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Original Research Article

Characteristics of convalescent plasma donors and their antibody titers in Bangladesh

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ABSTRACT

Background: Convalescent plasma is considered a promising therapy for severe COVID-19 disease. It is collected from the voluntary donors. Measurement of the antibody titer is necessary before transfusion to predict the outcome in the recipients. Characteristics of the convalescent plasma donors in Bangladesh and their antibody titers are not known.
Methods: Convalescent plasma was collected from the voluntary donors who survived the COVID-19 disease to transfuse to the severe COVID-19 patients under a randomized control trial. Total IgG antibody titer was measured in the donor plasma by indirect enzyme-linked immunosorbent assay. Data was collected in a preformed questionnaire before donor plasma collection.
Results: The median age is 32 (18-55) years. Fever, cough, sore throat, diarrhea was most common among 68.3% of the symptomatic participants and the remaining 31.7% were asymptomatic at the time when they were RT-PCR positive. Overall, 57.1% of participants had mild symptoms, 11.1% had moderate symptoms and none had severe symptoms. Participants' antibody titers were measured 41.68±14.072SD days after the RT-PCR positive date. Rapid qualitative test could not detect antibody in 11 (17.5%) potential donors. Of the remaining 52 (82.5%) antibody positive participants titer was measured in 43 participants and found 1:320 in 17 (27.0%) (n=63), 1:160 in another 17 (27.0%) (n=63) and 1:80 in rest of the 9 (14.28%) (n=63) Participants. The mean titer of the donors who were hospitalized during their illness (1:274.29) was statistically significantly higher (p=0.043, CI>95%). The mean titer was also higher in female than in male, symptomatic than asymptomatic participants and in donors of A positive blood group. However, these finding are not statistically significant. Antibody titer does not correlate with time of RT-PCR negativity from initial RT-PCR positivity, time from RT-PCR positivity to titer date, age and body mass index.
Conclusions: All RT-PCR positive COVID-19 patients subsequently may not develop antibody. Although antibody titer among hospitalized symptomatic patients was significantly higher, further study is needed to recommend optimal convalescent plasma donor criteria.

Keywords: Antibody titer, Convalescent plasma, COVID-19, Plasma donation

INTRODUCTION

COVID-19 (Coronavirus disease-2019) is a public health emergency of international concern. Convalescent plasma therapy has historically been used as a treatment of various infections especially during epidemics of respiratory infections.[1,2] It contains pathogen-specific neutralizing antibodies, which can neutralize viral particles, and treatment with convalescent plasma or hyperimmune immunoglobulins confers passive immunity to recipients.[3]

Preliminary evidence in humans and rhesus macaques has shown that reinfection with SARS-CoV-2 is not likely, with most (but not all) patients who recovered from

COVID-19 producing sufficient amounts of neutralizing antibodies to protect against reinfection.[4] This implies that convalescent plasma from people who have recovered from SARS-CoV-2 infection is capable of conferring passive immunity. A recently reported case series also indicated sufficient neutralizing antibody titers in convalescent plasma to neutralize SARS-CoV-2 in five COVID-19 patients, who all recovered after treatment.[5]

The efficacy of convalescent plasma therapy relies on robust antibody response in convalescent plasma donors. Measurements of antibody response among patients with COVID-19 demonstrate that the majority develop IgM and IgG within 2 weeks of symptom onset, with specificity towards receptor-binding domain (RBD) and spike protein viral epitopes correlating with virus neutralization.[6-8] In Bangladesh, there is no data regarding the COVID-19 convalescent plasma antibody titer. So, we plan to observe donor characteristics and their antibody titer in this report.

The general objective of the study was to observe the level of antibody formation against SARS CoV-2 virus among Bangladeshi people. Specific objectives were to find out the relationship of antibody titer with age, gender and blood group of participants. We also observed variation of antibody titer according to disease severity.

