



Research Article

Investigating the Correlation Between ABO Blood groups in COVID-19 Convalescent Plasma Donors and anti-SARS CoV-2 IgG Antibody Levels: A Retrospective study

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Abstract:

Background and Objectives: ABO blood groups have been associated with the pathogenesis of various diseases and their association with COVID-19 disease severity and anti-SARS CoV-2 IgG antibody response have been largely studied. This study aimed at determining the association between COVID-19 convalescent plasma (CCP) donor ABO blood groups and post-infection anti-SARS CoV-2 IgG antibody levels.

Materials and Methods: A total of 404 CCP donors were included in the study conducted from September 2020 to May 2021. Donor samples were tested for anti-SARS-CoV-2 IgG antibody level (S/CO value) using VITROS Anti-SARS-CoV-2 IgG chemiluminescence immunoassay which is a qualitative assay. Blood groups and number of donations with low (S/CO=1.0-4.42), medium (S/CO=4.62-18.45) and high levels (S/CO >18.45) of the antibody were compared using Chi-square test. Antibody levels during three stratified post-infection periods (0-60 days, 61-120 days and >120 days) in different blood groups were compared using Kruskal Wallis ANOVA test, while inter-blood group comparisons of antibody levels were performed by independent t-tests.

Result: Mean antibody level was the lowest in O group (17.22±9.2) followed by A (17.59±11.4), B (18.21±10.7) and AB (19.91±9.7) groups. Analysis of antibody levels according to blood groups was statistically insignificant (p value > 0.05). Antibody levels during stratified post-infection periods according to blood groups as well as inter-blood group comparisons of antibody levels were also not significant.

Conclusion: AB blood group had the highest but not significant anti-SARS CoV-2 IgG antibody levels. However, ABO blood groups do not influence antibody levels in CCP donors irrespective of post-infection period.

Keywords: COVID-19, convalescent plasma, ABO blood group, anti-SARS CoV-2 IgG antibodies, antibody levels, CCP donors

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INTRODUCTION

In mid-December 2019, COVID-19 caused by severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) emerged in China. WHO (under International Health Regulations) declared this outbreak as a “Public Health Emergency of International Concern” (PHEIC) in January 2020 and it was subsequently declared COVID-19 a pandemic in March, 2020 by WHO. According to WHO Coronavirus (COVID-19) dashboard, there have been 770 million confirmed cases globally which includes 6.9 million deaths, reported to WHO. This sudden pandemic has affected the global population. (WHO, 2023) Since there were a limited number of therapeutic and no prophylactic treatment options, convalescent plasma, in this case, known as COVID-19 convalescent plasma (CCP) was considered as an important mode of therapy to be given in early stages of the disease. This method of passive transfer of antibodies has been in use since the pre-antibiotic era for infections involving the respiratory system and has recently been applied for treating infections like Ebola, SARS, MERS and H1N1 (Hacibekiroğlu et al., 2021).

Blood groups have been associated with pathogenesis of various diseases. Hence it has been suggested that an association between ABO blood groups and COVID-19 severity is possible, with blood group A having highest severity while blood group O having the least (Cooling, 2015; Wu et al., 2020; Fan et al., 2020; Li et al., 2020).

The S1 subunit of the S protein surrounding the SARS-CoV-2 virus mediates its entry into the host cells by binding to the angiotensin converting enzyme-2 (ACE2). (Cevik et al., 2020). The IgG type rather than IgM anti-A isoagglutinins can inhibit the interaction between S protein and ACE2, thus blocking the virus entry. (Guillon et al., 2008; Focosi, 2020). Since IgG is more common than IgM in blood group O, it supports the occurrence of lesser severity of infection in blood group O individuals compared to non-O individuals. A study among COVID-19 infected patients with respiratory symptoms to find out the biological basis for association between ABO type and COVID-19 infection has demonstrated genetic susceptibility at the ABO group locus (GWAS, 2020).

