Seroprevalence of anti-severe acute respiratory syndrome coronavirus 2 antibody among healthy blood donors in a hospital-based blood center in Eastern India during the COVID-19 pandemic

Sudipta Sekhar Das, Subhas Chandra Bera, Rathindra Nath Biswas

Abstract:

BACKGROUND: Few international studies have reported the prevalence of anti-severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies in healthy, asymptomatic blood donors. These findings have definitely raised queries regarding blood safety and transfusion-transmitted coronavirus disease (COVID)-19. We conducted this first anti-SARS-CoV-2 seroprevalence survey among the healthy blood donors in Eastern India.

MATERIALS AND METHODS: The study included 611 healthy blood donors who donated whole blood (WB) in our blood center. For detailed analysis, social and demographic details of all donors like gender, age, weight, occupation, and place of residence were included. Donor eligibility criteria for WB donation were followed as per existing national guidelines. Residual serum samples leftover after screening the mandatory infectious markers were tested for the presence of anti-SARS-CoV-2 IgG directed against domain S1 and S2 of the SARS-CoV-2 spike protein using automated enhanced chemiluminescence technology following the manufacturer's instructions.

RESULTS: The mean overall seroprevalence of anti-SARS-CoV-2 antibody in blood donors was observed to be 4.4% (95% confidence interval 3.8–4.9) with a monthly increasing trend. Seroprevalence adjusted for sensitivity and specificity of the assay was 4.1%. The mean S/Co values of reactive donor samples were observed to be 2.99 and 3.42 in June and July 2020, respectively (P = 0.013). No significant variation in seroprevalence rate was observed among donor variables like donor age, gender, profession, and educational qualification. A higher significant prevalence of antibody was observed among voluntary donors and donors residing in suburban areas (P < 0.05). Among the ABO blood groups, no statistical significance of seroprevalences was observed among the various ABO blood groups.

CONCLUSION: We conclude that despite many limitations in the current study, we found 4.4% seroprevalence of anti-SARS-CoV-2 antibody in the asymptomatic, healthy, epidemiologically, and medically screened blood donors. These data are definitely the tip of an iceberg and signify much higher seroprevalence in the normal population and indicate that protective measures like masking and social distancing should remain implemented for a long term.

Keywords: Anti-severe acute respiratory syndrome coronavirus 2, blood donors, COVID-19, immunoassay, serology, seroprevalence
The coronavirus disease named as COVID-19 caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) rapidly affected the entire globe significantly. The World Health Organization declared the outbreak as a pandemic. Significant morbidities and mortalities have been observed in India affecting almost all states and districts. By August 10, 2020, the country observed a total of 22,68,675 confirmed cases with 45,257 deaths. In many instances, the infected individuals are asymptomatic and they become a potential source of disease transmission. Previous authors have investigated the seroprevalence of SARS-CoV-2 in asymptomatic groups including the major report from the Diamond Prince Cruise ship. Likewise, a study in the USA found a 2.8% seroprevalence of SARS-CoV-2 in their population. In India, the newspaper headlines reported that in Delhi, by early July, 23% of those surveyed had developed IgG antibodies and in Mumbai, by around the same time, 57% of those surveyed in slums and 16% in nonslum areas had developed these antibodies. Similarly, in Kolkata, the Indian Council of Medical Research survey investigated a seroprevalence of 14%. With regard to international data, London and New York estimated seroprevalences of 17.5% and 23%, respectively, in late April 2020. Although COVID-19 infection is confirmed by polymerase chain reaction (PCR), workers recently suggested a positive identification of anti-SARS-CoV-2 IgG antibodies as an acceptable approach to confirm infection.

Blood donors are healthy, asymptomatic individuals who donate blood selflessly and altruistically. Despite being healthy, authors reported 1.7% seroprevalence in blood donors in Denmark, 2.7% in The Netherlands, 4.6% in Spain, 3.8% in Brazil, and 0.9% in Germany. Worker also commented that evaluating the trends in the prevalence of viral infections in blood donors is essential not only for estimating the effectiveness of strategies for blood safety but also for enhancing them, reducing the potential risk of infection by blood transfusion.

