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Original article

Seroprevalence of SARS-CoV-2 among potential convalescent plasma donors and analysis of their deferral pattern: Experience from tertiary care hospital in western India

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ABSTRACT

Background and objectives. – Seroprevalence estimation of COVID-19 is quite necessary for controlling the transmission of SARS-CoV-2 infection. Seroprevalence rate in recovered COVID-19 patients help us to identify individual with anti-SARS-CoV-2 antibodies and its protective nature. The objective of present study was to evaluate seroprevalence of SARS-CoV-2 among potential convalescent plasma donors and analysis of their deferral reasons.

Materials and methods. – A total 400 potential convalescent plasma donors were enrolled over five-month period for this prospective study. Inclusion criteria were lab confirmed COVID-19 recovered patients and 14 days of symptoms free period. All prospective plasmapheresis donors were tested for IgG SARS-CoV-2 antibody through chemiluminescent microparticle immunoassay, CBC, serum protein, blood grouping along with other required test for normal blood donation as per Drugs & Cosmetics Act. After pre donation testing and medical examination if donor was found to be ineligible for plasmapheresis was deferred. Seroprevalence rate was calculated by positive IgG antibody test results among the potential plasma donors.

Results. – Seroprevalence rate was 87% for IgG SARS-CoV-2 antibodies in prospective convalescent plasma donors (recovered COVID-19 patients). There was no significant difference in seroprevalence rate between different sub-groups with respect to gender, age, blood groups, Rh factor, mode of treatment, day of Ab testing and repeat plasma donation. Most common reason for their deferral was absent IgG SARS-CoV-2 antibodies (13%) followed by absenteeism of eligible screen donors (6.7%), low Hb (1.7%) and poor veins for plasmapheresis (1.7%). Till five-month study period none of the plasmapheresis develop symptoms of reinfection with COVID-19.

Conclusion. – In all, 13% recovered patients did not develop IgG antibodies after SARS-CoV-2 infection. SARS-CoV-2 IgG antibodies persist for quite some time and are protective against reinfection. More long-term serology studies are needed to understand better antibody response kinetics and duration of persistence of IgG antibodies.

1. Introduction

Corona virus pandemic started from Wuhan, China in December 2019 as a cluster of severe acute respiratory syndrome (SARSs) like cases majority of whom were exposed to Huanan seafood market [1]. The disease started in China but rapidly spread to various other countries due to easy person to person transmission and absence of herd immunity and was characterized as a Pandemic by World Health Organization (WHO) on 11 March 2020 [2]. The virus responsible for the pandemic has been named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) as it shares 80% similarity with SARS-CoV that was responsible for 2002-2003 Pandemic. The current outbreak of infections with SARS-CoV-2 is termed Coronavirus Disease 2019 (COVID-19) [3]. After exposure with virus most people develop antibodies within two to three weeks [4,5]. Acute phase of infection of COVID-19 is detected by

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RT-PCR based molecular test while serological test detects antibodies after acute infection and can identify cases which were asymptomatic or missed by RT-PCR test.

Past reported studies mentioned potential benefits of convalescent plasma therapy in H1N1 influenza virus's pandemic, SARS CoV-1 epidemic and MERS-CoV epidemic led us to try it in COVID-19 disease [6–8]. This therapy is based on principle that plasma of convalescent patients who have recovered from COVID-19 infection may contain neutralizing antibodies which may help to neutralize virus in an infected recipient. In India plasma therapy was recommended for off label use in July based on presence of IgG antibodies (Abs) [9].

This led to sudden increase in demand of convalescent plasma across different states and cities in India. Previously our blood centre did not have an apheresis facility but within short span of time the infrastructure and machine were set up, manpower appointed and licence for apheresis was obtained. The next big challenge for us was to recruit plasma donors from limited number of recovered COVID-19 patients. We developed a dedicated team for the recruitment of plasma donors.