Several authors attempted to find out a correlation between anti-SARS CoV-2 IgG antibody levels in COVID-19 convalescent plasma (CCP) donors and their ABO-type. However, they could not find any convincing results as the number of cohorts related to COVID-19 were very less. Some of the previous studies have reported the relation of ABO blood type with COVID-19 infection, where non-O blood groups were linked with an increased risk of disease compared to blood group O (Li et al., 2020; Cheng et al., 2005). However, some studies have been unsuccessful to confirm such type of relationship (Žiberna et al., 2022; Almadhi et al., 2021).

In this study the effect of donor characteristics (age, gender, ABO blood group, interval between SARS-CoV-2 recovery and CCP collection) on anti-SARS CoV-2 IgG antibody levels in the CCP product was determined. This study aimed at determining the association between CCP donor ABO blood groups and anti-SARS CoV-2 IgG antibody levels.

MATERIALS AND METHODS

Study Setting and Design

This was a retrospective observational study conducted at the Department of Transfusion Medicine and Blood Bank of a tertiary care center in Eastern India, from September 2020 to May 2021.

Study Population

People who had recovered from COVID-19 were appealed to come forward and donate convalescent plasma at our centre. A drive for plasma donation was conducted via mass media (newspapers and radio) as well as social media platforms (Facebook and twitter) while some donors were contacted through emails and text messages (SMS, Whatsapp and telegram). Donors were selected as per guideline of ministry of health and family welfare, Standards of Blood banking and Drug and Cosmetics (second amendment) Rules, 2020 (cdsco.,2020).

Eligible donors included

- a. COVID-19 recovered men or nulliparous women
- b. Between 18 to 65 years of age
- c. Weight more than 50 kg
- d. Were confirmed case of COVID-19, tested by a RT-PCR test, and have had mild symptoms of COVID-19, at least fever and cough. The symptoms must have completely resolved:
 - i. 28 days before donation; or
 - ii. 14 days before donation, with two negative RT-PCR test results for SARS CoV-2 from nasopharyngeal swabs collected 24 hours apart.

All routine screening tests, including ABO and Rh blood grouping, complete blood counts, screening for infections like HIV, hepatitis B, hepatitis C, syphilis, and malaria and total serum protein were conducted according to the Drugs and Cosmetics (second amendment) Rules, 2020 (cdsco.,2020).

Exclusion criteria

- a. All those who were not eligible to donate blood as per guidelines(cdsco.,2020).
- b. Multiparous females.
- c. Those who did not gave consent to participate in the study.
- d. All transfusion transmitted infections (TTI) sero-reactive donors.
- e. All donors with positive irregular antibody screen.

Serological Tests

ABO and Rh blood group

Blood grouping was performed manual by conventional tube technique (CTT). For cell (forward) grouping commercial anti-A, anti-B and anti-D antisera were used [Eryclone®, Tulip Diagnostics (P) Ltd, India]. For serum (reverse) grouping, in-house prepared pooled A cells, B cells and O cells were used. (Olsson et al., 2020; DGHS., 2022) Agglutination reactivity was graded from 0 to 4+. Agglutination reactions of weak to 2 + were verified by repeat testing.

Indirect Coomb's Test (ICT)

ICT was performed by CTT using donor's serum and in-house prepared pooled O cells (Olsson et al.,2020; DGHS.,2022).

Antibody Screening

Samples with a positive ICT result were then tested by a 3-cell panel for antibody screen (ID-DiaCell I-II-III Asia, Bio-Rad Laboratories, Inc., California, USA) according to the manufacturer's instructions using CTT. Agglutination reactivity was graded from 0 to 4+. Agglutination reactions of weak to 2 + were verified by repeat testing.