Our being a blood center attached to a 750 bedded tertiary care hospital, we observed reasonable blood donations in the COVID-19 pandemic. We conducted a seroprevalence survey among the blood donors who were otherwise healthy and asymptomatic. To date, this is the first study in Eastern India addressing the seroprevalence of SARS-CoV-2 in asymptomatic blood donors.

Study participants
Donor eligibility criteria for WB donation were followed as per departmental standard operating procedures (SOP) in accordance with the Drugs and Cosmetics (D and C) Act 1940 and rules 1945 therein. Moreover to ensure optimal donor and staff safety; the guidelines provided by the National blood transfusion council (NBTC) of India in the light of COVID pandemic were strictly followed and executed. Social distancing, masking, hand hygiene, and cough etiquette were maintained strictly and ethically in the blood donation premises. In addition to the mandatory written questionnaire (donor card) and brief health screening, an extra questionnaire with pertinent questions related to donor travel history, contact history, and COVID-19 symptoms was developed and put in use to exclude the at-risk donors. As per departmental SOP developed in the light of the COVID-19 pandemic, all donors were educated and advised to contact the blood center in case of any postdonation illness including signs and symptoms of COVID-19 or in case of close contact with COVID-19. All donors in the study group were asymptomatic, afebrile with no known historical epidemiology of the disease within the last 28 days. Once accepted to donate blood, all blood donors were included in the study, once they accepted to donate blood and signed the informed consent form for blood donation and testing.

Sampling
Serum samples with defined barcode collected from all donors for testing of the mandatory infectious disease markers were also used for the SARS-CoV-2 antibody test. Donors who were seroreactive for any of the infectious markers like anti-HIV 1 and 2, HBsAg, anti-HCV, syphilis, and malaria were excluded from the study.

Serology
Residual serum samples leftover after screening the mandatory infectious markers were tested for the presence of anti-SARS-CoV-2 IgG directed against domain S1 and S2 of the SARS-CoV-2 spike protein using the automated VITROS ECiQ immunodiagnostic system based on enhanced chemiluminescence technology and approved kit controls and calibrators (Ortho Clinical...
Diagnostics, USA). The tests were intended for the qualitative detection of IgG antibodies to SARS-CoV-2 in human serum and all tests were performed following the manufacturer’s instruction.[24]

Briefly, the automated immunometric technique involves a two-stage reaction. In the first stage, antibodies to SARS-CoV-2 present in the sample bind with SARS-CoV-2 spike protein coated on wells. In the second stage, horseradish peroxidase (HRP)-labeled murine monoclonal anti-human IgG antibodies are added in the conjugate reagent. The conjugate binds specifically to the antibody portion of the antigen-antibody complex. After the washing step, the bound HRP conjugate is measured by a luminescent reaction and the light signals are read by the system. The amount of HRP conjugate bound is indicative of the amount of SARS-CoV-2 IgG antibody present. The manufacturer claims a specificity of 99.7% and a sensitivity of 90.0% for samples taken more than 15 days’ postsymptom onset.[24]

**Interpretation of the result[24]**

Results are calculated by the device automated Integrated systems.

\[
\text{Result} = \frac{\text{Signal for test sample}}{\text{Signal at Cutoff (S/Co value)}}
\]

S/Co value of <1 suggests that the sample is nonreactive for anti-SARS-CoV-2 IgG and a value ≥1 suggests a reactive sample.

**In-house control**

Serum samples of COVID-19 patients who were reverse transcription-PCR positive and free from symptoms for ≥28 days were used as in-house positive control in the assay. All these samples were reported reactive for anti-SARS-CoV-2 IgG antibody from the microbiology department of the hospital. These positive controls were used for the validation of the test runs.

**Statistical analysis**

Statistical analysis was done using the SPSS statistical package (IBM, 2015, Armonk, New York, USA). We adjusted the prevalence for test sensitivity at 90% and specificity at 99%, following the manufacturer’s estimates. The adjusted prevalence of IgG antibodies to SARS-CoV-2 in blood donors was reported as percentages with 95% confidence intervals (CI). Quantitative variables were calculated as mean ± standard deviation and a \( P < 0.05 \) was considered statistically significant. Social and demographic variables of blood donors were compared between reactive and nonreactive groups. Qualitative variables and quantitative variables were analyzed using the Chi-square test and paired Student’s \( t \)-test, respectively.

