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Original Article

Role of Therapeutic Plasma Exchange in Improving Inflammatory and Thrombotic Biomarker Levels in Covid 19 Patients: Experience from Tertiary care Hospital of Balochistan

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ABSTRACT

Introduction: Two proposed molecular mechanisms associated with Covid-19 induced lung injury are microthrombogenesis and inflammatory cytokine release. To reduce the inflammatory reaction is the key to reduce mortality and morbidity. Due to the lack of specific treatment, various treatment modalities including Plasmapheresis have been applied.

Objective: Our objective is to evaluate the Role of Therapeutic plasma exchange (TPE) in improving Inflammatory and thrombotic biomarker levels in Covid-19 patients.

Material and Methods: It is a Prospective longitudinal study, conducted at Combined Military Hospital Quetta from Jan to Dec 2021.All the PCR Confirmed Covid-19 positive patients who received Plasmapheresis as therapy during the duration of the study were included. Parameters under study were Total leukocyte count (TLC), C Reactive proteins (CRP), Ferritin, Lactate dehydrogenase (LD), Procalcitonin (PCT), IL-6, and Alanine transaminase (ALT).Samples were collected before and after every session. 40 ml /kg bodyweight of the patient was exchanged with FFPs derived from the blood of healthy donors. Biochemical parameters were analyzed on automated C501 and Cobas e411 analyzers .D-dimers were performed on manual latex kit. Data were analyzed using SPSS 21

Result: Data distribution was checked using Kolmogorov- Smirnov test. Categorical variables were expressed as frequencies while for continuous variables mean and SD was calculated. Wilcoxon signed-rank test is used for comparing the levels of different biochemical and hematological markers on different days before and after Plasmapheresis. Levels of IL-6, PCT, CRP, and Ferritin were high before and after Plasmapheresis. Their levels reduced significantly within 07 days ($p < 0.05$).There was no significant difference between Total leukocyte and platelet count.

Conclusion: Therapeutic plasma exchange is an avenue for future clinical trials. It can be utilize as a treatment in patients with raised cytokine and thrombotic markers levels.

Keywords: …Biochemical, Covid -19, Hematological, Plasmapheresis.

Introduction

Corona virus disease 2019 caused by SARS-COV-02 emerged in Wuhan, China in Dec 2019.Till now immeasurable human impact has already been caused by SARS-CoV 2 pandemic. According to a study conducted in China, 17 % of Covid -19 patients require ICU admission and mechanical ventilation.[1] Pulmonary involvement as the primary target organ can give a spectrum of symptoms ranging from mild to severe.[2] Two proposed molecular mechanisms associated with Covid 19 induced lung injury are microthrombogenesis and inflammatory cytokine release. [3]

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Severe infections are characterized by raised levels of inflammatory markers, and a deranged coagulation profile. [1] High Mortality in Covid is attributed to macrophage activation syndrome. It is characterized by fever spikes, deranged liver functions, raise CRP, ferritin levels and coagulopathy linked with DIC.[4] Rate of Mortality can be predicted by measuring levels of inflammatory markers produced as a result of cytokine reaction.[5]

Natural immune response in SARS Covid 2 results in antigen-containing cell lysis by NK cells. Raised level of Type 1 Interferon levels is a very important initial response to inhibit viral replication followed by the release of cytokines.[6]

Treatment targeting the systemic response to reduce the inflammatory reaction is the key to reduce mortality and morbidity .[3] The virus causes a decrease in lymphocytes and an increase in cytokine

production. Therefore therapy that can act at both of these levels can prove to be effective in the treatment of Covid -19 .[6]

Due to the lack of specific treatment, various treatment modalities including Plasmapheresis have been applied.[7]History shows safety of convalescent plasma usage in the treatment of different infectious diseases like MERS- B Syndrome, Myasthenia gravis .[7, 8]