Seroprevalence of SARS-CoV-2 among general population, blood donors and among health care workers was 0.73%, 4% and 11.94% respectively which was varying in different regions of different countries and in different time lines [10–12]. Till now only few studies reported seroprevalence among recovered COVID-19 patients. Seroprevalence of SARS-CoV-2 among potential convalescent plasma donor (recovered patients) is important to know the number of persons developing antibody after COVID-19 infection. For any successful blood/plasma donor recruitment program, analysis of donor deferral pattern is very crucial. The policies of recruitment and retention of blood donors depend on the donor deferral pattern. It is also important to assess the stability of SARS-CoV-2 antibodies to estimate for how long an individual might be protected from re-infection and to know how long this antibody might persists. We thus planned this study with an aim to know the seroprevalence of SARS-CoV-2 among potential convalescent plasma donor and analyse the reasons for their deferral. We also evaluated the stability of anti-SARS-CoV-2 antibodies in repeat convalescent plasma donors.

2. Materials and methods

This study was conducted in the Department of Blood bank, at Goa Medical College a tertiary care referral centre in western India after obtaining ethical approval from the institutional ethics committee and written informed consent was obtained from the convalescent plasma donors.

2.1. Study design

This study was prospective longitudinal observational study conducted on convalescent plasma donors (through apheresis) for a period of 5 months from August 2020 to December 2020. In the present study, we enrolled 400 prospective convalescent plasma donors over five-month period. All prospective plasma donors were enrolled during study period (universal sample size). The donors were selected on the basis of donor selection criteria as per the Drugs & Cosmetics Act, Government of India and Indian council of medical research (ICMR) guidelines [13,14]. Inclusion criteria for plasma donors were lab confirmed COVID-19 recovered patients (RT-PCR confirmed diagnosis of covid-19), 14 days symptoms free period after COVID-19 and presence of IgG antibody of COVID-19. Recovered COVID-19 patients' data were collected from hospital records and record of health services. Targeted donors were approached through phone calls. If they were willing to donate plasma and satisfied general eligibility criteria for plasma donation, they were called for screening in blood bank. Appointments were given to 8-10 prospective donors in a day for pre donation screening and health check-up. Each donor was assigned a unique identification code along with the sample IDs. The donors were tested for Complete blood count (CBC), Transfusion transmitted infection (TTI) (hepatitis B virus, hepatitis C virus, HIV, malaria, and syphilis), serum protein, blood grouping and IgG antibody of SARS-CoV-2. Final eligibility was ascertained through history, medical examination, laboratory tests and presence of suitable veins for apheresis. Suitability of vein for apheresis was decided by trained transfusion medicine doctor. After confirming final eligibility, 500 ml COVID-19 convalescent plasma (CCP) was collected by plasmapheresis (Spectra Optia USA). If the donor was not found to be eligible for plasma donation, then the donor was deferred after proper counselling. Donors who were deferred (for plasmapheresis) due to unsuitable vein were asked to donate plasma through whole blood donation but not included in this study. For repeat plasmapheresis donation, donors were allowed to donate again after 14 days if IgG antibody against SARS-CoV-2 was still present and satisfied the all above eligibility criteria.

2.2. IgG antibody against SARS-CoV-2

Every prospective plasmapheresis donor was tested for IgG antibody against SARS-CoV-2 through chemiluminescent microparticle immunoassay (CMIA) (Abbott Healthcare Pvt. Ltd, USA) according to manufacturer's protocol. CMIA assay is designed to detect qualitative IgG antibody to nucleocapsid protein of SARS-CoV-2. Result reported as reactive if cut off index (COI) $\geq$ 1.4 and non-reactive if cut off index COI < 1.4.

2.3. Seroprevalence of SARS-CoV-2 among potential convalescent plasma donor and reason for their deferral

The proportion of potential donors who developed antibodies after being positively tested for COVID-19 was calculated. Seroprevalence rate was seen as a percentage of donors who were positive for SARS-CoV-2 IgG antibody. Similarly, Seroprevalence rate was analysed with respect to gender, age, blood group, Rh factor, mode of treatment and repeat donors. Total number of deferred donors and for each reason deferral was analysed in term of percentage.