SARS Cov-2 Igg Antibodies Test

The surface of the virus SARS CoV-2 causing COVID-19 is covered by glycosylated S protein which mediate their entry into the host cells by binding to the cell receptor angiotensin converting enzyme-2 (ACE2). S protein consists of S1 and S2 subunits. Receptor Binding Domains (RBD) of the virus is situated in the S1 subunit which binds it to the ACE2 receptor. The neutralising antibodies (Nabs) targeting the S protein prevent the entry of virus into the host cells. These Nabs are the main ingredients in the convalescent plasma used to treat COVID-19 patients.

Donor samples were tested for SARS-CoV-2 IgG antibody using VITROS Anti-SARS-CoV-2 IgG chemiluminescence immunoassay (Ortho Clinical Diagnostics {OCD}, Raritan, NJ, USA) on VITROS ECiQ 3600 (Ortho Clinical Diagnostics Inc.) according to manufacturer's instructions. Binding antibody levels from sera were tested. The Ortho-Clinical IgG CLIA is a qualitative assay based on a recombinant form of the SARS-CoV-2 spike subunit 1 protein (Theel et al.,2020).

As per the study done by Joyner et al, the cut offs for the antibody, based on 20th and 80th percentiles of distribution of S/CO ratios, and were classified according to levels of protective antibodies in CCP which is indicated in table 1, (Joyner et al.,2020).

Table 1. Cut offs and levels of protective antibodies

VITROS® Anti-SARS CoV-2 IgG S/CO	Level of protective antibodies
1.0-4.62	Low level
4.62-18.45	Medium level
>18.45	High level

CCP Donation

Plasma donations were performed on a cell separator (Com.Tech, Fresenius Kabi, Germany). From each donor 400ml of plasma was collected which was split into two parts of 200ml each for patients. Serum samples were stored at or below -30 for neutralisation test to be performed in due course of time.

Statistical Analysis

Data was analysed using SPSS software. Mean, percentage and standard deviation were calculated. Blood groups and number of donations with low (S/CO=1.0-4.42), medium (S/CO=4.62-18.45) and high levels (S/CO >18.45) of the antibody anti-SARS CoV-2 IgG were compared using Chi-square test. The antibody levels during three stratified post-infection periods (0-60 days, 61-120 days and >120 days) in different blood groups were compared using Kruskal Wallis ANOVA test, while inter-blood group comparisons of antibody levels were performed by independent t-tests. A p-value of <0.05 was considered as statistically significant.

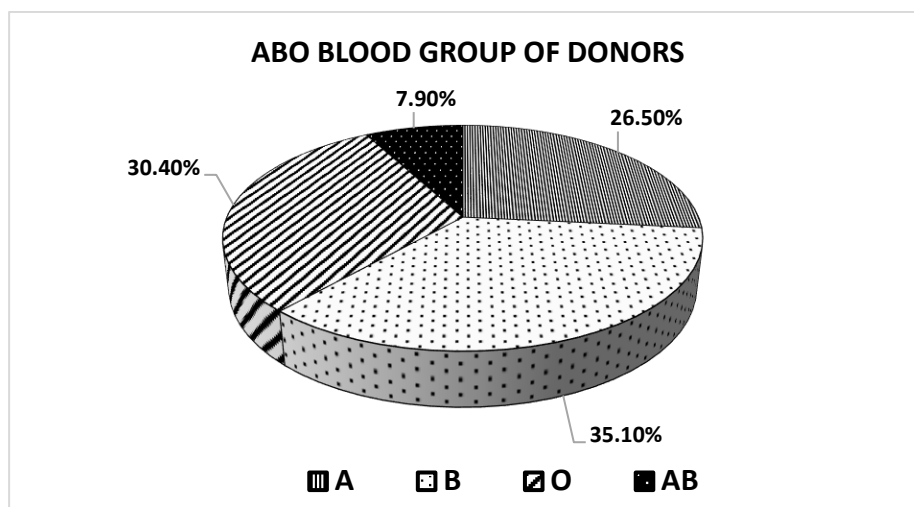
Ethical Approval

All donors who gave consent to participate in the study were included. The study was approved by the institutional review committee (IRC) and the institutional ethics committee (IEC).

