), Diazist Company). To determine specificity, 700 negative samples collected before the COVID-19 outbreak in 2018 were provided from the repository QCL of IBTO. The sensitivity and specificity of the test were calculated as 98.97% and 99.42%, respectively. None of the samples were reactive in infection screening tests, including HBV, HIV, syphilis, or HTLV 1,2.

### 2.5. Statistical analysis

Descriptive statistics were reported as frequency and percentage for qualitative variables and Mean ± SD (Median (IQR) for the quantitative variable.

Three steps estimated the prevalence of COVID-19 in the population. By using these three steps, we aim to estimate the prevalence of COVID-19 more precisely.

In the first step, the proportion of positive test results was reported as crude seroprevalence. To balance blood donors' gender

and age groups with those of the general population and extrapolate seroprevalence estimation from the blood donation samples to the general population, the crude seroprevalence was weighted based on the gender and age groups of the general population in each province. The Horvitz-Thompson method, by considering sampling weights, was used. Sampling weights were calculated based on the distribution of gender, age group (18–30, 31–45, over 45 years), and province population over the country (according to the results of the latest national census in Iran (Extracted from https://www.amar.org.ir/, 2016).

Finally, we adjusted the results of weighted prevalence by test performance. Sensitivity (98.97%) and specificity (99.42%) were calculated based on our laboratory facilities before the laboratory work on the study subjects described in the serological assay. Bayes rule applied to estimate the adjusted seroprevalence ($\pi$) as a function of crude seroprevalence ($p$), sensitivity ($r$), and specificity ($s$) according to the formula: $\pi = [p - (1 - s)/r - (1 - s)]$ [14]. The asymptotic method was used to construct 95% confidence intervals for the prevalence of COVID-19.

Univariate and multivariable logistic regression analyses were used to determine any potential specific association between SARS-CoV-2 infection and different independent variables, including gender, age groups, educational levels, ABO blood groups, family size, history of blood donation, history of contact with COVID-19 patients, and history of COVID-19 testing. Finally, odds ratio (OR) and adjusted odds ratio (AOR) with their 95% confidence intervals were reported as the effect size of logistic regression. For evaluating the change in the prevalence of COVID-19 over time in the whole country, the chi-square test was adjusted by sampling weights along with Bonferroni adjustment. The multiple imputation method was used for imputing the missing. The statistically significant level was set at 0.05. All statistical analyses were conducted using R software using mice, juice, and survey packages.

### 2.6. Ethics statement

This study was approved by the Ethical Committee of High Institute for Research and Education in Transfusion Medicine, Tehran, Iran (code No: IR.TMI.REC.1399.017).

### 3. Results

#### 3.1. Socio-demographic characteristics of participants

In total, 10,689 eligible blood donors aged 18–67were recruited for this study. Of this number, 337 subjects were excluded; 47 subjects did not have sufficient samples, and 290 had borderline serologic results. Therefore, the study population was limited to 10,352 individuals, including 3840 3697 and 3152 participants who enrolled in the third weeks in September, October, and November in 2020. The median age was 37 (IQR, 30–46), 36 (IQR, 29–46), and 37 (IQR, 29–46) for participants at the three-time points, respectively.

Overall, participants of the male gender (81.4%), family size ≤4 (76.7%), and no contact with COVID-19 patients (89.1%) were more frequent than other groups in comparison, and participants with the age range of 30–45 years (45.8%), education levels of diploma (40%), blood groups O (39.1%), first time (42.1%), and negative history of COVID-19 (85.6%) were the most frequent groups compared to other groups in comparison. Table 1 shows a descriptive analysis of participants' characteristics at the three-time points of the study.

#### 3.2. Prevalence of IgG antibody against SARS-CoV-2

The applied serological assay showed that 2577 (24.89%) out of 10,352 participants had developed IgG antibodies against the SARS-CoV-2 S antigen, and 7775 (75.11%) did demonstrate any trace of this antibody in their blood. Crude, population weight-adjusted, and weighted estimate adjusted for test performance seroprevalence rates according to the time points (September 3rd week, October 3rd week, and November 3rd week) are shown in Table 2.