As high mortality rate has been seen in Covid 19 induced Acute Respiratory Distress Syndrome and cytokine storm, early intervention to control immune dysregulation is crucial. Plasmapheresis has the definitive advantage of alleviating the viral load without any side effects as compared to polypharmacy. To produce maximum effect to neutralize the virus, the convalescent plasma should contain high titers of antibodies.[8]

Knaup et al have confirmed therapeutic plasma exchange to be effective in reducing levels of inflammatory markers in patients with severe sepsis .[9]

Use of blood products for treatment dates back to the 1800s.[6] Therapeutic plasma exchange removes proteins including antibodies paraproteins, complement and clotting factors, and even inflammatory ,mediators from plasma . It has been used in past for treating H1N1.[10]. Therapeutic plasma exchange along with removing these particles reduces viral load too[11, 12]. Therapeutic plasma exchange can reduce mortality by improving endothelial integrity. In this way their effects can be neutralized.

Therapeutic plasma exchange has the advantage of immediate effects as compared to pharmacological therapies[13] Keith et al have proposed TPE as a potential treatment for reducing inflammation, cytokine storm, coagulation and endothelial dysfunction.[14] Luo et al have shown a decrease in Levels of IL- 6 and CRP post Therapeutic plasma exchange . [15]

Two basic techniques used for plasmapheresis are based on centrifugation and filtration .The first one has a significant advantage of removing all particles irrespective of size while the other technique removes particles depending on their size[16].In our study, we used a centrifugation-based technique. Our study is an attempt to elaborate and quantify the effects of TPE on levels of inflammatory and thrombosis markers over a period of 01 week.

Materials & Methods

This prospective longitudinal study was conducted in tertiary care hospital of Quetta from June 2020 to Dec 2021. Parameters under study were TLC CRP, Ferritin, LD, PCT, IL-6, and ALT for improvement after Plasmapheresis. Permission was granted by the ethical review committee of the hospital. Written informed consent was taken from all participants. All the PCR confirmed cases of Covid 19 who received Plasmapheresis as therapy were included in the study. Plasmapheresis was done for 03 hours in each session .Samples were collected before and after every session. Spectra Optia and Fresenius were used in the process of Therapeutic plasma exchange using Fresh Frozen Plasma (FFPs) as a replacement solution.40 ml /kg bodyweight of the patient was exchanged with FFPs derived from the blood of healthy donors. Biochemical parameters were analyzed on automated C501 and Cobas e411 analyzers .D - dimers were performed on manual latex kit.

Results

Data were analyzed using SPSS 21. Data distribution was checked using Kolmogorov- Smirnov test. Categorical variables were expressed as frequencies while for continuous variables mean and SD was calculated. Friedman ANOVA test is used for comparing the levels of different biochemical and hematological markers on different days before and after plasmapheresis.

Table-1: Baseline Characteristics of Patients

Characteristics		
Average Age		
Male	48.9±15.4	
Female	49.1 ± 15.6	
Gender		
Male	109	77.3%
Female	31	22 %
Associated Morbidities		
Hypertension	29	20.6%
Diabetes	50	35.5 %
CLD	02	1.4%
CKD	14	9.9%
Diabetes and CKD	03	2.1%
Average Length of stay in hospital	14.8±5.5	
Overall Survival Rate		
Dead	21	15%
Alive	119	85%
Survival Rate with Different Comorbidities		
Diabetes	5(50)	10%
Hypertension	5(29)	17.2%
CLD	1(2)	50%
Diabetes and CKD	2(3)	66.7%
Nil	1(42)	2.4%
Mortality rate plasmapheresis started after 07 days	6/15	40%
Mortality rate plasmapheresis started within 07 days	15/125	12%

Table 1 shows that out of 140 patients, 109 (77.3 %) were male and the rest were females. 35.5% (50) patients were already suffering from diabetes followed by hypertension present in 29 patients. The average stay in hospital was 14.8 days .Overall mortality rate was 15% (21) Mortality was only 12% if Plasmapheresis started within 7 days of the appearance of symptoms and 40% for patients presenting later than this.

