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SEROLOGICAL EXPERIENCE OF CONVALESCENT PLASMA DONATION AND PROCESSING AT A DEDICATED COVID TERTIARY CARE INSTITUTE OF EASTERN INDIA

Immunology

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ABSTRACT

We describe our approach for prospective evaluation of convalescent plasma donation obtained from recovered COVID 19 patients (i.e. after achieving adaptive immunity against the virus) and was transfused to the critically ill COVID patient admitted to the intensive care unit as an addition to standard supportive care and antiviral agents as per MOHWF and ICMR -guidelines. As per the literature, convalescent plasma (CP) therapy is successfully used in the treatment of SARS, MERS, and the 2009 H1N1 pandemic with satisfactory efficacy and safety. [1-4] Till this period search of effective vaccine and specific antiviral medicines have been continuing. The study described only 0.2% of donation has achieved over the 92% recovered cases of COVID 19 during the current pandemic. From the screened donors, 80% of donors were seropositive for SARS-COV2 antibodies. Asymptomatic donors have higher antibody titer as compared to symptomatic donors. Recruitment of convalescent plasma donors with higher antibodies (>20 s/co) should be more preferable. They may potentiate plasma therapy more efficiently.

KEYWORDS

Convalescent Plasma (CP), Acute Respiratory Distress Syndrome (ARDS), Therapeutic Plasma Exchange (TPE), COVID-19.

1. BACKGROUND

The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which originated in Wuhan, China, has become a major concern all over the world. The pneumonia induced by the SARS-CoV-2 is named coronavirus disease 2019 (COVID-19). According to the W.H.O report of 31st December 2019, China had reported a cluster of cases ofpneumonia in Wuhan, Hubei Province. A novel coronavirus was eventually identified [5]. India's first case was reported on 30th January [6] 2020. According to the Worldometer reports of mid-September, of 2020 there are 3,111,493 cases of active cases of COVID 19 worldwide. As of now, this COVID-19 pandemic has taken more than 982,196 lakh human life [7]. This novel coronavirus is more contagious than other similarly related viruses causing syndromes like SARS, MERS, and H1N1. [8] To date no effective vaccine and specific antiviral medicines are available.

Severe acute respiratory syndrome coronavirus is a beta coronavirus that caused the pandemic. SARS-COV-2 is mainly transmitted through droplet and contact routes, and the virus infects the human cell via binding to angiotensin-converting enzymes receptor 2 (ACE)[10]. People who have been infected with SARS-COV-2 may express symptoms of acute respiratory illness, such as fever cough shortness of breath, etc., they can also be symptomatic, pausi-symptomatic, and asymptomatic. Studies have shown those who have symptoms developed antibodies in the second week[10] and developed convalescent immunity. [10] Convalescent plasma (CP) therapy, a classic adoptive immunotherapy, has been applied to the prevention and treatment of many infectious diseases for more than decades. The possible explanation for the efficacy of convalescent plasma therapy is that the antibodies from convalescent plasma might suppress viremia (antigen), induce free viral clearance and block new infection, and induce infected cell clearance.[11]Antibody rich convalescent plasma significantly reduced mortality compared with low quantity antibodies in COVID -19 patient.[12]high rich antibody plasma is well tolerated without any proven adverse effects.

2. Novelty

The use of convalescent plasma was recommended as an empirical treatment during the outbreak of the Ebola virus, and a protocol for the treatment of Middle East respiratory syndrome [MERS] with convalescent plasma was established in 2015.[11] This approach with other viral infections such as SARS-CoV, H5N1 avian influenza, and H1N1 influenza has also shown effectiveness in the transfusion of convalescent plasma.

Convalescent plasma having the highest titer could be the best choice of transfusion in the recipient.it has showed a positive correlation with the selected convalescent plasma donor's antibodies titer.

3. Sample Size

The study was conducted in the last four months (from middle June to September 2020) for experience of convalescent plasma donations.

