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Original Article

Pathology Section

COVID-19 Convalescent Plasma Collection: A Cross-Sectional Study From the Perspective of Transfusion Medicine

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ABSTRACT

Introduction: Convalescent plasma from cured symptomatic COVID-19 patients was one of the many treatment options rolled out during the COVID-19 pandemic. The high levels of viral neutralizing antibodies in plasma of those who had contracted the disease, and clinical improvement in viral pneumonias like Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) treated with convalescent plasma was the consideration behind this.

Aim: To study recruitment including demographic characteristics of plasma donors, and recording any adverse donor reactions.

Materials and Methods: This prospective cross-sectional study was conducted at the Department of Transfusion Medicine, Govt. Medical College, Kozhikode, Kerala, India from July 2020 to March 2021. All plasma donors who met the inclusion criteria as per the Kerala Government order permitting compassionate use of convalescent plasma were included in the study. Plasma collection was done using the Hemonetics MCS+ (MultiComponent System) Apheresis system and semiquantitative in-vitro determination of human antibodies, immunoglobulin class IgG against S1 domain of spike protein of SARS-CoV-2 was performed using EUROIMMUN anti-SARS CoV -2 ELISA.

Results: Among the124 convalescent plasma donors, more than 90% donors were males of the age group 18-40 years and with a prior history of blood donation (repeat donors). Ninety donors (73%) had symptomatic COVID-19, 34 (27%) were asymptomatic. COVID-19 antibody determination showed 82 (66%) positive cases, 32 (26%) negative cases and 10 (8%) cases with borderline values. COVID-19 antibody was positive in 65 (72.2%) of the donors who were symptomatic compared to 17 (50%) asymptomatic donors. Adverse reactions were noted in 40 cases (32.26%), of which the procedure was discontinued in two.

Conclusion: Donors with prior donation history and in the age group 18-40 years are more likely to donate. This study also showed a significant antibody response in symptomatic COVID-19 donors versus the asymptomatic. Low rate of serious adverse reactions amounting to stopping the procedure confirms its safety.

Keywords: Adverse donor reactions, Apheresis, Plasma donors

INTRODUCTION

A number of therapeutic strategies to deal with the COVID-19 pandemic were rolled out with an aim of offering supportive care like mechanical ventilation and oxygen therapy; and treating the disease like steroids, antivirals, interferon beta-1b, ribavirin and drugs like hydroxychloroquine [1,2]. Use of convalescent plasma from cured COVID-19 patients who had significant symptoms was also a treatment option that was considered. The thought behind this was that viral neutralizing antibodies in plasma of cured patients may be helpful to others who have contracted the disease and are in the early stages [3-7]. This was based on clinical improvement in previous viral pneumonias treated with convalescent plasma like SARS and MERS [8]. The US FDA had approved the usage of convalescent plasma from COVID-19 recovered individuals. As a formal proof of efficacy was lacking in India, it was recommended only as part of a clinical trial by Indian Council of Medical Research (ICMR) or when approved by the State government.

Kerala State Government gave permission for use of convalescent plasma in severe/ critical COVID-19 patients on compassionate grounds via order: Government of Kerala- No 31/F2/2020/Health dated 27/05/2020 [9]. This was followed by orders by the Director General of Health Services, Central Drugs Standard Control Organisation, Govt of India; File No X.11026/179/2020/BD dated 01/07/2020 [10].The criteria for both patient and donor selection, including the mode of collection and dose was mentioned in the orders issued.

With hospitals considered as harbingers of infection, convincing potential plasma donors about the safety of the apheresis procedure and its possible benefits to those with severe or life threatening COVID-19 was a major challenge to transfusion medicine. This study aimed at studying the practical aspects of donor recruitment in a pandemic, understanding demographic characteristics of donors and recording adverse donor reactions (if any) during the procedure.