The overall seroprevalence increased significantly through the three-time points, 19.59 (17.18–22.00) to 32.63 (29.93–35.33), respectively, P-value <0.001. The increase was not significant in seroprevalence from the first point in September to that of the second point in October (P-value = 0.166). Still, there was a significant increase from the first point in October to the second point in November (p-value <0.001), Fig. 1.

Variation was found in SARS-CoV-2 seroprevalence estimation between provinces' intra and inter-time points. Fig. 2 shows the change in the frequencies of IgG antibody levels against SARS-CoV-2 over three-time points.

The seroprevalence ranged from 5.45% (0.91–9.99) in Yazd province to 43.11% (31.36–54.85) in Guilan province in the first point. However, in the third month, the range increased from 15.25% (5.23–25.27) in Charmahal and Bakhtiari provinces to 57.84% (37.82–77.86) in Alborz province, Fig. 3.

No significant association was found between seropositivity prevalence estimates and gender, age group, family size, ABO blood type, blood collection center, and history of blood donation. AOR 0.76 (0.63–0.93) was found in an inverse association between seropositivity and educational level. A history of close contact with COVID-19 patients was associated with higher seropositivity, AOR 1.69 (1.35–2.11). A strong association was found between having a history of positive COVID-19 testing and higher odds of seropositivity, AOR 8.86 (5.38–14.60). However, there was no significant difference between seropositivity of COVID-19 in participants with a history of negative COVID-19 testing and participants with no history of COVID-19 testing, 23.08 (21.63–24.52) versus 26.30 (21.53–31.06), p = 44.1%, (Table 3).

### 4. Discussions

In this serial cross-sectional study, the nationwide population weight-adjusted and test performance-adjusted prevalence of SARS-CoV-2 IgG antibody was estimated in blood donors in the third weeks of September, October, and November 2020 as 19.59% (17.18–22.00), 22.67% (20.70–24.65) and 32.63% (29.93–35.33), respectively. The findings indicate a significant upward trend of seroprevalence in three-time points in the consecutive months. The increased seroprevalence rate in these months was compared with the country's epidemic curve, rising over the same period. Comparing the prevalence obtained in this study with the official number of cumulative confirmed cases from the onset of the epidemic to the same months of the study, reported 854,361 on 22 November 2020 [15] shows that the number of infected patients is much higher than the official numbers reported based on the molecular assays.

This study estimated about one-third of the population's cumulative population immunity against SARS-CoV2 infection. Compared to the recent studies from Iran, the present study found much higher values of anti-SARS-CoV-2 IgG [16,17].

A study conducted on the general population in 18 cities of Iran between April and June 2020 showed that the seroprevalence of anti-SARS-CoV-2 IgG was, on average, 17.1% [16].

Another recent study in the Guilan province of Iran estimated the seroprevalence as about 20% in April 2020 [17], whereas in the present study, seroprevalence in the same province was significantly higher (36.57%, 35.38%, 35.37%, respectively). It may be argued that the time intervals between the onset of the epidemic