Demographic location- Bihar, southern bank of river Ganges in Eastern India

4. Objective

1. To assess the safety level of convalescent plasma donation for COVID -19 patients.
2. To assess, quality of obtained plasma from donors.

a. Study Design

It is a single centric, prospective study. All willing persons recovered from COVID-19 screened for donation as per ICMR Interim Guidelines (on confirmation by real-time RT-PCR assay). Donors are enrolled after meeting the following enrolment criteria.

b. The enrolment criteria

1. Age-18 to 65 years
2. Male/female (nulliparous)
3. Infected by COVID -19 (confirmed by RT PCR Positive report for COVID -19) after the cession of last symptom/resolution of last symptom/date of discharge from hospital/negative RT PCR Report - to completion of 28 days.
4. The patient should have had a history of cough and fever
5. Asymptomatic, donors who have positive antibodies for COVID -19, were also enrolled in the studies after, mandatory negative R T PCR Testing (to rule out active infection) at the time of donation.
7. The criteria of normal blood donation was followed.
8. Diabetics could also donate but insulin-dependent diabetics were excluded from the studies.
9. Donors who have had a positive COVID diagnosis for more than 4 months would be excluded from donation.
10. A donor who had donated again after two weeks also participated in the study.

c. The exclusion criteria

1. pregnant women
2. Breastfeeding women
3. Person who had received blood or blood products in a year.

d. Donation for Convalescent Plasma.

Convalescent Plasma (CP) donation was obtained from recovered COVID-19 patients who had established adaptive immunity against the virus, as it contains a large quantity of neutralizing antibodies that would be capable of neutralizing SARS-CoV-2 and eradicating the pathogen from blood circulation and pulmonary tissues[13].
The recovery criteria would be as followed:

- Normality of body temperature for more than 3 days, at time of donation
- Resolution of respiratory tract symptoms
- Negative swab results of SARS-CoV-2 by RT-PCR assay (at a 24-hour sampling interval).
- Convalescent Plasma donation will be not collected in multigravida female patients.

e. Convalescent plasma donors must be screened for the following.

- Donors must be free from infections like hepatitis, malaria,

HIV/AIDS, and venereal disease or others.
- The donor should have no history of unexplained weight loss, repeated diarrhea, swollen glands, or a continuous low-grade fever for 6 months.
- Donors should be free of the following major diseases such as heart disease, lung disease, chronic nephritis, tuberculosis, abnormal bleeding tendency, jaundice, typhoid, schizophrenia, cancer/malignant disease, allergic disease, epilepsy, diabetes on insulin, syncope's, endocrine disorder, and asthma.
- There should be no history of minor surgery such as dental extraction, ear piercing, or tattooing within one month or any major surgery within six months.
- There should be no history of drug ingestion such as antibiotics, steroids vaccine, aspirin, alcohol, or any other medication within 72 hours.
- General physical examination criteria include weight (>45kg) , Height (> 150 cm) , Heart rate (60 to 100 /min) Haemoglobin (>12.5 g/dl), SBP (100 to 139 mm Hg) DBP (diastolic 60 to 89 mm Hg, serum protein >6 gm.

f. Clinical information of all COVID-19 donors before convalescent plasma donation would be obtained. It includes essential, clinical, and laboratory data for every donor

(a). Essential data
- Demographic data.
- Days of admission from symptom onset, and presenting symptoms;
- Data about various treatments, including mechanical ventilation, antiviral therapies, and supportive drug for treatment.

(b). Clinical data
- Skin temperature,Blood pressure measurement, pulse, Respiratory rate, weight

(c). Laboratory data,
- white blood cell count, lymphocyte count, platelet, Serum protein value, Seroreactive status
, RT PCR, Serum antibody titer (IgG, antibodies);

5. Methodology

Collection and processing of convalescent plasma

All recently recovered COVID-19 patients, who were previously infected with SARS-CoV-2 in which their SARS-CoV-2-specific antibody (IgG) neutralizing antibody titer was above >6 only recruited as suitable donors. Results were interpreted in the VITROS chemiluminescence system.

