Abstract The current study aimed to estimate the seroprevalence of SARS-CoV-2 IgG (S-protein) antibodies along with neutralizing assay (RBD-domain) among the whole blood donors without any prior Covid-19 history or symptoms visiting Blood Centre at a Tertiary care institution, South India amidst the ongoing pandemic. During September 2020 to March 2021, 1034 whole blood donors were enrolled into the study and were screened for anti-SARS-CoV-2 IgG antibodies using Chemiluminescence assay followed by neutralizing antibodies using surrogate neutralization ELISA. The study reported seroprevalence of 49.4%, (95% CI 46.3–52.5) among whole blood donors, with test sensitivity and specificity adjusted prevalence of 54.9% (95% CI 51.5–58.3). Seroprevalence was similar across age groups, gender, voluntary/replacement donations, area of residence, ABO and Rh groups without any statistical significance. However higher IgG antibody responses were found to be elicited in the 30–45 years age group when compared with 18–29 years age group (p value 0.046). This study also analysed the mean neutralizing capacity of SARS-CoV-2 antibodies among 97 blood donors which was 71.9 (SD: + 21.03, range 15.5 to 97.3). Donor samples with SARS-CoV-2 IgG S/Co > 9.5 had significantly higher neutralising capacity (> 68%) when compared with donor samples of S/Co < 9.5 (p value 0.000). Real-time seroprevalence studies will help to know the herd immunity among the blood donors which will assist in knowing the Covid-19 transmission dynamics, distribution of immunity levels at a particular point in time, immunity gaps, development of novel therapeutics and prioritize the vaccination programmes to high risk individuals.

Keywords Covid-19 pandemic · SARS-CoV-2 · Neutralizing antibodies · Blood donors · Seroprevalence
plays a key role in decision making for effective implementation of public health measures [3].

**SARS-CoV-2 seroprevalence:** Seroprevalence studies reported among the general population varied widely across the globe. The accurate estimates of SARS-CoV-2 seroprevalence patterns in the general population remain essential for containment of the pandemic [4]. Population studies are a way to gain knowledge regarding the prevalence of asymptomatic and mildly symptomatic cases, to assess the potency and sustainability of the acquired immune response which is of paramount importance from a public health perspective [5]. Such individuals miss out in the classic symptom-based infection chain tracking (Test-Track-Trace), further play a role in silent spread of infection and also sustainability of the global outbreak. The seroprevalence estimates are also essential for establishing the manufacturing, delivery and deployment of vaccines on wider scale [6]. The SARS-CoV-2 antibodies against Spike proteins and RBD domain in terms of neutralizing capacity is a key correlate for the protection from Covid-19 infection. Many studies have reported antibody responses in Covid-19 using ELISA or other immunoassays, but there are limited studies using neutralizing antibody assays which are more precise.

The current study was the first to describe the seroprevalence of SARS-CoV-2 IgG antibodies (S-protein) along with neutralizing assay (RBD-domain) among the whole blood donors from India. This will contribute to the seroprevalence estimates and herd immunity among healthy, active and asymptomatic population. Real time surveillance of regular blood donors will help to monitor the waxing and waning of immunity levels over a period of time, further which guides us to understand the immune gaps and provide an opportunity to evaluate natural Covid-19 transmission dynamics after each passing Covid-19 pandemic wave.

**Methods**

**Study Setting**

The prospective observational study was conducted at a Tertiary Care Centre, South India on the blood donors visiting the Blood Centre. During the study period i.e., from September 2020 to March 2021, 1034 whole blood donors (S-Protein) were enrolled and tested for SARS-CoV-2 IgG antibodies. The study protocol was approved by the institutional ethics committee.

**Table 1**

| Total No of Whole Blood Donors | Enrolled n=1034 |
|-------------------------------|----------------|
| SARS-CoV-2-IgG Reactive | n= 511 |
| SARS-CoV-2-IgG Non-Reactive | n= 523 |
| Neutralization Assay ELISA | n= 97 |

**Fig. 1** Daily new Covid-19 Cases in India depicting First and Second wave. Source: https://www.worldometers.info/coronavirus/country/india/ accessed on May 31, 2021

**Fig. 2** Flow chart of the study
donors who visited Blood Centre to donate blood were enrolled (Fig. 2).

Subjects and Sampling

The Blood donors who were found fit for routine whole blood donation in accordance with regulations laid by the Government of India were accepted [7, 8]. Donors not fulfilling the criteria were deferred from blood donation. All the donors enrolled were healthy and had not given any history of illness/symptoms suggestive of Covid-19 (History of cold/cough/sore throat/fever/fatigue/loss of smell or taste) in self or in the family 2 weeks prior to the donation and no history of vaccination. Subjects were defined by sociodemographic details including age (18–65 years), gender, type of donation (Voluntary/Replacement), previous blood donation status (first time donor/repeat donor) and area of residence (Urban/ rural). No additional sampling was required except for the samples collected at the time of blood donation for mandatory tests as per our Country guidelines.