**Results**

The present work describes the seroprevalence of SARS-CoV-2 IgG antibody in 611 asymptomatic healthy blood donors in the month of June and July 2020. The mean overall seroprevalence rate was observed to be 4.4% (95% CI 3.8–4.9). Where 12 of the blood donors out of 293 (4.1%) were seroreactive in June 2020; this was 15 in 318 donors (4.7%) in July 2020 (\( P = 0.008 \)). The mean overall seroprevalence adjusted for sensitivity and specificity of the assay was 4.1% (95% CI 3.7–4.6) which was lower than the mean crude or unadjusted rate of 4.4% (\( P = 2.142 \)) [Table 1].

Table 1: Seroprevalence of anti-severe acute respiratory syndrome coronavirus 2 immunoglobulin G antibody in healthy donors, estimates adjusted for sensitivity and specificity (n=611)

| Periods and estimates | Sample size (n) | Reactive samples (n) | Prevalence (%) | 95% CI |
|-----------------------|----------------|---------------------|---------------|--------|
| **June 2020**         |                |                     |               |        |
| Unadjusted prevalence | 293            | 12                  | 4.1           | 3.7-4.6|
| Adjusted prevalence for sensitivity and specificity | 293 | 12 | 3.9 | 3.3-4.3 |
| **July 2020**         |                |                     |               |        |
| Unadjusted prevalence | 318            | 15                  | 4.7           | 4.2-5.3|
| Adjusted prevalence for sensitivity and specificity | 318 | 15 | 4.4 | 3.8-4.9 |
| **Total**             |                |                     |               |        |
| Unadjusted prevalence | 611            | 27                  | 4.4           | 3.8-4.9|
| Adjusted prevalence for sensitivity and specificity | 611 | 27 | 4.1 | 3.4-4.7 |

CI=Confidence interval

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July 2020 (P = 0.915). Seroprevalence of anti-SARS-CoV-2 IgG antibody was investigated on the basis of social and demographic variables of the blood donors [Table 3]. Although no significant unadjusted seroprevalence rate of SARS-CoV-2 IgG antibody was observed among the donor age group, gender, profession, and educational qualification, the statistical significance of seroprevalence was observed among voluntary and replacement donors (5.9% vs. 3%, \( P = 0.039 \)) and donors residing in urban and suburban areas (2.8% vs. 6.1%, \( P = 0.044 \)). Among the ABO blood groups, the highest mean seroprevalence of SARS-CoV-2 IgG antibody was investigated in “B” group donors (4.9%), followed by “A,” “AB,” and “O” (3.9%) groups, however no statistical significance of seroprevalences was observed among the various ABO blood groups (\( P > 0.05 \)).

**Discussion**

This is to our knowledge, the first study performed in Eastern India to determine the seroprevalence of anti-SARS-CoV-2 antibody in healthy asymptomatic blood donors. We systematically investigated the SARS-CoV-2 seroprevalence among the donors’ who visited the blood center from the urban and suburban areas of the metropolitan city of Kolkata, West Bengal, and donated WB. Our study clearly demonstrated an overall mean SARS-CoV-2 seroprevalence of 4.4% (95% CI: 3.8%–4.9%). This figure was in approximation with large studies done in Spain and Brazil,[18,19] but definitely higher than other studies conducted in various countries in Europe.[16,17,20] We observed an increasing linear trend in the seroprevalence along the study period with 4.1% in June 2020 and 4.7% in July 2020 (\( P = 0.008 \)) indicating a rise in the epidemic curve. Though the present study is not randomized and included all samples in defined time periods, this accounted for a demographically and socially heterogeneous healthy blood donor population, allowing a preliminary outlook of the prevalence of the antibody in asymptomatic individuals. To obtain a better estimate of the prevalence of anti-SARS-CoV-2 antibody in asymptomatic blood donors, we adjusted our values for the sensitivity and specificity of the serological assay considering the manufacture’s data and claims. We observed an adjusted seroprevalence of 4.1% in our blood donor population. Owing to high reagent/test cost and their interrupted supply, we could include only 611 blood donors in this small study. However, such antibody tests with reliable performance are essential both for clinical diagnosis and epidemiological studies. Moreover, population-based serological surveillance is a critical approach to assess the prevalence of SARS-CoV-2, as well as to estimate herd immunity.[25] As we considered only those donor samples which were free from antibodies to HIV and hepatitis viruses, therefore assay interference due to potential antibodies against surrogate virus proteins which may present in samples of some individuals could be prevented. Since the blood donors are always between 18 and 65 years old and 52% of donors in the present study were young healthy adults (<35 years old), therefore they overrepresented the study group with underrepresentation of elderly people. In addition, females who only comprised of 15.7% of the total blood donors underrepresented the study population.