RESULTS

A total of 404 CCP donors were included in the study out of which 396 (98%) were males and 08 (02%) were females. The mean age of the donors was 33.53±9.18 years. B was the most common blood group among donors. The proportion of donors with different ABO blood group is shown in figure 1.

Figure 1. ABO blood group of donors



Donor demographic characteristics according to ABO blood groups were analysed which is summarised in table 2

Table 2. Donor Demographic Characteristics According To ABO Blood Groups

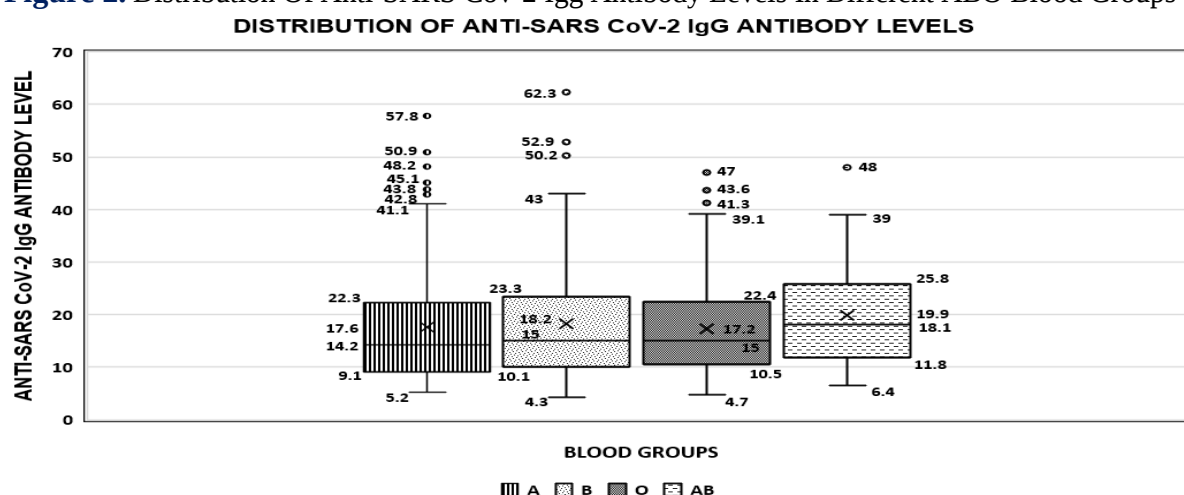
CHARACTERISTICS	OVERALL	BLOOD GROUP 'A' (N=107)	BLOOD GROUP 'B' (N=142)	BLOOD GROUP 'O' (N=123)	BLOOD GROUP 'AB' (N=32)
MEAN AGE $\pm$ SD (years)	33.53 $\pm$ 9.18	33.86 $\pm$ 9.77	33.78 $\pm$ 9.08	33.29 $\pm$ 9.1	32.12 $\pm$ 7.97
AGE GROUP:[n (%)]					
<30 years	162 (40%)	39 (36.4%)	59 (41.5%)	52 (42.3%)	12 (37.5%)
30-50 years	220 (54.4%)	61 (57%)	76 (53.5%)	64 (52%)	19 (59.4%)
>50 years	28 (6.9%)	12 (11.2%)	07 (4.9%)	08 (6.5%)	01 (3.1%)
GENDER:[n (%)]					
MALE	396 (98%)	107 (100%)	140 (98.6%)	120 (97.6%)	29 (90.6%)
FEMALE	08 (02%)	00 (0%)	02 (1.4%)	03 (2.4%)	03 (9.4%)
HEALTHCARE WORKER:[n (%)]					
YES	21 (5.2%)	06 (5.6%)	05 (3.5%)	07 (5.7%)	03 (9.4%)
NO	383 (94.8%)	101 (94.4%)	137 (96.5%)	116 (94.3%)	29 (90.6%)

The donors were divided into three age groups (18-30 years, 30-50 years and 51 -60 years) of which most of them belonged to age group 30-50 years [n=220 (54.4%)] and in them the most common blood group was B [n=76 (34.5%)]. The most common blood group among males was B [n=140 (35.4%)] while that among females were both O and AB [n=03

(37.5%)). Among healthcare professionals, the most common blood group was O [n=07 (33.3%)].