Ethics Statement

Enrolment of donors to the study was purely voluntary and non-remunerated. Samples of the whole blood donors who have provided additional written informed consent for the study were included and tested. The Institutional Ethics Committee, ESIC Medical College Hospital reviewed and approved the study protocol.

Serological Testing

The whole blood donor samples were screened for SARS-CoV-2 IgG antibodies against Spike protein using a SARS-CoV-2-IgG antibody kits by Chemiluminescence immunoassay (Vitros, Ortho Clinical Diagnostic, USA) as per the manufacturer’s instructions. Briefly, the immunometric assay detects SARS-CoV-2 IgG antibodies bound to SARS-CoV-2 spike protein coated on the kit wells and uses horseradish peroxidase (HRP)-labelled murine monoclonal anti-human IgG antibodies as conjugate. The bound HRP conjugate is measured by luminescent reaction. Although the amount of HRP conjugate bound is indicative of amount of SARS-CoV-2 IgG antibody present, the linearity of results has not been demonstrated as per manufacturer.

Samples with Signal cutoff (S/Co) value < 1.00 the results are interpreted as specimen sample non-reactive/ negative for SARS-CoV-2 IgG antibodies (S-Protein).

Neutralization Assay

SARS-CoV-2 Surrogate Virus Neutralization ELISA Test (sVNT Kit, GenScript, USA) was used to detect circulating neutralizing antibodies against SARS-CoV-2 that block/neutralize the interaction between receptor binding domain of the viral spike glycoprotein (RBD) with ACE2 cell surface receptor. The kit is blocking ELISA detection tool which mimics the virus neutralization process. The absorbance of the sample at the end of the test is inversely dependent on the titre of anti-SARS-CoV-2 neutralizing antibodies. Test results were validated using kit positive and negative controls. Inhibition rate of the Neutralizing antibodies was calculated using the formula and expressed in percentage.

Cutoff interpretation: ≥ 30% indicates the detection of SARS-CoV-2 neutralizing antibody.

Validation and evaluation: the clinical performance of the GenScript cPass SARS-CoV-2 Neutralization Antibody detection kit was validated using the Comparator Plaque Reduction Neutralization Test (PRNT) utilizing the SARS-CoV-2 virus (WA01/2020 isolate) as per manufacturer evaluation.

Data Collection and Statistical Analysis

Donor demographic details included age, gender, residential area, Type of donation (Voluntary/Replacement), donation status (first time donation/repeat donation), area of residence, history of Covid or related symptoms in self and/or family, quarantine, travel and vaccination history were retrieved from the blood centre records. Data was analyzed using Microsoft Office Version 16.0 and SPSS 22.0.

Sero-prevalence of Covid-19 antibodies was measured by crude rates, weighted prevalence, population adjusted prevalence and adjusted prevalence for test sensitivity at 96.33% and specificity at 99%. Association between the sero-prevalence of anti-SARS-CoV-2 antibodies and following demographic variables- age, sex, area of residence, type of donation, donation status and Covid related history was assessed by Chi-square pearson/Fisher exact test. Correlation between variables was estimated using bivariate Pearson Correlation at 95% significance.
Results

Demographics of Study Population

A total 1034 blood donor samples were tested, mean age of the donors was 28.8 years with age range of 18–58 years. Majority of the study participants were males 1027 (99.3%), 614 (59.3%) were replacement donors and 420 (40.6%) were voluntary donors. Among the study participants 414 (40%) were group O donors, 297 (29%) were group B, 247 (24%) were group A and 76 (7%) were group AB, out of these 963 (93.1) were Rh D positive and 71 (6.9%) were Rh D negative.

Serological Test Results

Among 1034 donor samples of whole blood donations tested, 511 (49.4%) were reactive for SARS-CoV-2-IgG antibodies against S-protein. The overall prevalence was found to be 49.4%, (95% CI 46.3–52.5) and test sensitivity and specificity adjusted prevalence was 54.9% (95% CI 51.5–58.3). Mean antibody level (Signal Cutoff – S/Co) among the reactive cases was 5.78 AU/mL (range 1.0–16.2).