Principle of chemiluminescence -

VITROS ECi/ECiq/3600/5600/XT(CHEMILUMINESE EQUIPMENT) Immunodiagnostic integrated system **immunometric technique** involved two-staged technique. In 1st stage antibodies to SARS-CoV-2 present in the sample bind with SARS-CoV-2 spike protein coated on wells. The unbounded sample is removed by washing the solution. In the 2nd stage horseradish peroxidase (HPR)-labeled murine monoclonal anti-human IgG antibodies are added to conjugated reagent. Conjugate binds to the antibody portion of the antigen-antibody complex. If complexity is not present the unbounded conjugate is removed by washing. The bounded HPR conjugate is measured by its luminescence. The light signal is analyzed by the system, the amount of HPR conjugate bound is indicative of SARS-CoV-2 antibodies present in the test sample. The following testing criteria were followed during the sample testing.

Incubation time- 37 minutes, Time to first result- 48 minutes ,Test temperature -37 °c, Reaction sample -volume 20 Ul required.

REACTION SCHEME

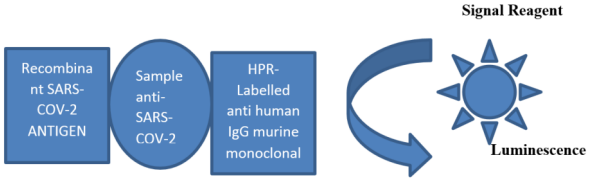


Fig-1

Result calculation - signal for test sample/signal at Cut-off (Cut-off value)

Interpretation of result

Result(S/C)	<1.00	>1.00
Result text	Not reactive	Reactive

Sample tested in different solution, e.g. [immaculate serum,] [equal dilution of Serum: A.C.D solution (1;1)], [Serum in EDTA solution]. [Equal dilution of Serum with normal saline (N.S)(1:1)]and [Processed unit]. All results had been evaluated.

RESULTS

Total 387 donors enrolled for plasma donation at AIIMS Patna till mid-September. Out of 387 donors, 310 donors (80%), were reactive for SARS-COV-2 antibodies. Among these 310 donors, 137 (44%) were symptomatic, whereas 56% of donors (173) were asymptomatic. Among symptomatic donors, 99% were in mild categories, (they were also screened for moderate and severe categories). Most of the symptomatic donors had taken- Hydroxychloroquine, montelukast, and paracetamol. The average day of donation from the cession of symptoms was 32 days. Among asymptomatic donors, 70% didn't consume any prophylactic medication while 30% had taken Hydroxychloroquine, montelukast, and PCM. Some of the asymptomatic donors were paucisymptomatic, they had complaints of mild myalgia. The mean of Haemoglobin among symptomatic donors was 13.4 g/dl while in asymptomatic donors it was 14.1g/dl. The mean platelet count was greater than 1.5 lacks. The mean weight for the donors was 58 kg.11 female donors who were fulfilling the criteria came forward for plasma donation. The maximum no of donations was between the age group of 20 to 40(220). Among the donors 19.8% (77) donors deferred **(Fig -7)** while 41.6% were non -reactive for SARS COV-2 IgG. According to the quantity antibodies categorization (S/CO) index, 8% of the donors were categorized in low titer, 60% in medium titer, and the remaining 32% high titer category[14]. **(Fig 2)**. 46% of donors were having SARS-CoV-2 antibodies higher than 15 (S/CO) while 32% were having more than 20 (S/CO) indexes. The result of the five samples showed SARS-COV-2 antibodies greater than 28 (S/CO) indexes. The mean of five immaculate antibodies sample was 28.6; these samples showed a positive correlation (0.87) with processed units (collected plasma). The coefficient of Variance was 0.024 and 0.034 in the immaculate serum and processed units respectively. The lower value of the coefficient of variance signifies a lower risk of error at a high (S/CO) index. 85% of the donated convalescent plasma was transfused in recipients and no significant adverse event was noted in donors and recipients. The most common convalescent plasma blood group was B positive (47%) followed by O positive (29%), A positive(18%), and AB(6%) positive.13 donors had made twice a donation two weeks apart in the present study.