Low seroprevalence for SARS-CoV-2 determined in few studies may be due to the imposition of preventive,

| Samples and controls result interpretation | June 2020 | July 2020 | Total | \( P \) |
|------------------------------------------|-----------|-----------|-------|--------|
| **Donor samples**                         |           |           |       |        |
| Nonreactive                               |           |           |       |        |
| Sample size (\( n \))                    | 281       | 303       | 584   | 0.627  |
| S/Co (mean±SD)                           | 0.08±0.12 | 0.11±0.15 | 0.09±0.14 |        |
| Reactive                                 |           |           |       |        |
| Sample size (\( n \))                    | 12        | 15        | 27    | 0.013  |
| S/Co (mean±SD)                           | 2.99±1.02 | 3.42±0.89 | 3.23±0.97 |        |
| **Controls**                             |           |           |       |        |
| Kit positive control                      |           |           |       |        |
| Sample size (\( n \))                    | 11        | 13        | 24    | 0.731  |
| S/Co (mean±SD)                           | 3.29±0.20 | 3.38±0.19 | 3.35±0.19 |        |
| Kit negative control                      |           |           |       |        |
| Sample size (\( n \))                    | 11        | 13        | 24    | 0.852  |
| S/Co (mean±SD)                           | 0.007±0.006 | 0.008±0.007 | 0.008±0.007 |        |
| In-house positive control                 |           |           |       |        |
| Sample size (\( n \))                    | 11        | 10        | 21    | 0.915  |
| S/Co (mean±SD)                           | 6.99±2.53 | 7.16±2.88 | 7.07±2.63 |        |

S/Co=Signal at Cutoff, SD=Standard deviation
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nonpharmaceutical, interventions at an early stage of the epidemic, and conducting these studies in the essential lockdown periods.[16,17,20] A study by Flaxman et al. indicated that these lockdowns contributed considerably to the containment of virus spreading and therefore may have saved many lives.[26] Their model calculation estimating the current SARS-CoV-2 seroprevalence for different European countries revealed the lowest prevalence for Germany (0.85%) and Norway (0.46%), while higher values were estimated for Belgium (8%) and Spain (5.5%).[26] The present study observed 27 healthy blood donors reactive for anti-SARS-CoV-2 antibody. These donors may have been recently infected and would subsequently have reached higher IgG antibody levels against SARS-CoV-2. However, this assumes that they had an asymptomatic SARS-CoV-2 infection since blood donors represent a selection of apparently healthy individuals lacking any physically detectable symptoms.[23] Scientists from Europe have shown that serum IgG levels remained partially negative in COVID-19 patients with a mild disease progression, whereas severe cases, independently of age, had significantly increased serum IgG levels.[27] In addition, Long et al. monitored 285 recovered COVID-19 patients who tested positive for anti-SARS-CoV-2 IgG antibodies within 19 days after symptom onset, but seroconversion was found delayed in patients with milder symptoms.[28] We also feel that not all healthy convalescents seem to express detectable levels of anti-SARS-CoV-2 IgG antibodies and that there is missing evidence on antibody persistence.