**Table 1**
Characteristics of participants in each time points of the study, Iran, September–November 2020.

| Characteristics, n (%) | September 3840 (100%) | October 3697 (100%) | November 3152 (100%) | Total 10,352 (%) |
|---|---|---|---|---|
| Gender, | | | | |
| Male | 3179 (85.3) | 2837 (78.9) | 2406 (79.5) | 8422 (81.4) |
| Female | 550 (14.7) | 760 (21.1) | 620 (20.5) | 1930 (18.6) |
| Age Group | | | | |
| ≤30 | 984 (26.4) | 1077 (29.9) | 864 (28.6) | 2925 (28.3) |
| 30–45 | 1755 (47.1) | 1606 (44.6) | 1396 (46.1) | 4757 (45.8) |
| >45 | 990 (26.5) | 914 (25.5) | 766 (25.3) | 2670(25.7) |
| Education Level | | | | |
| Under diploma | 995 (26.7) | 936 (26) | 834 (27.6) | 2765 (26.7) |
| Diploma | 1433 (38.4) | 1502 (41.8) | 1206 (39.9) | 4141 (40) |
| Graduated | 1301 (34.9) | 1159 (32.2) | 986 (32.6) | 3446 (33.3) |
| Family size | | | | |
| ≤ 4 | 2881 (77.3) | 2748 (76.3) | 2314 (76.5) | 7943 (76.7) |
| > 4 | 848 (22.7) | 849 (23.6) | 712 (23.5) | 2409 (23.3) |
| ABO blood Type | | | | |
| A | 1119 (30) | 1110 (30.9) | 904 (29.9) | 3133 (30.3) |
| B | 935 (25.1) | 822 (22.9) | 681 (22.5) | 2438 (23.6) |
| O | 1417 (38) | 1401 (38.9) | 1229 (40.6) | 4047 (39.1) |
| AB | 258 (6.9) | 264 (7.3) | 212 (7) | 734(7) |
| History of Blood Donation | | | | |
| First-time | 1313 (35.2) | 1741 (48.4) | 1308 (43.2) | 4362 (42.1) |
| Repeated | 843 (22.6) | 592 (16.5) | 604 (20) | 2039 (19.7) |
| Regular | 1573 (42.2) | 1264 (35.1) | 1114 (36.8) | 3951 (38.2) |
| History of Contact with COVID-19 patients | | | | |
| Yes | 397 (10.6) | 355 (9.9) | 375 (12.4) | 1127 (10.9) |
| No | 3332 (89.4) | 3242 (90.1) | 2651 (87.6) | 9225 (89.1) |
| History of COVID-19 testing | | | | |
| Negative | 3366 (90.3) | 3261 (90.7) | 2649 (87.5) | 9276 (89.6) |
| No history | 255 (6.8) | 244 (6.7) | 233 (7.7) | 732 (7.1) |
| Positive | 108 (2.9) | 92 (2.6) | 144 (4.8) | 344 (3.3) |

**Table 2**
Crude, weighted and adjusted seroprevalence of SARS-CoV-2 IgG (based on sampling weights and test performance) over the country in three study rounds, Iran, September- November 2020.

| Month | Crude (95% CI) | Weighted (95%CI) | Weighted & adjusted for test Performance |
|---|---|---|---|
| September | 20.38 (19.9–21.67) | 19.85 (17.48–22.22) | 19.59 (17.18–22.00) |
| October | 24.10 (22.71–25.50) | 22.88 (20.94–24.83) | 22.67 (20.70–24.65) |
| November | 31.39 (29.74–33.05) | 32.68 (30.02–35.34) | 32.63 (29.93–35.33) |

Weighted prevalence according to Iranian population aged 18–69 years, participant's province, age groups, and gender. Adjusted prevalence based on the test sensitivity and specificity.

and the time of studies and different methodological methods used could justify these differences.

In addition, our estimated seroprevalence was much higher than the reported results among blood donors in other countries such as the Netherlands (1.7%), the North of France (2.7%), and Brazil (3.6%) [7–9]. Besides the reason mentioned above, this could be justified by the differences in epidemic management strategies in different studies. Moreover, our result corroborates the massive effect of the SARS-CoV-2 epidemic in Iran compared to other countries.

This study's data endorses pandemics' evolution in three sequential time points in different provinces.

Yazd province, in the center of Iran, had a low prevalence at the first time point. However, in the third point, this status changed to a moderate majority and was substituted by Chaharmahal and Bakhtyari province. Also, in the first time point of the study, only Guilan (a northern Iranian province) had the highest SARS-CoV-2 prevalence.