Table 2: Total Number of Plasmapheresis Done in Patients

No of patients	No of Plasmapheresis
16	1
25	2
30	3
36	4
27	5
1	6
4	7
1	10

Table 2 shows the number of plasmapheresis done in each patient. Maximum (36) patients had 04 sessions of plasmapheresis while 33 patients required more than 04 sessions of Plasmapheresis.

Table 3 : Levels of inflammatory markers, acute phase reactants ,hematological parameters and markers of liver injury, platelet count before and after Plasmapheresis

	Day1	Day - 3	Day -7	Z1,Z2	P1,P2
CRP	71.5 (45 – 141.7)	53.5 (34–124)	39 (21-92.7)	-6.7,-6.8	0.000, 0.000
Ferritin	713 (474 – 1422.2)	637 (411.7–1376.25)	507 (317.5 – 982.2)	-6.765, -6.614	0.000, 0.000
LD	477.5 (364.2 – 630.5)	431.5 (321-570.2)	398 (265.5 – 478)	-6.939, -6.936	0.000, 0.000
Lymphocyte	13 (9-20)	15 (10-19)	16 (11-21)	-5.674, -5.289	0.000, 0.000
IL-6	7.6 (4.2 -17.2)	6.7 (3.5–12.3)	4.4 (2.1 – 8.7)	-8.106, -7.809	0.000, 0.000
TLC	7.6 (5.5 -12.0)	7.6 (5.6–11.9)	7.5 (5.5 – 11.8)	-.709, -0.38	0.478, 0.704
Hemoglobin	13.1 (12.1 – 14.7)	13.1 (11.9–14.5)	13.1 (11.3 – 14.5)	-4.254, -2.889	0.000, 0.004
ALT	49 (31.2- 66)	43 (29- 60.7)	36.5 (26- 45)	-4.949, -4.516	0.000,0.000
Platelet	185 (156.7–226.2)	188 (155.5-227)	194 (159.2 – 231.7)	-.037, -1.884	0.971, 0.060
PCT	2.08 (0.9–4.82)	0.9 (0.38 – 2.7)	0.10 (0.06 – 0.89)	-8.604, -8.424	0.000, 0.000

Table 3 show that after Post hoc analysis done with Wilcoxon signed-rank test was applied using $p<0.05$ which showed that there was a statistically significant difference between levels of inflammatory markers and markers of cell injury measured before and after plasmapheresis and done on 7th day post plasmapheresis . Levels of IL-6, PCT CRP, Ferritin were high before plasmapheresis and after plasmapheresis, their levels reduced significantly within 07 days ($p < 0.05$).There was no significant difference between TLC and platelet count.

Out of 119 alive patients, 89 patients had deranged D-dimers level .100% of patients who couldn't survive had deranged D- dimers level .Among patients who survived there was a significant improvement in the level of D- dimers after plasmapheresis .Among patients who survived the D- dimers levels improved from 95 % of patients to 57.1 % on day 3 and 24.3 %on day 7 .100 % of patients had deranged PT/PTTK levels in the non-surviving group while in survivors 93.8 % patients had deranged PT/PTTK which improved significantly to 43.7 % on 3rd and 13.4 % on 3rd and 7th post plasmapheresis days .

Discussion

The pandemic of novel Corona virus SARS_–CoV -2 is continuously spreading globally .Several randomized trials are in progress to find the definitive treatment .At present several supportive treatments including the use of antivirals, and steroids are being practiced.

Administration of Therapeutic plasma exchange for the correct duration ,calculation of volume to be replaced ,proper infection control , continuous monitoring of patient's vitals ,monitoring of PTT/PTTK after each session is important to avoid complications in patients .[17]No significant complications associated with Therapeutic plasma exchange in both elderly and young patients which is comparable to study Deng et al [18].None of the patients in our study had hypotension ,hypocalcaemia or any bleeding episode post therapeutic plasma exchange .