MATERIALS AND METHODS

This was a prospective cross-sectional study conducted at the Department of Transfusion Medicine, Government Medical College, Kozhikode, Kerala, from July 2020 to March 2021. Approval was required from Institutional and State medical boards and the Institutional Ethics committee, which was obtained, vides Ref. No GMCKKD/RP2020/IEC/490. Informed written consent from patient or relative was required for transfusion.

Inclusion and exclusion criteria for donors were based on the order issued by the Government of Kerala [9] and the order by the Director General of Health Services, Central Drugs Standard Control Organisation, Govt of India [10]. As per these, the donors had to meet all the donor eligibility criteria put forth in the latest amendment of Drugs and Cosmetics (D&C) Act 1940 and Rules 1945 [11]. Both males and females of weight >55 kg were eligible donors. The additional criteria which made them a candidate for donating convalescent plasma was preferably symptomatic COVID disease COVID-19 Disease Categories as per the COVID-19 Interim treatment guidelines for Kerala State No.31/2020/ Health dated 24th March 2020 is given in [Table/Fig-1]. COVID positive diagnosis was defined as either positive antigen test or Polymerase Chain Reaction (PCR). The donors should also have been COVID negative either by a 28-days prior antigen test or two

Category A	Mild Sore throat/Cough/Rhinitis/Diarrhea
Category B	Fever and/or Severe Sore throat/Cough/Diarrhea OR • Category A plus 2 or more of the following • Lung/ heart/ liver/kidney/neurological disease/ hypertension/ hematological disorders/ uncontrolled diabetes/cancer/ HIV-AIDS • Long term steroids/immunosuppressive drugs • Pregnancy • Age >60 years OR Category A plus Cardiovascular disease
Category C	• Breathlessness, Chest pain, Drowsiness, Fall in blood pressure, Hemoptysis, Cyanosis • Children with influenza like illness with red flag signs(somnolence, high/ persistent fever, inability to feed well, convulsions, dyspnea/ respiratory distress etc) • Worsening of underlying chronic conditions

[Table/Fig-1]: COVID-19 disease categories.
(COVID-19 Interim treatment guidelines for Kerala State No.31/2020/Health dated 24th March 2020)

negative (24 hrs apart) real time PCR performed 14 days prior to donation. The date of donation should fall within 4 months (120 days) of testing positive. Asymptomatic donors who fell within the time frame of acceptance were also included in the study subject to them fulfilling a positive COVID antibody test, post donation.

For getting donors, patients discharged from first line treatment centers of the district were contacted over phone from the Department of Transfusion Medicine, Govt. Medical College Kozhikode, Kerala.They were informed about the procedure and the probable benefit to a sick patient.

This was then successfully shifted to a multidisciplinary approach involving active involvement from the state government, district administration; health authorities and media resulting in passing on required information about convalescent plasma collection and therapy to various registered donor groups and also to the general population.

Blood samples were drawn from eligible donors for pre-donation testing which included - blood grouping, Complete Blood Count (CBC), serum protein, and testing for transfusion transmitted diseases. Donor acceptance criteria was haemoglobin >12.5 g/dL, platelet count > 1,50,000/µL, normal total WBC count, and serum protein >6g/dL.

Serologies for five transfusion-transmitted diseases were done [HIV, Hepatitis B and C (all tested by chemiluminescence immunoassay in Abbott Architect i1000sr Immunoassay Analyser), syphilis by Rapid Plasma Reagin method and malaria by the pan malaria rapid diagnostoic card test].

Exclusion criteria: Donors not meeting the criteria of eligible donor as put forth in the latest amendment of D&C Act and Rules, a COVID diagnosis of more than 4 months, and donors with history of transfusion of blood or blood products in last eight weeks.