In this study, participants with a history of positive COVID-19 testing had significantly higher seroprevalence than those with a history of negative COVID-19 testing or participants with no history of COVID-19 testing, AOR, 8.86 (5.38–14.60) $P < 0.001$.

In our study, the seroprevalence of the infection was lowest in participants with the highest educational levels, AOR 0.76 (0.63–0.93), which is in line with a study conducted on Brazilian blood donors [9]. This finding was expected because higher educational-level people are more likely to adhere to COVID-19 instructions and guidelines.

This study had several significant outcomes:

First, specific IgG antibodies were not detected in about one-fourth of the participants with a history of positive COVID-19 testing. Our study showed that IgG antibodies against SARS-CoV-2 waned over time after resolving the infection. The declining levels of IgG antibodies against SARS-CoV-2 over time or defective production of specific IgG antibodies after exposure to the virus can be one explanation for this finding [18,19]. In addition, defective antibody production against SARS-CoV-2 due to the severity of the disease, race/ethnicity, obesity, and consumption of immunosuppressive drugs are reported in a large cohort of patients who were infected in the past [20].

Second, participants with a history of negative COVID-19 testing had a trace of IgG antibodies against the virus in their circulation. False negative COVID-19 testing by PCR/chest CT scan may explain these findings [21,22].

Third, seropositivity was also detected in a group of participants with no history of being tested for COVID-19. This emphasizes the importance of COVID-19 laboratory diagnostic investigations in managing the infection in the country. However, detection of IgG antibodies in a large group of participants with a history of negative COVID-19 testing, and those with no history of COVID-19 testing,

F. Ranjbar Kermani, A. Arabkhazaeli, P. Eshghi et al.
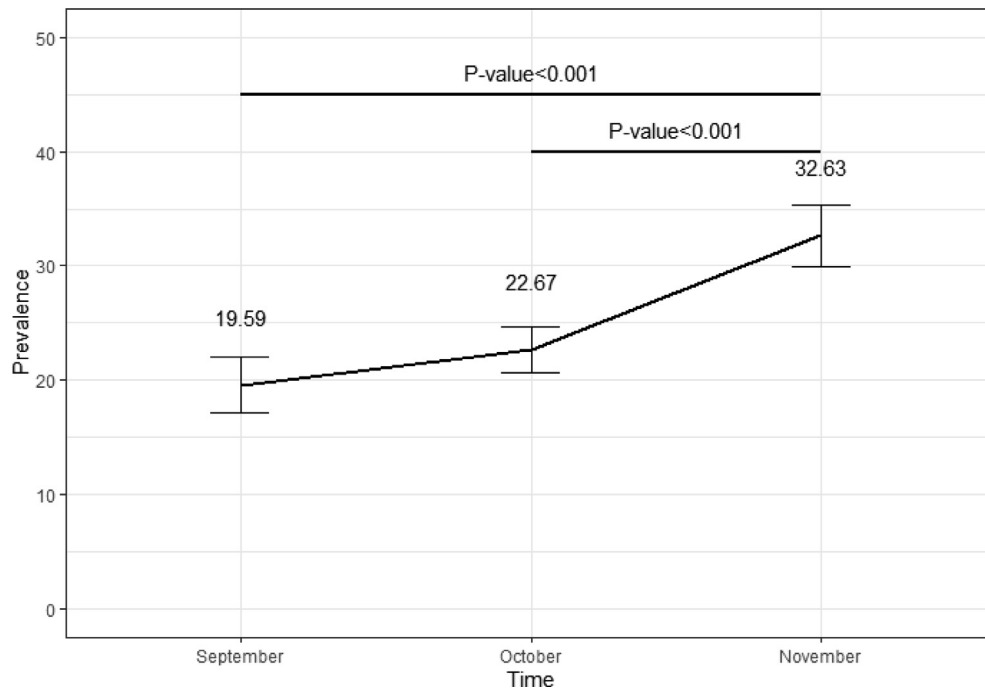
**Fig. 1.** Seroprevalence of SARS-CoV-2 IgG antibody over three study rounds, Iran, September–November 2020.
Note: Seroprevalence is shown as a percentage. The line represents trend of the Seroprevalence over the study periods.
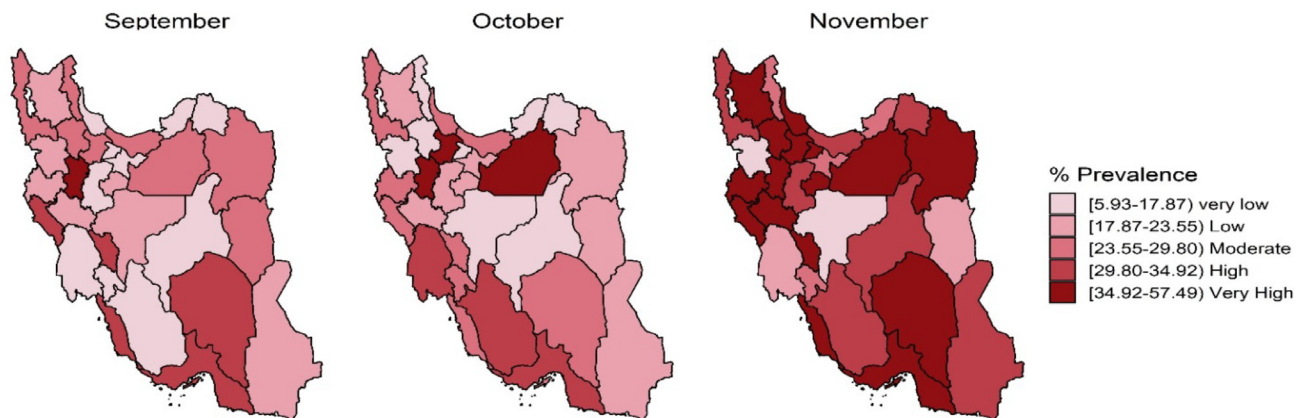