METHODS

During this pandemic US food and drug administration issued emergency use authorization (EUA) for the use of convalescent plasma.[9] A committee formed by the directorate general of health services (DGHS) of Bangladesh Government designed a trial protocol for the collection of donor plasma and transfusion of convalescent plasma to the eligible patients. Ethical permission for that trial named "Convalescent plasma therapy in severe and critically ill COVID-19 patients: a randomized clinical trial to observe the efficacy and safety" was taken from the ethical review committee (ERC) of Dhaka Medical College. The donor plasma collection part of the study was conducted at Dhaka medical college hospital (DMCH). We used the existing hospital infrastructure and personnel of the transfusion medicine department and COVID-19 dedicated medicine department of DMCH. The study was conducted from 15th May to 30th July, 2020. The voluntary convalescent plasma donors presented at the transfusion medicine department were our study population. Informed written consent was taken from voluntary donors. We consecutively included 63 participants according to selection criteria. Donors of age greater than 18 years with a prior diagnosis of COVID-19 by positive RT PCR test who recovered completely from COVID-19 according to recovery criteria were included in this study. The recovery criteria were-Resolution of symptoms at least 14 days before donation and two consecutively negative results of nasopharyngeal swab SARS CoV-2 of real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assay (based on investigational COVID-19 convalescent plasma-guidance for Industry, U.S. food and drug administration, April 2020). The donors who were unwilling to provide consent for the study, who did not meet all allogeneic blood donor eligibility criteria, and whose blood donation was unsuccessful due to difficulty of venous access were excluded from the study. At first qualitative rapid kit test was done to observe the presence of antibodies. Convalescent plasma was then collected by apheresis from COVID-19 recovered antibody-positive donors. Five ml of the donated plasma was sent for titer measurement. Data was collected in a preformed questionnaire. All statistical analyses were done using SPSS statistical software version 23 (IBM Corp).

Antibody titer detection method

In this study, indirect enzyme-linked immunosorbent assay (ELISA) designed for total IgG antibody detection was used. According to the ELISA manufacturer's instructions, the plasma sample was serially titrated in the order of 1:1, 1:20, 1:40, 1:80, 1:160 and 1:320. Dilution was achieved by adding plasma from unexposed donors. This methodology had been adopted from a Chinese study.[10]

RESULTS

The mean age of the participants is 32.14 years and the median age is 32 (18-55) years. Of them, 92.1% male and 7.9% are female. The majority of the donors are health care workers (doctors-44.4%, nurses-3.25). Among other professional's service-12.7%, businessman-11.1%, student-11.1%, policeman-6.3%, are also common. 87.3% of participants came from urban areas, 9.5% from suburban areas and 3.2% from villages. Among different blood groups, B positive donors were most common (33.3%). Other blood groups were O positive-31.7%, A positive-23.8%, AB positive-9.5%, O negative-1.6%. We found BMI range of the participants to be 25.444±3.1506. Among the participants, 95.2% mentioned that they intake a moderate amount of protein (at least 2 pieces of fish or meat) daily. Rest of them intake proteins more than 3 times a day. 95.2% of the participants were graduate or higher educated. 68.3% of the participants were symptomatic and 31.7% were asymptomatic at the time when they were RT-PCR positive. Fever, cough, sore throat, diarrhea were the most common symptoms. Overall, 57.1% of participants had mild symptoms, 11.1% had moderate symptoms and none had severe symptoms. 2 participants had hypertension, 1 had diabetes mellitus and chronic kidney disease others did not have any co-morbid conditions. During the period of RT-PCR positivity, most participants (79.4%) got home management. The rest of them were managed at COVID-19 dedicated hospitals, 19.0% at wards and 1.6% at high dependency units. No participants needed ICU management. Participants' RT-PCR test became negative17.56±5.891 SD days after positive RT-PCR test. Their titers were measured 41.68±14.072 days after RT-PCR positive date. Among 63 participants rapid qualitative test could not detect antibody in 11 (17.5%) patients, of them 4 was asymptomatic, 6 having mild

symptoms and 1 moderate symptom. Remaining 52 (82.5%) participants demonstrated positive rapid qualitative antibody test and titer was measured in 43 of them. The titer of the rest of 9 (14.28%) participants could not be measured due to lack of resources at the study site. The titer was measured 1:320 in 17 (27.0%) participants, 1:160 in another 17 (27.0%) and 1:80 in rest 9 (14.28%) participants (Table 1).

Table 1: COVID-19 convalescent plasma donor characteristics.