Anti-SARS CoV-2 IgG antibodies were measured by qualitative assay (VITROS anti-SARS CoV-2 IgG chemiluminescence immunoassay, Ortho Clinical Diagnostics) whose results are based on sample signal-to-cut-off ratio (S/CO) that indicates the approximate levels of these antibodies. The value <1 is considered negative while ≥ 1 is considered positive. The values were recorded according to blood groups and their distribution is shown in figure 2.

Figure 2. Distribution Of Anti-SARS Cov-2 Igg Antibody Levels In Different ABO Blood Groups



Mean antibody level was the lowest in O group (17.22 ± 9.2) followed by A (17.59 ± 11.4), B (18.21 ± 10.7) and AB (19.91 ± 9.7) groups.

To determine the association of anti-SARS CoV-2 IgG antibody levels with age and gender of the donors, Spearman's rho and t-value were calculated respectively. The association was found to be insignificant for both the characteristics with p-value more than 0.05 (p-value=0.11 for age; p-value=0.32 for gender).

The protective levels of anti-SARS CoV-2 IgG antibody levels categorised as low (S/CO=1-4.62), medium (S/CO=4.62-18.45) and high (S/CO>18.45) were compared with ABO blood groups using Chi-square test and the result was found to be statistically not significant (Chi-square value=3.07; p-value=0.79) as shown in table 3.

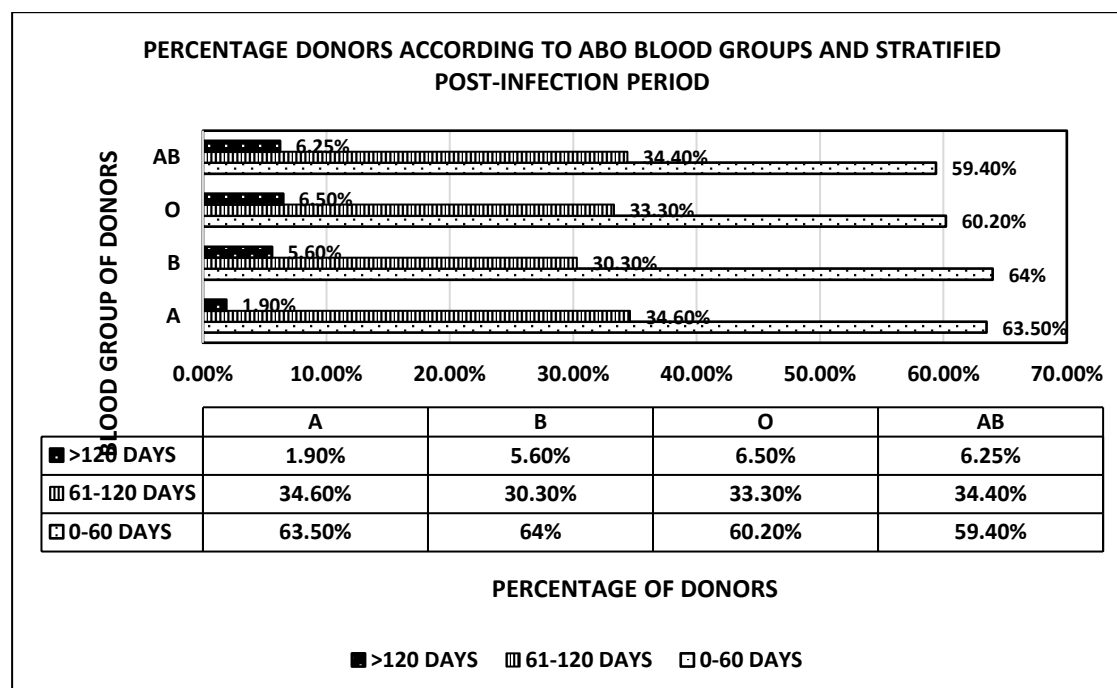
Table 3. Levels of SARS Cov-2 Igg Antibodies Measured By Quantitative Test According To Blood Groups