**Fig. 2.** Distribution of adjusted seroprevalence of SARS-CoV-2 IgG (based on sampling weights and test performance) in provinces over three study rounds, Iran, September–November 2020.
Note: The seroprevalence was categorized based on the first, second, and third quintiles.

can lead to underestimation of COVID-19 officially reported statistics at the national level. Moreover, those people who might carry and transmit the infection strongly highlight the importance of preventive behaviors at the population level.

To the best of our knowledge, it is the first study for seroprevalence estimation of SARS-CoV-2 IgG antibodies in the country at the national level. Since blood donors are more accessible than the general population, the prevalence of SARS-CoV-2 IgG antibodies in these individuals can be used as a panorama of the epidemic in the community. Although the people of blood donors is a criteria-based cohort and may not be a complete picture of the general population, similar studies in other countries indicate that the results of the prevalence of SARS-CoV-2 IgG antibody in blood donors did not substantially differ from those in the general population [23].

We designed an equally quota sampling method to best estimate the seroprevalence of COVID-19 among the Iranian population. We weighted the crude seroprevalence based on the distribution of gender and age group in each province population over the country (described in the method section). In addition, we re-calculated the sensitivity and specificity of the EUROIMMUN test and adjusted the seroprevalence estimate based on test performance.

This study encompasses potential limitations. First, the age group distribution of blood donors (18–65 years) does not capture the population under 18 years old exposed to SARS-CoV-2. Second, since blood donors are more accessible than the general population, the presented prevalence of SARS-CoV-2 IgG antibodies estimation would be underestimated.
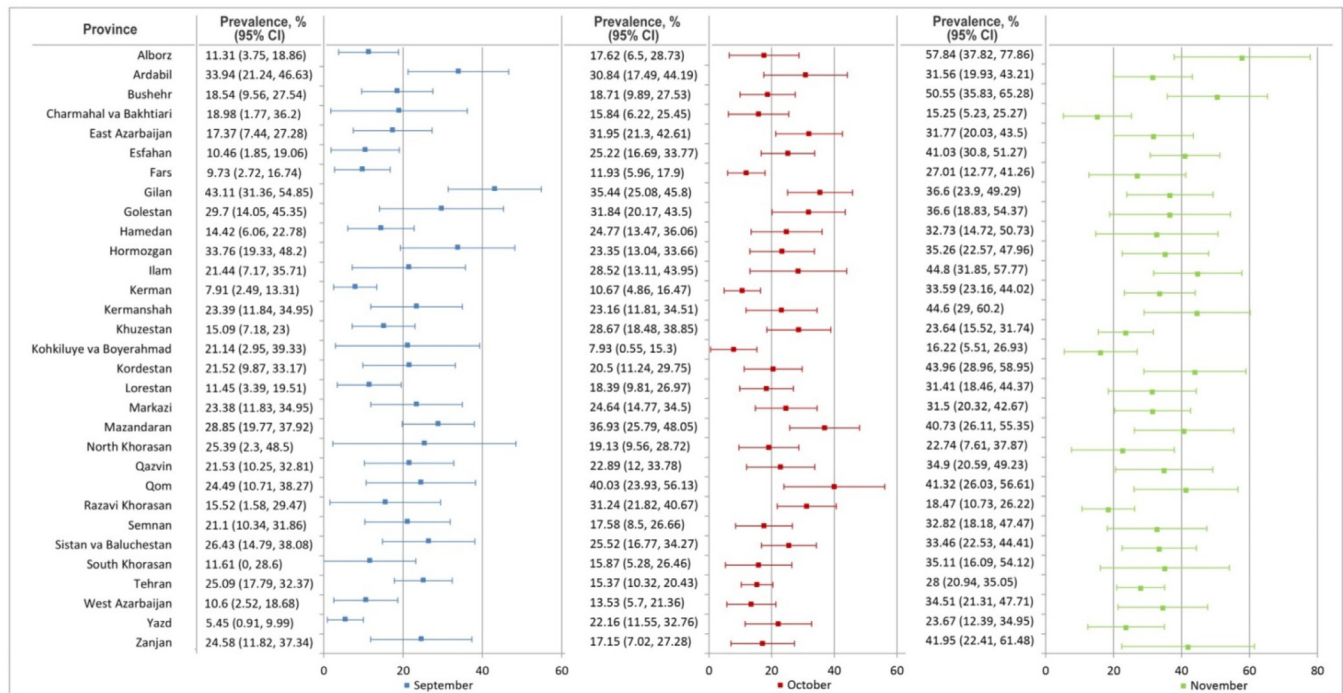
F. Ranjbar Kermani, A. Arabkhazaeli, P. Eshghi et al.

**Fig. 3.** Forest plot of adjusted seroprevalence of SARS-CoV-2 IgG (based on sampling weights and test performance) in provinces over three study rounds, Iran, September–November 2020.