Characteristics	Number (%)
Number of participants	63
Sex	
Male	58 (92.1)
Female	5 (7.9)
Age (Years) mean (SD)	32.14 (7.492)
BMI mean (SD)	25.444 (3.1506)
Clinical classification	
Asymptomatic	20 (31.7)
Mild symptoms	36 (57.1)
Moderate symptoms	7 (11.1)
Severe symptoms	0
Symptoms	
Fever	36 (85.7)
Cough	28 (66.6)
Sore throat	12 (28.6)
Diarrhea	10 (23.8)
Shortness of breath	7 (16.6)
Body ache	4 (9.5)
Anosmia	3 (7.1)
Headache	2 (4.7)
Myalgia	2 (4.7)
Eye ache	1 (2.4)
Weakness	1 (2.4)
Redness of eye	1 (2.4)
Running nose	1 (2.4)
Comorbidities	
Hypertension	2 (3.2)
Diabetes mellitus	1 (1.6)
Chronic kidney disease	1 (1.6)
Home management	50 (79.4)
Ward management	12 (19)
HDU	1 (1.6)
Interval between RT-PCR positive date and RT-PCR negative (days)-mean (SD) (min-max)	17.56 (5.891) (7-37)
Interval between RT-PCR positive date and plasma donation (days)-mean (SD) (min-max)	41.68 (14.072) (24-106)
Antibody not detected	11 (17.5)
Antibody detected	52 (82.5)
Titer measured	43
1:320 (n=43)	17 (39.5)
1:160 (n=43)	17 (39.5)
1:80 (n=43)	9 (20.9)
ABO-n (%)	
A positive	15 (23.8)
A negative	0
B positive	21 (33.3)
B negative	0
AB positive	6 (9.5)
AB negative	0
O positive	20 (31.7)
O negative	1 (1.6)
Antibody titer (1:)-mean (SD) (range)	206.51 (97.563) (1:80-1:320)

Mean titer was higher in female patients than in male patients but this is not statistically significant (p=0.775). The mean titer of the non-hospitalized participants was 1:193.33 and hospitalized participants was 1:274.29 which is statistically significantly higher (p=0.043, CI>95%). The mean titer of the asymptomatic participant donors was1:196.92 and symptomatic donors was 1:210.67. Although the mean titer was higher in the donors who had symptoms during the period of their RT-PCR positivity it is not statistically significant (p=0.677, CI>95%). The mean titer measured more than 30 days after the initial RT-PCR positive date was 1:213.33, which is higher than the mean titer (1:190.77) measured before 30 days after the initial RT-PCR positive date, but it is not statistically significant (p=0.493, CI>95%). The mean titer done in participant donors whose RT-PCR was negative more than 15 days after the initial positive RT-PCR test was higher (1:223.45) than the mean of titer (1:171.43) of those participants whose RT-PCR became negative before 15 days of initial positivity. However, this finding is also not statistically significant (p=0.102, CI>95%). Mean antibody titer was highest in donors of A positive blood group among the groups having more than one donor. AB positive and O positive was second and third respectively. The difference of mean antibody titer between blood groups was statistically significant (p=0.006), but antibody titer deference within groups was not significant (Table 2).

Pearson correlation detected a weak positive (0.253) correlation between antibody titer level and time of PCR negativity from initial RT-PCR positivity. Very weak positive (0.012) correlation was found with time from PCR positivity to titer date while weak (-0.183) and very weak (-0.095) negative correlation was found with age and body mass index. However, all the correlations found here are statistically not significant (p>0.05) (Table 3).

Table 2: Comparison of antibody titer levels (n=43).

Variables	Number	Mean titer (1:)	Standard deviation	P value
Gender (a)				
Male	39	205.13	96.760	0.775
Female	4	220.00	120.000	
Hospitalization (a)				
No	36	193.33	96.333	0.043*
Yes	7	274.29	78.072	
Clinical symptoms (a)				
Asymptomatic	13	196.92	106.410	0.677
Symptomatic	30	210.67	95.084	
Time from RT-PCR date to RT-PCR PCR negativity(a)				
15 days and less	14	171.43	87.956	0.102
More than 15 days	29	223.45	98.861	
Time from RT-PCR date to titer date (a)				
30 days and less	13	190.77	110.940	0.493
More than 30 days	30	213.33	92.376	
Blood group (b)				
O positive	13	178.46	87.354	0.006*
A positive	9	302.22	53.333	
B positive	16	175.00	93.381	
AB positive	4	180.00	100.664	
O negative	1	320.00	-	

a- independent sample t-test was done to compare means, b- ANOVA was done to compare mean

Table 3: Pearson correlation between antibody titer and age, BMI, time from RT-PCR positive date to PCR negative date and titer date (n=43).