Blood group	Low level (1-4.62) N (%)	Medium level (4.62-18.45) N (%)	High level (>18.45) N (%)	Chi-square value	p-value
A (n=107)	0 (0%)	71 (66.35%)	36 (33.64%)	3.07	0.799
B (n=142)	02 (1.4%)	84 (59.15%)	56 (39.43%)		
O (n=123)	0 (0%)	80 (65%)	43 (35%)		
AB (n=32)	0 (0%)	18 (56.25%)	14 (43.75%)		

Also, an independent t-test was used to compare anti-SARS CoV-2 IgG antibody levels among the blood groups. P-values were calculated to compare blood groups O and A (p-value=0.39), O and B (p-value=0.21), O and AB (p-value=0.07), A and B (p-value=0.33), A and AB (p-value=0.15) as well as B and AB (p-value=0.20). These inter-blood group comparisons were not significant. The post-infection period for donors was divided into three phases, 0-60 days, 61-120 days and >120 days.

The percentage of donors with different ABO blood groups during these three stratified post-infection periods is depicted in figure 3.

Figure 3. Percentage Of Donors According To ABO Blood Groups In The Stratified Post-Infection Period



Lastly, the comparisons of antibody levels during stratified post-infection periods according to blood groups were done by using Kruskal Wallis ANOVA test which is shown in table 4. This comparison was also found to be statistically insignificant.

Table 4. Comparison of SARS CoV-2 IgG antibody levels in donors of different ABO types according to post-infection period.

Days after recovery from COVID-19	Mean antibody level [S/CO]	Blood group 'A' [S/CO]	Blood group 'B' [S/CO]	Blood group 'O' [S/CO]	Blood group 'AB' [S/CO]	p-value
0-60 days	17.9 [IQR=4.27-57.8] (n=252)	15.2 [IQR=5.24-57.8] (n=68)	15.1 [IQR=4.27-53.9] (n=91)	15.7 [IQR=4.7-47] (n=74)	22 [IQR=6.45-48] (n=19)	0.279
61-120 days	18.5 [IQR=6.28-62.3] (n=132)	12.8 [IQR=6.28-50.9] (n=37)	15.2 [IQR=7.68-62.3] (n=43)	13.4 [IQR=6.28-44] (n=41)	14.6 [IQR=6.93-39] (n=11)	0.338
>120 days	13.3 [IQR=6.21-43] (n=20)	14.14 [IQR=9.09-19.2] (n=02)	8.82 [IQR=6.21-43] (n=08)	9.59 [IQR=6.67-19.1] (n=08)	17.6 [IQR=11.8-23.4] (n=02)	0.433

DISCUSSION

Severity of COVID-19 has been associated with an individual's characteristics such as age and gender (Klein et al.,2020; Biswas et al.,2021).Maximum donors in our study belonged to age group 30-50 years with a frequency of 54.4%. In a study by Mahapatra, majority of the donors with positive anti-SARS CoV-2 antibodies belonged to 21-40 years (37.2%) while only 29.7% of the donors belonged to age group 40-60 years (Mahapatra., 2022). Male gender has been found to be more susceptible towards acquiring COVID-19 infection. (Klein et al, 2020) They require longer course of hospital stay and have higher rates of mortality in comparison to females (Mahapatra., 2022; Qin et al., 2020). In our study, number of male CCP donors was more than females (98% Vs 2%) similar to the study by Mahapatra (99.32% Vs 0.68%). (Mahapatra et al, 2022) Mean anti-SARS CoV-2 antibody levels were also higher in males (S/CO=17.95) as compared to females (S/CO=14.23) similar to previously published data (Robbiani et al., 2020). These higher levels can be related to more severe infection in males mounting increased inflammatory responses leading higher recruitment of B cells, thus increased antibody production.