COVID-19 antibody was determined post-donation. For this 2 ml of serum separated was stored in deep freezers at -40°C for assessment which was carried out in batches. Semiquantitative in-vitro determination of human antibodies of the immunoglobulin class IgG against S1 domain of spike protein of SARS-CoV-2 was performed using EUROIMMUN anti-SARS CoV -2 ELISA [sensitivity 94.4%, 10 days after onset of infection] in serum/ plasma. The reagent wells of the ELISA were coated with an S1 domain of the spike protein of SARS-CoV-2 expressed recombinantly in the human cell line HEK 293. The extinction of the calibrator defines the upper limit of the reference range of non-infected persons (cut-off) recommended by EUROIMMUN. Values above the indicated cut-off are considered positive, those below as negative. Results were evaluated semi quantitatively by calculating a ratio of the extinction of patient sample over the extinction of the calibrator. According to the kit manufacturer's recommendation, the result interpretation was as ratio <0.8 as negative, ≥0.8 to <1.1 borderline and ≥1.1 as positive [12].

A standard performa was used for data collection and included basic characteristics like age, sex, occupation, address, and whether they were first time or repeat donors. Details about COVID diagnosis included positive and negative dates, method of testing and symptomatology. The performa also had questions on adverse events experienced during donation, if any. Consent was taken for donation. Plasma was collected by apheresis using the Hemonetics MCS+ (MultiComponent System) Apheresis system. This allowed preferential collection of plasma in higher volumes and unnecessary loss of red cells compared to whole blood collection.

As per the government orders, [9,10] 400 mL plasma was collected from the accepted donors and aliquoted into two 200 mL bags, frozen within 8 hours at -40°C. Dosage of convalescent plasma was 2 doses, 200ml each administered 24 hrs apart and ABO compatible. Pooling of plasma was not permitted. A donor after successful plasma donation could donate again after 2 weeks provided total donation in 1 month did not exceed 1000 mL.

All adverse donor reactions were monitored by classifying as local and systemic reactions. Local reactions included hematomas, infections and allergy. Systemic reactions included reactions of the vasovagal spectrum including loss of consciousness, citrate reactions, seizures and hyperventilation. Contact number of the blood centre was provided for reporting delayed reactions.

The patients for whom this treatment was offered were adults >18 years of age with severe/ life threatening COVID. Severe COVID was defined as one or more of the following- Respiratory rate ≥30/min/ blood oxygen saturation ≤93% on room air/ratio of partial pressure of arterial oxygen to fraction of inspired oxygen >300/lung infiltrates >50% within 24-48 hrs. Life threatening COVID was defined in the government order [9] as one or more of - respiratory failure/septic shock/multiorgan dysfunction or failure. Age <18 years and known hypersensitivity to blood products were to be excluded.

STATISTICAL ANALYSIS

Where required computer assisted software SPSS-17.0 was used for statistical analysis. Chi-square test of independence with p-value ≤0.05 was taken as statistically significant.

RESULTS

During the 9-month study period, there were a total of 137 donor registrations, of which 124 were accepted as convalescent plasma donors. All the deferred 13 donors were males, out of which 4 had a COVID positive report of more than four months prior, and 9 did not meet the standard donor criteria. A summary of the basic donor characteristics of the 124 accepted donors is given in [Table/Fig-2].

Sex	Male: 123 (99.19%)	Female: 1 (0.81%)
Age	18-40 yrs: 117 (94.35%)	40-60yrs: 7 (5.65%)
Residency	Indian: 114 (91.93%)	Non-resident Indian: 10 (8.07%)
First timer/Repeat	First Timer: 40 (32.25%)	Repeat: 84 (67.75%)
COVID antibody OD ratio	Positive | Borderline | Negative 82 (66%) | 10 (8%) | 32 (26%)	

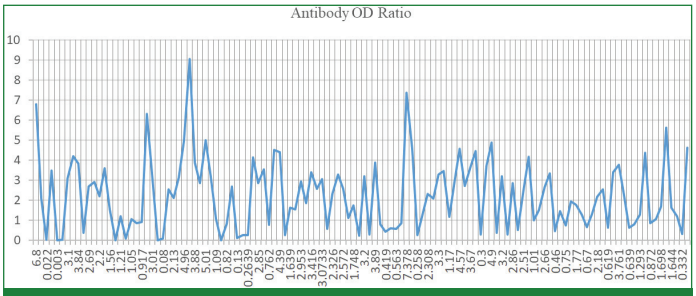
[Table/Fig-2]: Convalescent plasma- donor characteristics.
OD: Optical density