Therapeutic plasma exchange can be a useful approach for increasing positive outcomes removing cytokines and toxins from the human body with Covid 19 .Similar results were shown by Khamis et al[11] .TPE is an effective intervention as proved by many studies

Almost all of our patients had ARDS ranging in severity from mild to severe .21 out of 140 patients died even after receiving TPE along with other supportive treatment. Leading cause of death in 5 out of 150 patients who underwent TPE was ARDS and cytokine storm .Early initiation of TPE can remove the inflammatory cytokines and toxins that have a role in triggering cytokine-related immune injury .In this way, multiple organ damage including progressive lung injury, renal and hepatic impairment can be avoided .[19]

Therapeutic plasma exchange with normal plasma improves coagulation status and cytokine response .Reductions in inflammatory markers including CRP, Ferritin, Interleukin-6, and Procalcitonin were observed in all patients .Similar effects have been reported by Shi et al 2020.[20]Decrease in levels can be attributed to the use of antivirals and other supportive treatments but the instant reduction in levels of inflammatory markers has been reported by Xu et al[21] too which is similar in our study.

According to American plasma association guidelines, the timing of TPE should be decided on an individual basis .[16] Our study showed that mortality and morbidity were reduced in patients in whom TPE was started within 2 to 7 days of onset of symptoms. So the key to success with TPE is to start it in early stages.

Decrease in Covid related complications with timely use of TPE is encouraging as seen in our study though this single-center study has its limitations. Randomized control trials going on in different parts of the world can confirm the beneficial outcomes of TPE. High incidence of death in patients with co morbidities like diabetes, and hypertension has been suggested to be related to a decrease in patient's capacity to tolerate raised levels of cytokines resulting in Cytokine storm-like condition. [22]In our study the proportion of patients with co morbidities who died despite Plasmapheresis is higher than normal patients.

Covid -19 positive patients who succumbed to death suffered from consumptive coagulopathy shown by the presence of thrombocytopenia and comparatively elevated levels of D dimers .Wang et al reported D-dimers levels higher in non survivors than survivors .[23]In a meta-analysis conducted on 1779 patients Thrombocytopenia has been reported to be marker of severe clinical disease.[24]

Conclusion

We suggest that Therapeutic plasma exchange should be utilized as a treatment in patients with raised cytokine and thrombotic markers levels. Therapeutic plasma exchange is a venue for future clinical trials.

Competing interests

The authors of the study have no conflict of interest.

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References

1. Guan W, Ni Z, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2020;382(18):1708-1720.doi:10.1056/nejmoa2002032
2. 2.Duan K, Liu B, Li C, Zhang H, Yu T, Qu J, et al. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. Proc Natl Acad Sci U S A 2020;117(17):9490–6.
3. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, et al. COVID-19: Consider cytokine storm syndromes and immunosuppression.The Lancet 2020;395: 1033-1034
4. McGonagle D, Ramanan AV, Bridgewood C. Immune cartography of macrophage activation syndrome in the COVID-19 era. Nature Reviews Rheumatology. 2021 Mar; 17(3):145-57.
5. Ragab D, Salah Eldin H, Taeimah M, Khattab R, Salem R (2020) TheCOVID-19 Cytokine Storm; What We Know So Far. Frontiers in Immunology11: 1446.