Table-2 Comparison of s/co index of SARS-COV-2 antibodies among donors

CCP interpretation	VITROS IgG S/Co	Criteria for study	Number of CP Donation
Low titre	1.0-4.62	>6-10	(N1=24)
Medium titre	4.62-18.45	10-20	(N2=186)
High titre	>18.45	>20	(N3=100)

Statistical analysis

All raw data related to the study was analyzed with the help of statistical tools. Statistical assimilation of p-value extracted and statistical correlation and Chi-square test results were found out **(Table 3).** Statistical software used included SPSS 24.0**(Table-4, 5, 6).** The correlation coefficient between serum and different solutions established **(Fig-5)** results show all variables are positively correlated.

Table-3 The chi-square statistic is 0.0855. The p-value is .770009 which is not significant at p<0.05.

	Symptomatic donors	Asymptomatic donors	Marginal row
SARS COV-2 IgG >15	55	89	144
SARS COV-2 IgG >20	44	66	110
Marginal level	99	155	254 (grand total)

Table-4 Variation in[IgG] quantities among different solutions.

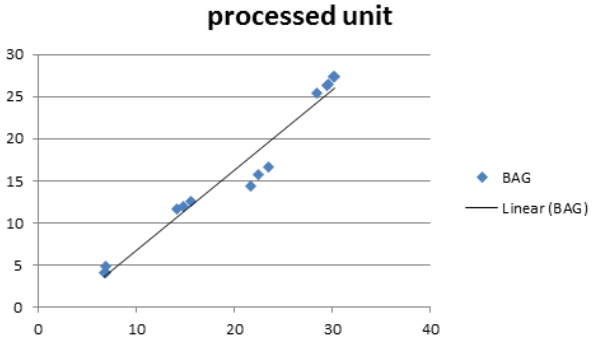
variation in IgG quantities of different solution	SERUM(A)	ACD(B)	N.S(C)	EDTA(D)	processed units (E)

Standard deviation	9.10	8.46	8.41	7.59	8.86
Mean	20.03	13.69	13.78	14.73	16.35
C.V	0.45	0.62	0.61	0.51	0.54

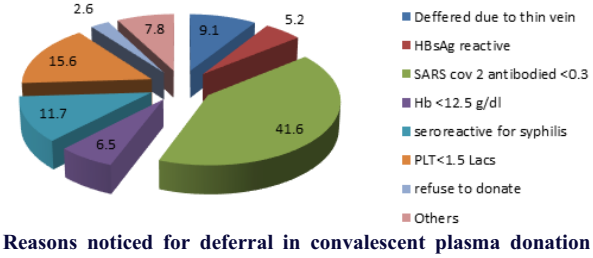
[*ACD-Acid citrate dextrose] [N.S-Normal Saline] [EDTA-EthyleneDiamineTetra Acetic acid]

Table-5 Correlation coefficient between serum and different solution

Serum(A) and ACD(B)	0.98
Serum(A)and processed unit(E)	0.98
Serum(A)and N.S(C)	0.98
Serum(A)and EDTA(D)	0.99
ACD(B) and processed unit(E)	0.99
ACD(B) and N.S(C)	0.99
N.S(C) and processed unit(E)	0.99
Interpretation	Positive correlation



Serum (A) and processed unit (E) 0.98 graphical correlation (Fig-6)



Reasons noticed for deferral in convalescent plasma donation (70)19.8% (Fig-7)

g. Method followed for plasma collection

- The donor's plasma was collected after 40 days of post-onset of illness.
- Written informed consent was obtained from each patient.
- Plasma Preparation Procedure and Quality Control run (as per SOP).
- Apheresis was performed using [**COBE SPECTRA**] and [**COM.TEC**] cell separator.
- Convalescence plasma was collected from all potential donors.
- Around 200- to 400-mL ABO-compatible plasma samples harvested from each donor depending on age and body weight.
- The CP treated with UV light treatment for 30 min in the medical plasma virus inactivation cabinet
- Each sample was divided and stored as 200-mL aliquots at -40 °C without any detergent or heat treatment.
- At the time of shipping, it was thawed in a plasma bath.