The donor profile showed 114 (91.93%) donors to be Indian residents. As there was only 1 female donor, males accounted for 99.19%. Though 84 (67.75%) of the donors had donated blood prior, none had experience with apheresis donation. All donors weighed more than 55 kg, mean weight being 66.7 kg.

Of the 124 donors, 34 cases (27%) were asymptomatic and the rest 90 (73%) had Category A symptoms. None had required hospitalisation and were managed either at home or in the first line treatment centers run by the government for those who had inconvenience for home isolation.

COVID antibody testing was done in batches post-donation and only antibody positive plasma was issued to patients.

Of the 124 cases tested, 82 cases (66%) tested positive, 32 cases (26%) tested negative, and 10 cases (8%) tested borderline, in the EUROIMMUN anti-SARS CoV-2 ELISA. [Table/Fig-3] shows the antibody OD ratio. The positive OD ratio values ranged from 1.17 to 9.07, the mean value being 3.13. The negative OD ratio ranged from 0.003 to 0.74, mean value was 0.34. The borderline values ranged from 0.803 to 1.09 with mean 0.937. [Table/Fig-4] shows the OD ratio with day of collection after turning COVID positive.The highest OD ratio observed was of 9.07 in a symptomatic donor .The sample was collected on the 40th day of turning COVID positive. The lowest OD ratio observed was 0.003; it was seen in 4 donors. One was asymptomatic and his sample was collected on the 56th day of turning positive. All the other three donors were symptomatic with samples collected on 53rd, 57th and 61st day of a positive report.



[Table/Fig-3]: Antibody OD ratio.
OD: Optical density

	OD ratio	Day of collection
Positive	1.17-9.07 (Mean-3.13)	40-120 days (Mean-71.09)
Borderline	0.803-1.09 (Mean-0.937)	47-106 days (Mean-72.3)
Negative	0.003-0.74 (Mean-0.34)	38-120 days (Mean-70.53)

[Table/Fig-4]: OD ratio in relation to the day of collection.
OD: Optical density

Borderline values were seen in 4 cases of asymptomatic and 6 of the symptomatic group. The findings are summarized in [Table/Fig-5].

Donors	COVID IgG antibody OD ratio in donors		
	Positive (≥1.1)	Negative (<0.8)	Borderline (≥0.8 to <1.1)
Symptomatic 90 (73%)	65 (72.22%)	19 (21.11%)	6 (6.67%)
Asymptomatic 34 (27%)	17 (50%)	13 (38.24%)	4 (11.76%)
p-value			

[Table/Fig-5]: COVID IgG spike protein antibody determination in symptomatic versus asymptomatic donors.
Chi-square statistic in antibody positive and negative group- 4.6977; p-value 0.030204; At p-value <0.05, the result is significant; OD: optical density

Adverse donor reactions were monitored. Two (1.6%) of the procedures were stopped midway-one due to vasovagal syncope and the other due to hematoma formation. Among the remaining 122 successful cases, the only adverse event noted was mild circumoral tingling/numbness related to hypocalcemia in 38 donors (30.6%). Most of these; 29 (76%) were noted by donors in the performa given post donation, only 9 donors (24%) reported circumoral numbness while the procedure was ongoing. However, all the 9 cases were successfully managed and procedures completed by slowing the return flow in the apheresis machine. Eighty-four (67.8%) cases were uneventful.

DISCUSSION

The nine-month period from July 2020 to March 2021, when convalescent plasma was collected in the department was highly challenging for donor recruitment as apheresis donation was unfamiliar among the donors and they also feared their own health would be compromised in the donation process. Active involvement from state and district administration and health authorities in passing on information to general public played a major role in getting donors.

