ORIGINAL ARTICLE

Effects of Convalescent Plasma Therapy on TNF-α and IFN-γ in Randomized COVID-19 patients at Haji Surabaya General Hospital

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ABSTRACT

Introduction: The preferred treatment for COVID-19 cases is Convalescent Plasma. In COVID-19 patients, cytokine storms are caused by high levels of cytokines including: Inflammatory cytokines such as (IL-10, IL-12, IL-27, IL-21, TNF-α and IFN-γ , IL-2, and IL-6), are triggered by T cell responses. The goal of this research is to determine Does administration of convalescent plasma affect the TNF-α and IFN-γ cases of COVID-19 sufferers. **Materials and methods:** The experimental research method uses (a post-test only control group design). The study material in the form of serum from the blood of COVID-19 patients as many as 38 samples selected randomly with details of COVID-19 patients as many as 19 samples were not given convalescent plasma treatment and 19 samples of COVID-19 patients receiving convalescent plasma therapy. **Results:** This study showed that there was no effect on TNF-α levels before and after convalescent plasma administration (P> 0.05), while IFN-γ levels showed an effect between before and after convalescent plasma administration (P< 0.05). **Conclusion:** Although in this study there were differences in the results of statistical analysis of TNF-α and IFN-γ levels in convalescent plasma treatment of COVID-19 patients, this study proved to be able to improve the clinical condition of COVID-19 cases.

Keywords: COVID-19, Convalescent plasma, IFN-γ, TNF-α

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INTRODUCTION

The world health organization (WHO) labeled the (COVID-19) sickness caused by SARS CoV-2 a pandemic on March 11, 2020. (1). Given the lack of alternative prevention and specialist treatment in the coronavirus disease COVID-19 pandemic, convalescent plasma transfusions be the best value choice. Convalescent plasma could be a cheap and widely available treatment for COVID-19 (2). Infections with SARS-CoV or MERS-CoV are often associated with lung tissue inflammation, Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are caused by an increase in proinflammatory cytokines. TNF-α a potent pro-inflammatory cytokine in various ways can affect macrophages in their activities. TNF-α releases cells such as neutrophils, monocytes, endothelial cells, astrocytes, adipocytes, smooth muscle cells, and activated lymphocytes.(3). Tumor Necrosis Factor /TNF-α is produced in response to trauma, infection, or the onset of numerous chronic inflammatory illnesses.(4). The interaction between lung epithelial cells and macrophages that reduce soluble substances is the source of enhanced expression. the emergence of a cytokine storm, can be viewed as a side consequence of the ongoing pro-inflammatory cascade, emphasizing a cytokine storm's potential to develop cross-interaction with the impacted mucosal tissue, to sustain amplification automatically, resulting in a systemic rise in cytokine storm (3). IFN-γ is the only type II IFN in the family. Innate immunity (macrophages and NK cells) is further activated makes IFN-γ the main product (5). SARS-CoV-2-specific antigens are produced during viral attachment. antibodies or SARS-CoV-1 cross-neutralization can inactivate the virus, target cells and attach to them and opsonize for viral clearance via complement mechanisms and alveolar macrophage phagocytosis mediated by Fc receptors. After effectively infecting lung epithelial cells and recognizing the virus, type I interferon was released from the epithelial cells

almost immediately.As an antiviral molecule main host, IFN limit the spread of the virus, and play a role in immunomodulator to induce macrophage antigen phagocytosis, as well as limit NK cells from infected target cells and T cells or B cells. Inhibited IFN production has a direct effect on viral survival in the host (6). Healing plasma from cases affected by the SARS-CoV-2 virus can be used as a treatment without the occurrence of severe side effects, therefore, there may be benefit in testing the safety and efficacy of transfusion of recovered plasma in patients with SARS-CoV-2 (7). Therefore, research was carried out with the aim of knowing the effect of giving convalescent plasma to determine the levels of TNF-α and IFN-γ cytokines in COVID-19 patients using random sampling method.

MATERIALS AND METHODS

Population and sample

The purpose of this study was to determine how high the levels of TNF-α and IFN-γ in cases of COVID-19 without receiving convalescent plasma and receiving convalescent plasma therapy. The dose that was transfused was 500 ml at RSU Haji Surabaya which had been collected with a total sample of 38 samples. (19 = no convalescent plasma and (19 = received convalescent plasma) who met the inclusion criteria for COVID-19 patients including : minimum age of 18 years, According to WHO, the patient is in critical condition, from the results of RT-PCR testing in the diagnosis of COVID-19, oxygen saturation level below 93% at rest, PaO2 (Partial pressure of oxygen) and FiO2 (Oxygen concentration) < 300 mmHg (mmHg = 0.133kPa) (8).

This research is a quasi-experimental type of research with a post-test only control group design.The study has obtained permission from the Health Research Ethics Committee of RSU Haji Surabaya. The collected samples were examined using the Sandwich ELISA method and measured levels of TNF-α and IFN-γ with Elabscience reagent with production code E-EL-H0109 for TNF-α and E-EL-H0108 for IFN-γ. Measurement tool using Ryto Reader photometer.

Statistical analysis

The SPSS for Windows application was used to examine the data. The Shapiro-Wilk test was employed to determine normalcy. If the data were regularly distributed (P> 0.05), the paired T-test was used. The Wilcoxon non-parametric test is used if the data are not normally distributed (P< 0.05).