Distribution of SARS-CoV-2 Antibody

Seroprevalence of SARS-CoV-2 IgG (S-protein) antibody levels were distributed based on age groups, gender, type of blood donation, blood group and area of residence and analysed. No statistical significance was observed among the demographic variables (Table 1). Further, based on SARS-CoV-2 IgG antibody S/Co values were categorized as < 3.5, 3.5–6, > 6–12 and > 12 as per the guidelines issued from time to time during the pandemic where > 12 confers to the high titers of antibodies [9]. The distribution of the demographic variables across the Antibody S/Co categories were similar with no statistical significance. However statistically significant higher immunological antibody (IgG) responses were found to be elicited by the 30–45 years age group in comparison to the 18–29 years age group (p value 0.046) (Fig. 3).

Neutralization Assay

SARS-CoV-2 surrogate virus neutralization ELISA test was performed on 97 samples reactive for SARS-CoV-2 IgG (S-protein) antibodies with signal cut-off (S/Co) ranging from 3.06 to 16.2. The pattern of distribution of Neutralizing capacity of SARS-CoV-2 antibodies against the SARS-CoV-2 IgG results was analysed (Fig. 4). Distribution of Neutralizing antibodies with age, gender, blood groups among the samples tested were similar and did not show any statistical significance. Donor samples with S/Co > 9.5 had significantly higher neutralizing capacity (> 68%) than donor samples with S/Co < 9.5 (p value 0.000). Also, neutralising activity was directly in correlation with SARS-CoV-2-IgG CLIA OD values (Bivariate Pearson Correlation Coefficient p = 0.000).

Discussion

SARS-CoV-2 seroprevalence estimates in the community enables us to understand the total infections, including mild and asymptomatic individuals who may not get themselves tested and also guides the possible transmission interruption through depletion of susceptible individuals [6]. In a populated country like India, herd immunity threshold (> 50% of population as per estimates) may be achieved either by natural infection or by vaccination. Until then the transmission of Covid-19 viral infection in the population is expected to occur [10]. Heterogeneity in susceptibility to infection or exposure in individuals, pre-existing immunity in the population, use of infection control, containment measures and seroprevalence estimates might alter the required threshold for herd immunity [11, 12]. Return to normalcy in social living can be achieved with adequate herd immunity.

In this study, the adjusted and weighted seroprevalence of SARS-CoV-2 IgG antibodies among 1034 whole blood donors (September 2020–February 2021) was found to be 49.4%. Although the blood donors included to the study never had any history of symptomatic Covid-19 or positive diagnosis or contact history, a considerable number of blood donors i.e. 511/1034 (49.4%) were SARS-CoV-2-IgG seropositive, indicating the subclinical or asymptomatic nature of disease which missed the detection and hence containment. The asymptomatic Covid-19 infections can pose the risk of spread of infection to the vulnerable population [13]. Education of attitudes and practices among such population regarding asymptomatic infections is very essential step in containment of the spread of infection.

The main finding in this study is that, seroprevalence estimates are higher than other seroprevalence studies reported earlier to the first wave from India [6, 14–18] (Table 2, A). With phased relaxation of stringent lockdown measures after May 2020 and increased social activities, amid a backdrop of lack of adherence to covid appropriate behaviours such as wearing masks, social distancing etc.
would have led to increased exposure and hence increase in Covid-19 spread and thus seroprevalence. Seroprevalence estimate of 49.4% reported in our study was also higher than reported estimates from various countries [19–28] (Table 2, B) The higher seroprevalence estimates in comparison to other studies may be due to various factors like geographical distribution of Covid-19 pandemic, the timing of the pandemic spread, population statistics, early initiation of containment measures and antibody testing strategies in respective countries. Also our study was done later in comparison to the other studies and thus increased seroprevalence.

The seroprevalence estimates in the study across the demographics parameters like age groups, ABO blood groups, type of blood donation, and residential area remained similar with no statistical difference (p > 0.05). Although age-dependent pattern of disease severity in Covid-19 was discussed in previous studies [19, 29], overall seroprevalence among higher age group (46–65 years) was higher (51.1%) in comparison to other age groups but statistical significance was not established due to low turn-out of donors from 46 to 65 years age group who were at high risk during first wave of Covid-19 pandemic in India (Table 1). The mean of S/Co within each cut off range was compared across the different age groups of donors and no statistical significance was found. However higher immunological antibody (IgG) responses were elicited by the 30–45 years age group (p value 0.046). More studies may be required to understand the immunological differences among blood donors belonging to different age groups and it could potentially lay the groundwork for further research in vaccine development and therapeutics.
No significant difference in seroprevalence estimates across the ABO groups was observed in our study while Golinelli et al. (2020) showed association of group-A blood donors with higher seroprevalence of SARS-CoV-2 antibodies [30], and Guillon et al. (2008) reported that a monoclonal anti-A antibody was able to inhibit SARS-CoV