The present study used anti-SARS-CoV-2 IgG chemiluminescent immunoassay processed on an automatic analyzer. The immunodiagnostic pack contained test reagent, anti-SARS-CoV-2 IgG calibrator, and appropriate positive and negative controls. The test was intended for the qualitative detection of IgG antibodies to SARS-CoV-2 in human serum and used as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. The assay has been approved by both USA-FDA and is listed as CE marked. As per the manufacturer’s information, the assay uses the structural spike protein of SARS-CoV-2 as its antigen.[24]

In addition, the manufacturer claims that a nonreactive result can occur if the quantity of antibodies for the

### Table 3: Unadjusted seroprevalence of anti-severe acute respiratory syndrome coronavirus 2 immunoglobulin G antibody in healthy blood donors based on sociodemographic variables (n=611)

| Donor variables | Sample size (n) | Reactive (n) | Prevalence (%) | OR   | 95% CI | P   |
|-----------------|----------------|-------------|----------------|------|--------|-----|
| Gender          |                |             |                |      |        |     |
| Male            | 515            | 23          | 4.5            | 1.13 | 0.73-1.57 | 0.649 |
| Female          | 96             | 4           | 4.2            | 1.26 | 0.69-1.97 |     |
| Age group       |                |             |                |      |        |     |
| 18-35           | 318            | 15          | 4.7            | 1.17 | 0.71-1.63 | >0.05 |
| 35-50           | 220            | 10          | 4.5            | 1.31 | 0.86-2.01 |     |
| 50-65           | 73             | 3           | 4.1            | 1.43 | 0.95-1.91 |     |
| Donation type   |                |             |                |      |        |     |
| Voluntary       | 280            | 17          | 5.9            | 1.23 | 0.64-1.59 | 0.0391 |
| Replacement     | 331            | 10          | 3.0            | 1.19 | 0.71-1.53 |     |
| Profession      |                |             |                |      |        |     |
| Student         | 48             | 2           | 4.2            | 0.97 | 0.55-1.28 | >0.05 |
| Government/private employee | 131 | 6 | 4.6 | 1.11 | 0.70-1.42 |     |
| Health worker   | 264            | 12          | 4.6            | 1.15 | 0.63-1.39 |     |
| Unemployed      | 49             | 2           | 4.1            | 0.92 | 0.45-1.27 |     |
| Manual labor    | 119            | 5           | 4.2            | 1.14 | 0.66-1.41 |     |
| Education       |                |             |                |      |        |     |
| Undergraduate   | 219            | 10          | 4.6            | 1.21 | 0.69-1.54 | >0.05 |
| Graduate        | 199            | 9           | 4.5            | 1.16 | 0.58-1.62 |     |
| Postgraduate    | 193            | 8           | 4.1            | 1.37 | 0.86-1.89 |     |
| Blood group (ABO) |            |             |                |      |        |     |
| A               | 234            | 11          | 4.7            | 1.12 | 0.65-1.57 | >0.05 |
| B               | 154            | 7           | 4.9            | 1.29 | 0.77-1.72 |     |
| O               | 178            | 7           | 3.9            | 1.17 | 0.59-1.73 |     |
| AB              | 45             | 2           | 4.4            | 0.94 | 0.54-1.42 |     |
| Residence       |                |             |                |      |        |     |
| Urban           | 316            | 9           | 2.8            | 1.73 | 1.19-2.87 | 0.0441 |
| Suburban        | 295            | 18          | 6.1            | 1.43 | 0.82-2.13 |     |

CI=Confidence interval, OR=Odds ratio
SARS-CoV-2 virus present in the specimen is below the detection limit of the assay, or the virus has undergone minor amino acid mutation(s) in the epitope recognized by the antibody detected by the test. All in-house positive controls obtained from the department of microbiology were found to be reactive with a mean S/Co value of 7.07 and these controls could adequately validate the assays. Moreover, the manufacturer’s controls produced appropriate results as per their claims.