2.4. Stability of anti-SARS-CoV-2 antibodies in repeat convalescent plasma donors

Antibody was tested again in repeat convalescent donors at various time interval when they came for repeat plasma donation. Days of follow-up was calculated from the date of last symptoms. At the end of study period history of reinfection with SARS-CoV-2 (symptoms of repeat COVID-19 or any repeat positive RT-PCR test if done) was asked from all plasmapheresis donors (who donated convalescent plasma at our centre) telephonically.

2.5. Statistical analysis

The collected data were entered in excel (Microsoft office 365) coded and analysed. Analysis was carried out using Epi Info version 7.0 and SPSS version 23.0. All tests were considered significant if the *P* value was < 0.05. Paired student "t" test was used to calculate difference of means for quantitative variables. Pearson's chi square was used to evaluate qualitative data.

Table 1
Descriptive statistics of prospective plasma donors ($n = 400$) and seropositivity rate.

Parameter	Group	Total number	Positivity, *n* (%)	*P* value
Prospective plasma donors	Overall	400	348 (87%)	–
Gender	Male	380	331 (87.1%)	0.785
	Female	20	17 (85%)	
Age	< 30 years	173	148 (85.5%)	0.591
	30–50 years	211	185 (87.7%)	
	> 50 years	16	15 (93.7%)	
Blood group	O Group	124	108 (87%)	0.786
	A Group	123	107 (86.9%)	
	B Group	116	99 (85.3%)	
	AB Group	37	34 (91.9%)	
Rh factor	Rh positive	379	330 (87%)	0.857
	Rh Negative	21	18 (85.7%)	
Mode of treatment	Hospital	27	24 (88.8%)	0.522
	Covid care centre	186	158 (84.9%)	
	Home quarantine	187	166 (88.7%)	
Days of Ab testing after the date of last symptoms	< 30 days	11	10 (90.9%)	0.725
	30–60 days	339	293 (86.4%)	
	> 60 days	50	45 (90%)	
Repeat testing during follow up plasma donation	Two times[a]	9[a]	8 (88.9%)[a]	0.718
	Three times	2	2 (100%)	
	Four times	2	2 (100%)	
	Five times	2	2 (100%)	

[a] One donor donated only once. Repeat test was negative for SARS-CoV-2 antibody.

3. Results

A total 400 prospective plasmapheresis donors were included in the study. The mean age of donor was 32.94 ± 8.67 (20–57) years. Majority of the donors were male (95%). Overall, 348 donors were found to be reactive for anti-SARS-CoV-2 antibodies giving a seroprevalence rate of 87% (Table 1). Seroprevalence rate was compared with respect to gender, age, blood groups, Rh factor, mode of treatment, day of Ab testing from the date of last symptoms and repeat plasma donation (Table 1). There was no significant difference in seroprevalence rate between different sub-groups as *P* value was more than 0.05 (Table 1). No significant association was seen with either of blood groups or Rh factor in regard to SARS CoV-2 antibodies. Seroprevalence rate was similar between hospitalized patients Vs home quarantine patients (88.8% vs. 88.7%, $P = 0.52$). SARS-CoV-2 antibody was present even if antibody testing was done after 60 days of last symptoms of COVID-19 disease.

3.1. Deferral reasons of plasmapheresis donors

Although there was 87% seroprevalence rate for IgG SARS-CoV-2 antibodies, 25.5% of prospective plasmapheresis donors were deferred due to various reasons (Table 2). Most common reason for deferral was negative IgG SARS-CoV-2 antibody (13%) followed by absenteeism of eligible screen donors (6.7%), low Hb (1.7%) and poor veins for plasmapheresis (1.7%).

Table 2
Reasons for deferral of prospective plasmapheresis donors [$n = 102$ (25.5%)].