6. Cunningham AC, Goh HP, Koh D. Treatment of COVID-19: old tricks for newchallenges. Crit Care 2020; 24(1):91. https://doi.org/10.1186/s13054-020-2818-6
7. Brown BL, McCullough J. Treatment for emerging viruses: Convalescent plasma and COVID-19. Transfusion and Apheresis Science. 2020 Apr 20:102790.
8. Lin L, Lu L, Cao W, Li T. Hypothesis for potential pathogenesis of SARS-CoV-2 infection- a review of immune changes in patients with viral pneumonia. Emerg Microbes Infect 2020; 9(1):727–32.
9. Knaup H, Stahl K, Schmidt BMW, et al. Early therapeutic plasmaexchange in septic shock: a prospective open-label nonrandomizedpilot study focusing on safety, hemodynamics, vascular
10. barrier function, and biologic markers. Crit Care. 2018; 22(1):285. doi:10.1186/s13054-018-2220-9.
11. Schwartz J, Padmanabhan A, Aqui N, Balogun RA, Connelly-Smith L, et al.Guidelines on the use of therapeutic apheresis in clinical practice-evidence-based approach from the Writing Committee of the American Society for Apheresis: the seventh special issue. J Clin Apher 2016; 31(3):149–62.
12. Khamis F, Al-Zakwani I, Al Hashmi S, Al Dowaiki S, Al Bahrani M, Pandak N, Al Khalili H, Memish Z. Therapeutic plasma exchange in adults with severe COVID-19 infection. International Journal of Infectious Diseases. 2020 Oct 1; 99:214-8.
13. Hashemian SM, Shafigh N, Afzal G, Jamaati H, Tabarsi P, Marjani M, Malekmohammad M, Mortazavi SM, Khoundabi B, Mansouri D, Moniri A. Plasmapheresis reduces cytokine and immune cell levels in COVID-19 patients with acute respiratory distress syndrome (ARDS). Pulmonology. 2020 Dec 4.
14. Barelli S, Alberio L The role of plasma transfusion in massive\bleeding: Protecting the endothelial glycocalyx? Frontiers in Medicine 2018; 5:91. https://doi.org/10.3389/fmed.2018.00091
15. Keith P, Day M, Perkins L, Moyer L, Hewitt K, Wells A. A novel treatment approach to the novel coronavirus: an argument for the use of therapeutic plasma exchange for fulminant COVID-19. 2020. https://doi.org/10.1186/s13054-020-2836-4
16. Luo S, Yang L, Wang C, Liu C, Li D. Clinical observation of 6 severe COVID-19 patients treated with plasma exchange or tocilizumab. J Zhejiang Univ (Med Sci) 2020; 49(2):227-231.
17. Winters JL. Plasma exchange: concepts, mechanisms, and an overview of the American Society for Apheresis guidelines. Hematol Am Soc Hematol Educ Program 2012; 2012:7–12.
18. Yang XH, Sun RH, Zhao MY, et al. Expert recommendations on blood purification treatment protocol for patients with severe COVID-19: recommendation and consensus. Chronic Dis Transl Med 2020; 10.1016/j.cdtm.2020.04.002.
19. Deng Y, Liu W, Liu K, Fang YY, Shang J, et al. (2020) Clinical characteristics of fatal and recovered cases of coronavirus disease 2019 (COVID-19) in Wuhan, China. Chin Med J (Engl) 133: 1261-1267.
20. Qin C, Zhou L, Hu Z, et al. dysregulation of immune response in patients with COVID- 19 in Wuhan, China. Clin Infect Dis 2020; ciaa248.
21. Shi H, Zhou C, He P, et al. Successful treatment of plasma exchange followed by intravenous immunoglobin in a critically ill patient with 2019 novel coronavirus infection. Int J Antimicrob Agents 2020;105974, DOI:http://dx.doi.org/10.1016/j. ijantimicag.2020.105974.
22. Xu X, Han M, Li T, et al. Effective treatment of severe COVID-19 patients with tocilizumab. Proc Natl Acad Sci U S A 2020;117:10970–5.
23. Mangalmurti N, Hunter CA. Cytokine storms: understanding COVID-19. Immunity 2020. Jun 28.
24. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA. 2020;323(11):1061-1069. doi:10.1001/jama.2020.1585
25. Mei HHY. Characteristics, causes, diagnosis, and treatment ofcoagulation dysfunction in patients with COVID-19. ZhonghuaXue Ye Xue Za Zhi. 2020;41(0):E002. doi:10.3760/CMA.j.issn.0253-2727.2020.0002

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