During the pandemic a number of studies were done to find out whether ABO blood group affects the disease severity and/or susceptibility (Cooling., 2015; Li et al., 2020, Cheng et al., 2005; Almadhi et al., 2021). In the pre pandemic era, Cheng et al reported that group O individuals were less susceptible to SARS CoV infection in comparison to non-O individuals (Cheng et al., 2005). Majority of the CCP donors in our study belonged to blood group B which was similar to another study in India where type B donors had the highest frequency of anti-SARS CoV-2 antibodies (Mahapatra., 2022) However, a recent meta-analysis done by Golinelli et al showed that blood group A had this highest frequency (Golinelli et al., 2020).

Mean antibody titre was found to be the lowest in blood group O in our study figure 2. Similar finding was described by Gil-Manso et al and they hypothesised that the reason of higher antibody levels in non-O group individuals could be due to the need of more time to clear the virus, hence longer time of viral exposure leading to stronger immune response, regardless of the severity of the disease (Gil-Manso et al., 2022).

We tried to find out association between ABO blood groups and anti-SARS CoV-2 IgG antibody levels table 3. The results were not statistically significant similar to other studies worldwide (Dzik et al., 2020; Boudin et al., 2020). However, a study from Wuhan, China suggested a significant association of blood group A with COVID-19, but not with blood groups B, AB or O (Fan et al., 2020).

We compared the association of different ABO blood groups with anti-SARS CoV-2 IgG antibodies and this comparison was also not found to be statistically significant. After initial infection, the immune system of a person provides some level of life long protection against the same virus. This protection is provided by antibodies in the form of immunoglobulins produced by the B cells when the immune system is activated by virus entry into the body. These antibodies circulate in the body, recognise and bind to the viruses, neutralise them and thus prevent them from infecting other cells and cause their clearance from the body. However, the level of these antibodies declines with time. Anti-SARS CoV-2 IgG antibodies may decline as early as 3 months or may persist up to five months (Isho et al., 2020; Iyer et al., 2020). Some data has been published regarding persistence of this antibody for over 8 months to 1 year (Dan et al., 2021; Gallais et al., 2021). In our study, those donors who donated between 61 to 120 days post recovery from COVID-19 infection had the highest mean level of anti-SARS CoV-2 IgG antibodies table 4. Mahapatra et al showed this level to be the highest in those donors who donated within 60 days post recovery (Mahapatra., 2021). However, on calculating any correlation between antibody levels and duration between recovery from COVID-19 infection and CCP donation, there was no significant finding.

The study had a few limitations. Firstly, the sample size was small (404 participants). Secondly, there was a lack of control group with normal population who were asymptomatic yet were seropositive for anti-SARS CoV-2 IgG antibodies. Thirdly, we could not measure the neutralising antibody titres due to lack of resources.

In conclusion, we could not find out any significant association of anti-SARS CoV-2 IgG antibodies with ABO blood groups as well as with post-recovery periods. However, the higher mean levels of antibodies in non-O group individuals support the possibility of higher susceptibility and/or severity of infection in them and they should take more precautions. Since the highest mean antibody level was found in donors with AB blood group in our population, preferential recruitment of CCP donors of certain blood types can be done according to the antibody levels. CCP from such donors can be selectively used for manufacture of hyperimmune globulin products on commercial level.

Conflicts Of Interest:

None

Sources Of Funding:

None

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