**Table 3**
Seroprevalence of SARS-CoV-2 IgG in participants by different variables, Iran, September–November 2020.

| Characteristics | Crude (%) (95%CI[a]) | Weighed and adjusted [b](%) (95%CI) | OR[c] (95% CI) | P value | AOR[d] (95%CI) | P value |
|---|---|---|---|---|---|---|
| **Gender** | | | | | | |
| Male | 24.45(23.53–25.37) | 25.16(24.05–26.28) | 1.00(0.86–1.17) | 0.967 | – | – |
| Female | 26.84(24.86–28.82) | 25.10(22.52 27.69) | Ref. | | | |
| **Age group** | | | | | | |
| ≤30 | 26.6(25–28.2) | 24.63(22.11–27.16) | Ref | – | – | – |
| 30–45 | 24.36(23.14–25.58) | 25.51(23.25–27.77) | 1.05(0.87–1.26) | 0.613 | | |
| >45 | 23.97(22.35–25.59) | 25.15(22.70–27.61) | 1.03 (0.85–1.24) | 0.774 | | |
| **Educational level** | | | | | | |
| Under diploma | 24.05(22.46–25.64) | 24.74(22.22–27.25) | Ref. | – | Ref. | – |
| Diploma | 26.27(24.93–27.61) | 27.97(25.63–30.32) | 1.18(0.99–1.41) | 0.067 | 1.15(0.96–1.38) | 0.136 |
| Graduated | 23.91(22.49–25.34) | 22.11(19.78–24.43) | 0.86(0.71–1.05) | 0.134 | 0.76(0.63–0.93) | **0.009** |
| **Family size** | | | | | | |
| ≤4 | 24.59(23.64–25.53) | 25.13(23.54–26.73) | Ref. | – | – | – |
| >4 | 25.9(24.15–27.65) | 25.14(22.29–28.00) | 1.00(0.84–1.19) | 0.994 | | |
| **Blood group** | | | | | | |
| AB | 25.61(22.46–28.77) | 24.79(19.08–30.49) | 1.01(0.73–1.41) | 0.929 | 0.98(0.70–1.38) | 0.916 |
| B | 25.06(23.34–26.78) | 23.77(21.08–26.46) | 0.96(0.79–1.17) | 0.683 | 0.94(0.77–1.14) | 0.527 |
| A | 23.65(22.34–24.96) | 27.21(24.75–29.66) | 1.15(0.97–1.37) | 0.114 | 1.12(0.93–1.34) | 0.226 |
| O | 26.20(24.67–27.74) | 24.51(22.21–26.81) | Ref. | – | Ref. | – |
| **Blood donation status** | | | | | | |
| First-time | 25.97(24.67–27.28) | 25.35(23.21–27.49) | Ref. | – | – | – |
| Repeated | 24.42(22.56–26.29) | 24.22(21.11–27.33) | 0.94(0.77–1.16) | 0.563 | – | – |
| Regular | 23.94(22.61–25.27) | 25.37(23.11–27.63) | 1.00 | 0.989 | | |
| **Contact with COVID-19 patients** | | | | | | |
| Yes | 40.20(37.33–43.06) | 41.37(36.81–45.93) | 2.35(1.91–2.88) | – | <0.001 | **<0.001** |
| No | 23.02(22.17–23.88) | 23.13(21.69–24.57) | Ref. | Ref. | – | – |
| **History of COVID-19 testing** | | | | | | |
| Negative | 22.49(21.64–23.34) | 23.08(21.63–24.52) | 0.84(0.65–1.09) | 0.191 | 0.90 (0.70–1.17) | 0.441 |
| No history | 29.64(26.34–32.95) | 26.30(21.53–31.06) | Ref. | Ref. | – | – |
| Positive | 79.65(75.40–83.91) | 78.18(70.65–85.70) | 10.0(6.06–16.6) | <0.001 | 8.86(5.38–14.6) | **<0.001** |

The significant values were shown as bold format.
[a] CI: Confidence interval.
[b] Weighted seroprevalence based on gender and age groups of the population in each province adjusted by sensitivity (98.97%) and specificity (99.42%).
[c] OR = Odds ratio.
[d] AOR = Adjusted OR.

## 5. Conclusions

In conclusion, in this study, the estimated burden of the infection in the whole country in three time periods before the commencement of the COVID-19 vaccination program showed that approximately one-third of the population was exposed to the SARS-CoV2 disease. According to the COVID-19 seroprevalence estimates in this study, the number of infected individuals in the country was much higher than the number of officially announced confirmed cases. This reflects the limitations of the daily molecular test numbers, which public health strategists should consider at the Iranian Ministry of Health. Moreover, the seropositivity in about one-fourth of people with a negative history of contracting or being tested, who might carry and transmit the infection, strongly highlights the importance of preventive measures for COVID-19 at the population level.

## Declaration of Competing Interest

The authors declare that they have no competing interest.

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