DISCUSSION

U.S food and drug administration (USFDA) has approved the emergency use of convalescent plasma with their interventional guideline[13] .Studies describe transfusion of high threshold antibodies of SARS-COV-2 (18.45 s/co index as compared to 4.64 s/co index) effects significantly over mortality compared to lower quantity antibodies transfused. Present studies have shown 67.8% of donors had s/co index greater than 6, which is quite higher than M J Joyner studies[14]. Studies conducted by valentine et al show that convalescent plasma transfusion among 100 patients had an effective outcome in 28 days survival and ineffective breathing for those who were on mechanical ventilation. The present study collected 310 convalescent plasma donations; the valentine study collected 287 plasma donation.[15]

A similar study on convalescent plasma for COVID-19 was conducted by Avendano-Sola C et al, the multicentre study shows 0% patients mortality out of 38 patients in the intervention arm while the control arm had 6% mortality out of 14 patient. Plasma therapy was claimed as superior and standard care towards progression of mechanical ventilation or death in hospitalized patients with COVID-19.[16] while another study by Rogers R et al, shows no significant effect of convalescent plasma on overall mortality although it is a safe treatment.[17]

A review of clinical trial by Athavale A et al had also evaluated a potential role of convalescent plasma, Remisdesvir, and Favipiravir in COVID -19 treatment.[18]Finding a suitable plasma donation for convalescent plasma therapy may be pronounced a possible treatment in COVID -19 pandemics.

According to the Woldometer report till mid-September total 23,692,401 people have recovered from COVID infection in the worldwide. In India 5,732,518 cases have recorded and 91,173 death occurred, total no recovered population was 4,674,987(81.6%). Bihar, a state in India has shown 173.1 thousand confirmed cases of COVID-19 out of the confirmed cases 158.5 thousand (92.2%) patients recovered from COVID-19 whereas 874 fatalities occurred till mid-September. The donation of convalescent plasma is at merely 0.2% donation of all COVID-19 recovered cases, which was quite low compared to the expected probability of a 1-5% donation by Sapkota SD[19]. The scarcity of plasma donors may threaten in the plan of management of plasma therapy.

The success of plasma therapy completely relies on plasma donation from donors who have recovered, and have a high concentration of antibodies.

It was observed that the course of the pandemic badly affected the blood center storage of blood or blood product. Motivational support is needed to stabilize the balance of the demand and supply of blood and product. To enhance donations, AIIMS Patna has initiated traveling allowance support for plasma donors. Ideas that can ramp up the donation should be supported and converge with the present drives.

8. Outcomes

Hyper immunoglobulin therapy may offer more therapeutic benefits inpatient. Donor's enrolment with higher SARS-COV-2 antibodies might help in the revitalization of plasma therapy**.** Donors having a higher S/CO index of SARS-COV-2 antibodies had shown lower dilution in their processes unit of S/CO index of SARS-COV-2 antibodies comparatively donors who had lower S/CO index of SARS-COV-2 antibodies. Higher S/CO index has a minor significant difference in their collection unit. They should be more preferable for transfusion in directed needs. The selection of plasma donors with high rich antibodies may enhance plasma therapy. The study has supported Improvement in the plasma donation enrolment understanding. Regulatory review of troubleshooting origin may be done with the above conclusion. Despite the proven clinical efficacy of convalescent plasma therapy, there are certain limitations of these therapy e.g side effects and serious adverse effects also have been noticed. Assessment of a potential donor is also needed in the management of COVID-19.

9. Limitations of this study

Long term convalescent plasma donation risk evaluation required. Kinetics of antibody persistence may define adaptive immunity. More study is required in convalescent plasma donation. Look back/traceability of recipients/donors should in need.

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