Fig. 3 Comparison of SARS-CoV-2-IgG Antibody S/Co across the age groups. Considering various guidelines for Convalescent plasma therapy [9, 38], the whole blood donors with SARS-CoV-2 Ig antibody positivity were categorised based on the S/Co value as < 3.5, 3.5–6, > 6–12 and > 12. The mean of S/Co < 3.5 among the age groups 18–29 years, 30–45 years and 46–65 years was 1.9, 1.77 and 2.16 respectively, mean among 3.5–6 was 4.64, 4.62, 3.86; among > 6–12 was 8.41, 8.5, 7.27 and among > 12 was 12.7, 13.8, 13.05. The mean of S/Co within each cut off range was compared across the different age groups of donors and no statistical significance was found. However higher immunological antibody (IgG) responses were found to be elicited by the 30–45 years age group in comparison to the 18–29 years age group (p value 0.046)

No significant difference in seroprevalence estimates across the ABO groups was observed in our study while Golinelli et al. (2020) showed association of group-A blood donors with higher seroprevalence of SARS-CoV-2 antibodies [30], and Guillon et al. (2008) reported that a monoclonal anti-A antibody was able to inhibit SARS-CoV
S protein/ACE2 dependent adhesion [31]. Larger sample studies may be required to estimate the ABO group specific seroprevalence estimates. Also, the other studies included the general population while our study included a specific set of population—i.e., blood donors. In our study the mean neutralizing capacity of SARS-CoV-2 antibodies among 97 blood donors was 71.9 (SD ± 21.03 range 15.5 to 97.3). The distribution of neutralizing antibody capacity across age groups and ABO blood groups showed no significant difference. On assessing the Chemiluminescence assay S/Co and Neutralization Inhibition, linear association was observed (Fig. 4, %). Donor samples with S/Co > 9.5 had significantly higher neutralising capacity (> 68%) than donor samples with S/Co < 9.5 (p value 0.000). Our study findings are complying with US FDA guidelines, considering this as cut-off, 38 (39%) of donor samples with CLIA S/Co ≥ 9.5 had higher mean neutralization of 85.4% (range 47.7–97.3) than 59 (61%) of donors with CLIA S/Co < 9.5 (neutralization mean: 58.4%). Donor samples with S/Co ≥ 9.5 depicted as blue dots had significantly higher neutralising capacity (≥ 68%) than donor samples with S/Co < 9.5—orange dots (p value 0.000). Neutralising activity was directly in correlation with SARS-CoV-2-IgG CLIA OD values (Bivariate Pearson Correlation Coefficient p = 0.000) (colour figure online).

As pandemic progresses further, and with new variant/mutant SARS-CoV-2 emerging the real time surveillance of SARS-CoV-2 antibodies among the population as well as blood donors helps in estimating the disease burden, herd immunity and to strategize vaccination for at risk individuals [35]. Immunity gaps identified through multiple data sources, including serology, can be used to target specific interventions and improve routine programs to prevent future gaps.

Donors who recover from recent/ variant induced Covid-19 infections are likely to generate high titre antibodies capable of neutralizing variants [36]. Hence the variant Convalescent plasma could be a potential antidote for variant strains of SARS-CoV-2 [37, 38]. Further, high titre whole blood derived convalescent plasma may be utilised for Convalescent Plasma Therapy [39–42] in non-responders to Covid-19 vaccines, preparing hyperimmunoglobulins [43], monoclonal antibodies and lyophilized products [44].