The mean S/Co values of the reactive donor samples in June and July 2020 were observed to be 2.99 and 3.42, respectively ($P = 0.013$). The highest S/Co of 5.1 was found in a young donor who was otherwise asymptomatic with no health issue in the past 1 year. As per the updated blood center record on August 10, 2020, none of the donors complained of postdonation illness including signs and symptoms of COVID-19. Interestingly, the S/Co value of all nonreactive donors was observed to be ≤0.4. None of the samples in the current study produced equivocal results (S/Co: 0.8–1.08).

Among the total 611 donors understudy, only 4 (0.65%) females were found to be seroreactive. Although not statistically significant, seroprevalence in females was observed to be lower than male donors (4.2% vs. 4.5%). Similar data were published by authors among Brazilian blood donors. We observed a higher prevalence of the anti-SARS-CoV-2 antibody among the younger donors (<35 years) and this was predictable because they comprise the main workforce, more likely to move around, and are susceptible to greater exposure and infection.

A significant higher seroprevalence was investigated in voluntary donors because most of them were health-care workers and regular voluntary donors from corporate/private and government offices. The replacement donors were mostly family members of the patients with restricted movement and exposure. Profession wise, the prevalence of antibody was higher in health-care workers and office employees. This was expected because they formed the main donor pool in the essential lockdown period as well as in the initial months of the COVID-19 pandemic. In addition, many health-care workers were our hospital employees who volunteered to donate blood during the critical blood shortage in the pandemic. A higher seroprevalence was noticed among the less educated donors (4.6% vs. 4.1%). Although the authors could not obtain specific causes, low socioeconomic stratum, outdoor works, difficulties in maintaining social distancing, crowded household, and inadequate basic hygiene may be the result of such findings. A similar experience was shared by Filho et al. where a higher prevalence of anti-SARS-CoV-2 antibody was observed in less educated donors (2.8% vs. 4.7%, $P = 0.011$).

A study conducted by Lima et al. found that individuals with primary education considered themselves at lower risk for getting COVID-19 and were less engaged in voluntary quarantine than those with higher education levels. Authors in the recent past concluded that blood group “A” was associated with a higher risk for acquiring COVID-19 compared with “non-A” blood groups, whereas blood group “O” was associated with a lower risk for the infection compared with “non-O” blood groups. On the contrary in a large, multinstitutional review by Latz et al., there was no association noted between ABO blood type and COVID-19 disease severity defined as intubation or death. In the present study, although higher seroprevalence was observed in the “B” group, followed by “A,” the study is very small to comment on the association between ABO blood type and seroprevalence of anti-SARS-CoV-2 antibody in blood donors. Moreover, owing to the shortage of a particular blood type in a blood center, more group-specific donors tend to visit the center in a defined period.

Blood donors from suburban areas showed a higher prevalence of antibody compared to their urban counterparts (6.1% vs. 2.8%, $P = 0.044$). This may be due to the fact that people from suburban areas avail various means of transportation to reach the city for employment and at times they fail to maintain adequate social distancing and personal hygiene during transportation and work. Higher seroprevalence of anti-SARS-CoV-2 antibody in suburban donors was also published by other authors.

Since it is believed that SARS-CoV-2 is not transfusion-transmissible, therefore blood components prepared from seroreactive donors were stored for intended uses. As per departmental SOP, to enhance blood safety, all blood donors on the 14th day of donation were personally contacted over the telephone asking their well-being and health status. Blood components namely packed red blood cells and plasma units were quarantined for 14 days before issue. However, platelet concentrates due to short shelf life were issued within 3–5 days.

Conclusion

We conclude that despite many limitations in the current study, we found 4.4% seroprevalence of anti-SARS-CoV-2 antibody in the asymptomatic, healthy, epidemiologically, and medically screened blood donors. These data are definitely the tip of an iceberg and signify much higher seroprevalence in the normal population. We may also infer that there is a long way to achieve effective levels of natural herd immunity to SARS-CoV-2 and protective measures like masking and social distancing should remain implemented for a long term. Additional large, multicentric studies are needed to better define and evaluate (a) the seroprevalence of SARS-CoV-2 antibody in the blood donor population considering their social and demographic variables and (b) the epidemiology of COVID-19.
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Conflicts of interest
There are no conflicts of interest.

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