In the study by Cheng Y et al., [13] on convalescent plasma in Hong Kong during the SARS outbreak of 2003, plasma was collected within 7 days of afebrile state or 25% radiographic improvement and an upper limit was not mentioned, antibody titers were between titre range of 160-2560.The volume of convalescent plasma transfused was 279.3±127.1 mL (range, 160-640 mL). In the present study, plasma was collected within 14 days of a twice negative PCR report or 28 days after a negative COVID-19 antigen report. The upper limit to collecting plasma was 4 months after turning positive. The volume of plasma collected was 400 mL divided into two 200 ml doses.

Regarding studies on COVID-19, the study by Lu L et al., [14] showed maximum antibody response to SARS- CoV-2 infection at 5 weeks of diagnosis, it was time bound and more response was seen in severe disease. In the present study, antibody production in asymptomatic versus symptomatic donors was compared and it was seen that the symptomatic donors had significantly more antibody production than asymptomatic. Gharbaran A et al., [2] selected donors who were RT-PCR confirmed COVID-19 and asymptomatic for at least 14 days. Only plasma with antiSARS-CoV-2 neutralizing antibodies confirmed by a SARS-COV-2 plaque reduction neutralization test (PRNT) and a PRNT50 titer of at least 1:80 was used. A SARS- Cov2 neutralizing antibody level of 1:160 and symptom free period of 14 days was the donor recruitment factor in the study by Bloch EM et al., [7]. This study included donor's negative either by PCR (14 days) or antigen (28 days). SARS CoV-2 IgG antibody against S1 domain of spike protein was estimated, however, neutralizing antibodies were not.

Focosi D et al., [15] accepted donors who were SARS-CoV2 positive for upto 6 months, collecting 600 ml plasma at 14 day intervals and aliquoting it into 200 ml plasma bags. In the present study, donors were accepted only upto 4 months after turning positive. Only 400 mL was collected from each donor, which was aliquoted into 200 mL bags. All our donors donated only once.

Adverse reaction in apheresis donors was also noted. There was only one local and one systemic reaction (1.6%), which resulted in the procedure being discontinued; 38 (30.6%) had mild circumoral tingling/numbness. But for the majority 84 (67.8%),the procedure was uneventful, emphasizing the safety of the procedure from the donor viewpoint. In the multivariate analysis by Despotis GJ et al., [16] 159 donors (0.81%) experienced adverse reactions of which 70 (0.35%) were hemodynamic and citrate related, 73 (0.37%) venepuncture related and 23 (0.12%) non-specific.

Limitation(s)

Since the study was a prospective one on a new disease, the impact of the disease or requirement of convalescent plasma was unclear at the time of start of the study. The study subjects were planned as all the donors who would be accepted in a one year study period starting from July 2020, when convalescent plasma was permitted for compassionate use by the Kerala government. However, after March 2021, collection of convalescent plasma stopped as it was not considered a treatment option by then. This study, hence, included all accepted donors in the 9-month frame between July 2020 to March 2021.

COVID-19 antibody that was determined was human antibody of the immunoglobulin class IgG against S1 domain of spike protein of SARS-CoV-2. However, SARS-CoV-2 neutralizing antibody titers could not be determined as facility was unavailable at the time of study.

Follow-up of patients who received the convalescent plasma was beyond the scope of this study.

CONCLUSION(S)

This study, from a transfusion medicine perspective shows the importance of a multidisciplinary approach in an acute emergency like a pandemic, involving health department, state and district administration, and all forms of media to get the correct information passed on to potential plasma donors for plasma collection and inventory management. In the present study, >90% donors were resident Indian males of the age group 18-40 years and with prior history of blood donation. The study could also show more significant antibody response in symptomatic donors versus the asymptomatic. Low rate of serious adverse reactions amounting to stopping the apheresis procedure (2 donors, 1.6%) confirms its safety.

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