The limitations of our study were: we included participants who are blood donors in the age group 18–65 years, hence the obtained seroprevalence may not be representative of the general population. And asymptomatic donors were included, thus the seroprevalence may vary over time due to waning of antibody levels, and may underestimate the actual proportion of the population who might have been previously infected with SARS-CoV-2.
### Table 2 SARS-CoV-2 Seroprevalence studies

| Country/State, Author | Period              | Method                          | Study population                  | Seroprevalence |
|-----------------------|---------------------|---------------------------------|-----------------------------------|----------------|
| **A. Seroprevalence Studies from India** |                     |                                 |                                   |                |
| Delhi                 | April–July 2020     | SARS-CoV-2 IgG (Abbott)         | 1191 blood donors                 | 9.5%           |
| Pandey H. C.          |                     |                                 |                                   |                |
| Karnataka             | June 2020           | SARS-CoV-2 IgG ECLIA            | 509 adults                        | 8.5% (95% CI 6.9–10.8%) |
| Inbaraj L. R. et al.  | (2020)              |                                 |                                   |                |
| Delhi                 | June–July 2020      | Anti SARS-CoV-2 Total CLIA      | 3939 health care workers          | 13%            |
| Gupta et al. (2021)   |                     |                                 |                                   |                |
| Karnataka             | June–August 2020    | SARS-CoV-2 IgG ELISA            | 2912 household population         | 37.4–45.6%     |
| Mohanan M. et al. (2020) |                 |                                 |                                   |                |
| India                 | August–September 2020 | Anti-SARS-CoV-2-IgG (Abbott)  | 29,082 households                 | 10.8%          |
| Murhekar V. et al.    |                     |                                 |                                   |                |
| 12 cities India       | July–December 2020  | SARS-CoV-2 IgG CLIA             | 4,48,518 self-referred individuals | 31%            |
| Velumani A. et al. (2021) |           |                                 |                                   |                |
| Current Study         | September 2020–March 2021 | SARS-CoV-2 IgG CLIA (spike protein) | 1034 asymptomatic Blood Donors | 49.4% (95% CI 46.3–52.5) |
| **B. Seroprevalence Studies across the World** |                     |                                 |                                   |                |
| France                | March–April 2020    | Antibodies Neutralizing SARS-CoV-2 | 998 blood donors                  | 2.7 (n = 27)  |
| Gallian et al. (2020) |                     |                                 |                                   |                |
| Denmark               | April 6–May 9 2020  | Commercial Lateral Flow test for IgG/ IgM | 20,640 Blood Donors | 1.9% (95% CI 0.8–2.3%) |
| Erikstrup et al. (2020) |                 |                                 |                                   |                |
| Geneva                | April–May 2020      | Anti-SARS-CoV-2-IgG ELISA       | 2766 household participants       | 4.8% (95% CI 2.4–8.05%) to 10.8% (95% CI 8.2–13.9%) |
| Stringhini et al. (2020) |           |                                 |                                   |                |
| Spain                 | April–May 2020      | SARS-CoV-2 IgG (nucleoprotein) Abbott | 61,075 household participants | 5% (95% CI 4.7–5.4) |
| Pollan M. (2020)      |                     |                                 |                                   |                |
| Kenya                 | April–June 2020     | SARS-CoV-2 IgG ELISA            | 3098 blood donors                 | 5.6% (95% CI 4.8–6.5%) |
| Uyoga S. et al. (2021) |                 |                                 |                                   |                |
| Saudi Arabia          | May–July 2020       | SARS-CoV-2 IgG ELISA            | 1212 Asymptomatic Blood Donors    | 19.31 % (95% CI 17.1–21.6%) |
| Mahallahwi H. et al. (2020) |       |                                 |                                   |                |
| Pakistan              | June–July 2020      | ECLIA, ELISA (IgG, IgM)         | 380 healthy blood donors          | 33.6%          |
| Younas A. et al       |                     |                                 |                                   |                |
| Romania               | July–September 2020 | Anti SARS-CoV-2 Total (IgA, IgM, IgG) antibodies ECLIA | 2115 blood donors | 1.51% (95% CI 1.07–2.13%) |
| Tudor Rares et al. (2021) |           |                                 |                                   |                |
| Current Study         | September 2020–March 2021 | SARS-CoV-2 IgG CLIA (spike protein) | 1034 asymptomatic Blood Donors | 49.4% (95% CI 46.3–52.5) |
Conclusion

The prevalence of SARS-CoV-2 IgG antibodies among the asymptomatic blood donors was high and no significant difference was observed across age groups or ABO/Rh blood type. Neutralizing antibody assays will help to understand the immune responses to SARS-CoV-2 among healthy asymptomatic regular blood donors over a period of time. Further, identifying the seropositive blood units will help to monitor any benefits or serious adverse events to the recipients arising due to transfusion of such blood or blood components.

Real-time seroprevalence studies will help to know the herd immunity among the blood donors which will assist in knowing the Covid-19 transmission dynamics, distribution of immunity levels at a particular point in time, immunity gaps, development of novel therapeutics and prioritize the vaccination programmes to high risk individuals.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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