SN	Reason of deferral	Number of donors
1	Non-reactive (Negative) of IgG covid antibody	52 (13%)
2	Absenteeism of eligible screen donors (Not willing after pre donation screening)	27 (6.7%)
3	Low Hb	7 (1.7%)
4	Poor veins for plasmapheresis	7 (1.7%)
5	HCV reactive	4 (1%)
6	HBsAg reactive	2 (0.5%)
7	Under weight	1 (0.25%)
8	Syphilis reactive	1 (0.25%)
9	Gilbert syndrome	1 (0.25%)

3.2. Stability of anti-SARS-CoV-2 antibodies

In all, 15 plasmapheresis donors were tested for antibody again after various time interval when they came for repeat plasma donation (Table 3). Out of 15 donors, 14 (93.3%) were positive for SARS-CoV-2 antibodies during testing for repeat plasmapheresis. It was observed that antibodies against SARS-CoV-2 were present in five donors for more than 100 days after the last symptoms with the longest persistence of 109 days in one of the donors (Table 3). Till the end of study period, no plasmapheresis donor developed symptoms of repeat COVID-19 infection. Mean follow up period for repeat COVID-19 infection was 58 days.

4. Discussion

Estimation of seroprevalence is necessary for controlling the transmission of SARS-CoV-2 as well as to measure the burden of disease. It is also helpful for the government to develop policy for vaccination in vulnerable population. In our study seroprevalence in recovered COVID-19 patients was 87%. It was similar compared to the study of PLACID Trial from the India (83%) [14]. In another study by Luchsinger et. al. seroprevalence was 79.4% in convalescent plasma donors [15]. In our study 13% of the recovered patients with past RT-PCR confirmed diagnosis of COVID-19 did not develop SARS-CoV-2 antibodies (Abs). So, with these results we can say that all lab confirmed COVID-19 recovered patients did not develop SARS-CoV-2 antibodies due to many reasons. Early Ab testing, late Ab testing (after Ab disappear), false positive RT-PCR test, false negative Ab testing and poor sensitive test for antibody detection could be possible reasons. In the absence of SARS-CoV-2 antibodies these people may be susceptible for developing repeat COVID-19 infection.

In our study seroprevalence rate between male and female and different age groups of plasmapheresis donors were similar. This was in contrast to study by Ray et. al. where with the increasing age of donor, higher IgG content was noted [16]. This may be due to difference in qualitative and quantitative evaluation of SARS-CoV-

Table 3
Follow-up data of antibodies in repeat convalescent plasma donors.

S.No.	Sample ID = (Registration ID)	31–45 days[a]	46–60 days[a]	61–80 days[a]	81–100 days[a]	101–120 days[a]
1	ID0024		2.4		Neg.	
2	ID0029	9.3	9.2	9.5	8.2	8.1
3	ID0071	2.9	3.2		2.9	
4	ID0128		6.4	5.6	4.9	4.1
5	ID0129		6.3	5.3	4.6	3.1
6	ID0130		8.7	8.2	7.1	
7	ID0153	8.3	7.9	7.4	6.2	4.9
8	ID0165			7.4		2.3
9	ID0179	5.3		3.4		
10	ID0287	7.9	6.9			
11	ID0291		6.4		3.9	
12	ID0307	7.5		6.2		
13	ID0334		4.9		3.3	
14	ID0359	8.1	6.3			
15	ID0381	7.3	5.8			

[a] Day of antibody testing after the date of last symptoms.

2 IgG antibody in our study and study by Ray et. al. respectively. Comparison of seroprevalence rate was done between different blood groups and Rh factor of plasmapheresis donors and no significant association were noted. Similar finding was noted in other studies as well [12,17]. Previously blood group association with COVID-19 infection has been noted in some studies. Blood group A people have been found to be more at risk as compared to those with blood group O [1,18]. However, in our study no such association was observed and this could be an incidental finding in few studies without any possible explanation. Seroprevalence rate was similar between hospitalized patients (moderate to severe symptoms) Vs home quarantined patients (mild symptoms). This was in contrast to initial postulation that hospitalized patients (severe symptoms) developed high titre antibody. This finding is similar in study by Ray et. al. where plasma donors IgG content was not correlated with WHO clinical progression score for the COVID-19 disease [16]. Seroprevalence rate was compared between different day of antibody testing from the date of last symptoms and no corelation was found. By these findings we can say SARS-CoV-2 IgG antibodies start developing after 14 days of infection and persist for 2-3 months or longer. Similar findings were noted in by Siddiqui et. al. in seroprevalence and stability study [19].