RESULTS

Table I shows the mean TNF-α level in the control group (without convalescent plasma administration) was 2,673 and the mean of the treatment group (convalescent plasma administration) was 2,656. These results indicate that TNF-α levels in the treatment group were lower than the control group, so the test continued with the Shapiro-Wilk test. The results of the normality test with the Shapiro-Wilk test showed that the control had a significant value (P>0.05) and the treatment had a value (P<0.05). From one of the data in the group, there is data that has a p-value <0.05, which means that the TNF-α level data is not normally distributed, while the average IFN-γ level in the control group (without convalescent plasma administration) is 7.320 and the mean the treatment group (convalescent plasma administration) was 13,782. These findings indicate that the IFN-γ levels of the treatment group were lower than the control group, so the test was continued with the Shapiro-Wilk test. The results of the normality test with the Shapiro-Wilk test showed that the control had a significant result (P<0.05) and the treatment had (P>0.05). The data obtained from one group contains data that has a value (P <0.05) which means that the IFN-γ level data is not normally distributed, so the test is continued by using the Wilxocon test as well as the TNF-α

Table I: The mean and standard deviation of TNF- and IFN-γ levels

Group	TNF- (Mean Standard Deviation)	IFN -γ (Mean Standard Deviation)
Control	2.673 ± 0.389	7.320 ± 5.340
Treatment	2.656 ± 1.854	13.782 ± 7.938

Table II The results showed that there was no difference in TNF-α levels in the control (without convalescent plasma administration) and treatment (convalescent plasma administration) with a significance value of 0.840, where the significance was above 0.05 (P>0.05). In the results of IFN-γ there were differences in IFN-γ levels in the control without convalescent plasma administration) and treatment (convalescent plasma administration). The significance level with a value of 0.008 where the significance value is below 0.005 the value obtained (P<0.005)

Table II: Wilxoxon test result for TNF-α and IFN-γ levels

Group	TNF- α (Mean ± Standard Deviation	*p-value*	IFN-γ (Mean ±Standard Deviation)	*p-value*
Control	2,673± 0,3896		7.320 ± 5.340	0.008
Treatment	2,656 ± 1,854	0.840	13.782 ± 7.938	

DISCUSSIONS

Based on testing the results of control and treatment (mean: 2.673 vs 2.656), (standard deviation: 0.389 Vs 1.854) showed a decrease in TNF-α levels on treatment (convalescent plasma therapy) but not statistically significant with (P> 0.05). Results obtained

from This research revealed that there was no change in TNF-α levels in control (without convalescent plasma administration) and treatment (convalescent plasma administration) or it could be interpreted that convalescent plasma administration had no effect on TNF-α levels in COVID-19 patients with moderate symptoms. and heavy. TNF-α has a variety of functions, among which various functions are particularly important in regulating the production of the proinflammatory cytokine cascade. TNF-α is considered to be a major regulator of pro-inflammatory cytokine production. In the early stages of infection, TNF-α levels rise and remain high throughout the disease.(3). One of the functions of TNF- is that it has a very important role in regulating the production of proinflammatory cytokine cascades. (3). The increased expression of TNF-α is the result of macrophage-derived soluble factors interacting with lung epithelial cells in a cytokine storm state (3). The distribution of IFN-γ, was significantly higher in the treatment compared to the control plasma. Control and treatment results (mean: 7,320 vs 13,782), (standard deviation: 5,340 Vs 7,938) showed an increase in IFN-γ with convalescent plasma therapy and was statistically significant with (P< 0.05). The role of IFN-γ as the most important product of the Th-1-mediated immune response as a continuation of activation of innate immunity from macrophages and NK cells and regulates Th-1 effector mechanisms.Th-1 cell status is characterized by the production of interferon-γ (IFN-γ) and cell-mediated immune-inducing activity against intracellular pathogens. The release of these cytokines and chemokines induces recruitment of monocytes, macrophages, and neutrophils into the lung and subsequent secretion of proinflammatory cytokines and forms a feedback loop. (5). Anti-viral activity of IFN-γ in various stages using cellular functions. Neutralization of the virus is thought to reduce the inflammatory response so that the possibility of an exaggerated immune response developing into lung damage can decrease. In addition to neutralizing antibodies, convalescent plasma contains cytokines (pro-inflammatory and anti-inflammatory), neutralizing antibodies, clotting factors, defensins, pentraxins, and other undefined proteins.

Antibodies present in convalescent plasma can also exert a therapeutic effect through various mechanisms, including direct virus neutralization, antibody intermediaries that activate complement, Neutralization of cytotoxicity or cellular phagocytosis in the presence of antibodies.

The researcher realizes that this study still has many shortcomings due to the limited number of patients receiving convalescent plasma therapy. Clinical data of patients are needed that support the diagnosis of the severity of hospitalized patients so that the effects of this therapy can be known and as an effective therapeutic for COVID-19 patients.

CONCLUSION

Administration of convalescent plasma has no effect on TNF- levels in COVID-19 patients but does affect IFN-γ levels, it is necessary to conduct research on other cytokines in COVID-19 patients so that be a benchmark for the benefits that exist in convalescent plasma for COVID-19 patients

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