Variable		Time from RT- PCR positive date to titer date	Time from RT-PCR positive date to PCR negative date	BMI	Age
Antibody titer	Pearson correlation	0.012	0.253	-0.095	-0.183
	P value	0.940	0.102	0.545	0.239

DISCUSSION

In this study, we found that 17.5% of participants did not develop a detectable antibody against SARS CoV-2 although most of them were symptomatic during their RT-PCR positivity. The characteristics of these patients were similar to other patients. It is not clear whether they recovered without generating detectable antibodies or the

antibody level had been decayed to an undetectable level. Some studies also found a similar finding and assumed that other immune responses, including T cells or cytokines, may have contributed to the recovery of these patients.[11-13]

In one study, the most common self-reported symptoms at the onset of illness of the donors were fever (39, 80%) and cough (30, 61%). Other symptoms included shortness of breath, muscle aches, and diarrhea.[14] This finding is consistent with our study.

We found significantly higher titer among hospitalized participants than those who got home treatment. Titer was also higher among female than male, symptomatic than asymptomatic participants but it was not statistically significant. However, our findings are consistent with several other studies.[15-18]

Higher titer was found in participants those RT-PCR became negative more than 15 days later of the initial positivity. This is consistent with the study that showed a stronger antibody response is associated with delayed viral clearance.[19] Titer was higher in participants those titers done more than 30 days after initial positivity. This finding is roughly similar to the study that found the S-RBD-specific IgG antibody reaches higher levels after 4 weeks from the onset of COVID-19 symptoms.[20]

A weak positive correlation was found between antibody titer level and time of PCR negativity from initial RT-PCR positivity. A very weak positive correlation was found with time from PCR positivity to titer date while weak and very weak negative correlation was found with age and body mass index respectively. However, all the correlations found here are statistically not significant ($p>0.05$).

Mean antibody titer was highest in donors of A positive blood group among the groups having more than one donor. The difference of mean antibody titer between blood groups was statistically significant. One study showed that AB donors had higher anti-RBD titer than O negative donors ($p=0.048$) and higher anti-spike titer than O negative ($p=0.015$) or O positive ($p=0.037$) donors. It also demonstrated higher average anti-RBD and anti-spike antibody titers to be associated with plasma donors who were older, male, had higher BMI, had a fever and had been hospitalized.[21] Comparable to this study is, Symptomatic patients and hospitalized patients developed higher antibody titer in our study but contrary to this study is our study showed female participants, younger participants and lower BMI participants showed higher antibody titer. Another study showed that S-RBD-specific and N-specific IgG antibodies did not significantly correlate with ABO blood type.[14] In our study we could not measure the anti-S-RBD and anti-spike antibody titer; we measured total IgG against SARS CoV-2, this may be a cause of deference with those studies.

Limitations

This study has several limitations. First, our sample size was low. Second, we could not perform an antibody titer of all participants despite the detectable antibody in the qualitative test due to lack of resources. Third, we could not perform the neutralization assay that requires virus culture which needs to be conducted in laboratories with higher biosafety levels.[22,23] We could only detect total anti-SARS-CoV-2 antibodies by ELISA. The relationship between total anti-SARS-CoV-2 antibodies and neutralizing anti-SARS-CoV-2 antibodies remains unclear.[24]

CONCLUSION

All RT-PCR positive COVID-19 patients subsequently may not develop the antibody. Antibody titer among symptomatic hospitalized patients was significantly higher. Antibody titer was higher among patients with long viral clearance time and whose titer was done later than one month after initial RT-PCR positivity. However, further study is needed to recommend optimal convalescent plasma donor criteria.

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