In our study qualitative IgG antibody detection was done through chemiluminescent microparticle immunoassay (CMIA) (Abbott Architect) which detect antibody against the nucleocapsid protein of SARS-CoV-2. There were many other platforms to detect antibody against SARS-CoV-2 like Roche Anti-SARS-CoV-2 total assay (IgA, IgM, and IgG), Ortho-Clinical Diagnostics VITROS AntiSARS-CoV-2 IgG test through CLIA method that detects antibodies against the RBD of the spike protein, Ortho-Clinical Diagnostics VITROS AntiSARS-CoV-2 which measures total antibody {including IgA, IgM, and IgG (S1)} to SARS-CoV-2 and rapid card test to detect total IgG and IgM antibody against SARS-CoV-2. In study by Setia et. al. performance evaluation for above mentioned methods and seroprevalence of SARS CoV-2 in Frontline Healthcare Workers were done. They reported overall sensitivity for Abbott was 71%, Roche 96%, Ortho (both total and IgG(S) 99% and rapid card test 56%. They concluded assay performance depends on assay design (total IgM & IgG antibodies versus IgG alone), choice of antigen, and time of sample testing from the onset of disease [20]. It is currently not known whether the detection of antibodies that bind these proteins (nucleocapsid protein, RBD of the spike protein) predicts neutralizing activity or protection against infection [21].

Non-reactive (Negative) SARS-CoV-2 IgG antibodies was the main reason for plasmapheresis donor deferral followed by absenteeism of eligible screen donors. The probable reason for absenteeism could be that initially we had only one apheresis machine and due to huge number of donors, few donors had to wait for whole day for their turn to donate plasma. Due to this problem many potential donors did not turn up for plasma donation after pre donation testing (screening). After a month the institute procured another apheresis machine and then this problem was resolved. Low Hb and poor veins for plasmapheresis were other reasons for plasmapheresis donor deferral. Similar reasons for deferral were noted in other studies as well [22,23].

Till now there is limited data published on the SARS-CoV-2 antibody stability from India. We could follow up few of the seropositive plasmapheresis donors for antibody testing again during repeat plasmapheresis. In our study only one donor become negative for SARS-CoV-2 IgG antibody during testing for repeat plasmapheresis. In few of our plasma donors, antibody was present for more than 100 days after COVID-19 infection and this was the maximum duration of follow up that could be done. We can suggest these antibodies as protective as none of our plasmapheresis donor develop COVID-19 infection (symptoms of reinfection) again till the end of five months study period. This finding was similar with review by Roy et al. where they conclude reinfection with SARS-CoV-2 seems unlikely [24]. More such studies are required to gain more confidence about protective nature of these antibodies. There are few limitations of this study. Major limitation of our study was use of qualitative method for antibody detection and unavailability of precise quantitative test for IgG antibody titre or neutralizing antibody titre. Another limitation was long term follow up of donors for antibody stability and reinfection against SARS-CoV-2 were not done. RT-PCT test was not done to check for reinfection and only telephonically history was asked.

5. Conclusion

We conclude that all patients do not develop IgG antibodies after SARS-CoV-2 infection (Seroprevalence rate 87%). Negative SARS-CoV-2 IgG antibodies was the main reason for plasmapheresis donor deferral. Once developed these (SARS-CoV-2 IgG) antibodies persist for quite some time (varying period) and provide protection against reinfection. This study could be useful to understand the infection recovery and re-infection pattern. Long term follows up evaluation of durability and protective nature of this antibody may help us to identify population at risk against SARS-CoV-2 reinfection.

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Disclosure of interest

The authors declare that they have no competing interest.

Ethical approval

Ethical approval was taken by Institute ethical committee before start of the study.

Donor consent

Consent was taken from all participant convalescent plasma donors.

Contribution of authors

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All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

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