ORIGINAL ARTICLE

Effect of Convalescent Plasma Therapy on IL-10 and IL-12 Levels in COVID-19 Patients at Haji Surabaya General Hospital Randomly

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ABSTRACT

Introduction: IL-12 and IL-10 are cytokines with different mechanisms in the pathogenesis of COVID-19. Differences in IL-12 and IL-10 levels in patients receiving convalescent plasma and non-convalescent plasma recipients need to be known because the total levels of IL-12 and IL-10 also determine the clinical condition of the patient. **Materials and Methods:** This study used 40 randomly selected blood serum samples with details of 20 samples of COVID-19 patients without convalescent plasma therapy and 20 samples of COVID-19 patients. The COVID-19 patients at the Haji General Hospital in Surabaya provided the patient serum that was utilized. **Results:** Based on the findings, there were differences in IL-10 levels between the control group and convalescent plasma therapy recipients ($P<0.05$). On IL-12 levels had no difference between the control and treatment groups ($P>0.05$). **Conclusion:** According to statistical estimates, convalescent plasma treatment made a difference to IL-10 but not IL-12 levels in COVID-19 patients.

Keywords: COVID-19, IL-10, IL-12, Convalescent plasma

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INTRODUCTION

The number of pro-inflammatory and anti-inflammatory cytokines is different in each COVID-19 patient with mild, moderate and severe symptoms. Various variations in the symptoms of COVID-19 patients also correlate with high levels of several pro-inflammatory cytokines such as IL-1, IL-2, IL-6 , TNF-α, IFN-γ, IP-10, GM-CSF, MCP-1, and IL-10 (1). In severe COVID-19 patients there is an increase in the levels of IL-1, IL-12, IL-6, IFN-γ and TNF-α (2). Interestingly, IL-12p70 and IL-10 levels can be the main biomarkers of the clinical condition of COVID-19 patients suffering from hypertension to predict the severity of the patient's disease (3). In addition, IL-10 and IL-12 levels also decreased concomitantly in COVID-19 patients taking opioid drugs (4).

Interleukin 10 and IL-12 have different mechanisms in the pathogenesis of COVID-19. In the pathogenesis of COVID-19, IL-10 may play a role as a proinflammatory and immune-activating cytokine. IL-10 also has a role in the severity of COVID-19 patients, especially with regard to patient mortality. When there is an increase in the production of IL-10 in COVID-19 patients, it will trigger hyperinflammation due to T cell proliferation, thus worsening the patient's condition (5).

Cells infected with the COVID-19 virus will rapidly produce IL-12, IL-1α, IL-6 and IL-1β (6). IL-12 is an antiviral cytokine that has low levels in asymptomatic and mildly symptomatic patients. As a result of SARS-

CoV invasion of the respiratory tract, studies conducted in a Chinese population showed that IFN-γ, IL-12, IL-1β and IL-6 can induce an innate inflammatory response, thereby activating Th1 cell-mediated immunity due to cell stimulation. Cytotoxic NK and T (CTLs) (7). in COVID-19 patients with mild or asymptomatic symptoms have high levels of IL-12 (8).

Blocking IL-10 in COVID-19 patients using virus neutralizing antibodies provides the possibility to reduce disease severity and patient mortality (5). Therapy containing viral neutralizing antibodies is present in convalescent plasma. The use of convalescent plasma can suppress viremia in COVID-19 patients and is ideal in cases of SARS-CoV and MERS-CoV (9). Convalescent plasma therapy has a mechanism of action similar to IVIG administration, IVIG administration can reduce IL-12 production and increase IL-10 production (10). Treatments targeting the SARS-CoV-2 immune response, such as convalescent plasma, IVIg, and monoclonal antibodies, have been effective in the treatment of COVID-19 (11). Research on IL-10 and IL-12 levels in COVID-19 patients has been carried out in several countries (12, 13, 14). Research must continue to be carried out considering the effectiveness of convalescent plasma therapy against variants of the corona virus that continues to develop and differs in each country (15).

MATERIALS AND METHODS

Population and sample

The population in this study were Covid-19 patients at Haji General Hospital Surabaya. The total sample was 38 samples, namely the group without convalescent plasma therapy (control) (n=19) and the group with convalescent plasma therapy (n=19). The administration of convalescent plasma with a dose that has been determined by WHO is 500 ml. In this study, the sample criteria include inclusion and exclusion criteria, namely: Inclusion criteria for COVID-19 patients The inclusion criteria are as follows (16) :

1. Informed consent has been signed.
2. Must be at least 18 years old
3. COVID-19 diagnosis based on RT-PCR testing
4. At rest, oxygen saturation should be less than 93 percent.
5. Oxygen partial pressure (PaO2)/ Oxygen concentration (FIO2) < 300 mmHg (mmHg = 0.133kPa).

COVID-19 patients must meet requirements in order to be included in the study :

1. Condition of being pregnant or breastfeeding
2. Patients with previous allergic reactions to transfusion
3. ICU patient who is critically unwell
4. Patients who have had surgery within the prior 30 days
5. Cancer patients undergoing active treatment (i.e., radio therapy or chemotherapy)
6. HIV diagnosis in people infected

Cytokine Assay

The collected samples were examined using the ELISA method with Elabscience reagents having production codes E-ELH0103 for IL-10 and E-ELH0150 for IL-12.

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 normally 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 show that Both the control and the treatment have significant values with p values below 0.05. The Wilxocon test is used to continue the test since data from one of the group's data has a P value of 0.05, indicating that the data on TNF levels are not normally distributed.

Table I: Normality test levels IL-10 and IL-12

Levels	Groups	Sig
IL-10	Control (Without therapy Convalescent Plasma)	0.234
	Treatment (With therapy Convalescent Plasma)	0.453
IL-12	Control (Without therapy Convalescent Plasma)	0.003
	Treatment (With therapy Convalescent Plasma)	0.008

Table II show that the mean value of the control group was smaller than the treatment so that there was an effect on convalescent plasma therapy on IL-10 levels.

Table II: Paired t-test levels IL-10

Groups	IL-10 levels (Mean SD)	p-value
Control	8.86 ± 4.58	0.01
Treatment	17.73 ± 10.19	

Table III show that The significance level for the Wilcoxon test is more than 0.05 (p>0.05). Convalescent plasma delivery did not affect IL-12 levels in COVID-19 patients, according to the research data, which acquired a significant value of 0.263, which means that H0 is rejected and H1 is accepted, indicating that there is no influence between control and treatment.

Table III: Willcoxon test levels IL-12

Groups	IL-12 levels (Mean SD)	p-value
Control	26.06 ± 21.09	0.263
Treatment	40.46 ± 41.54	

DISCUSSION

The overall value of IL-10 levels of COVID-19 patients in this study was in the above normal range (13) which was 0–4.91pg/mL. IL-10 levels that have an effect when given convalescent plasma therapy according to the study (12, 17), also showed that the levels of IL-10 in COVID-19 patients before and after convalescent plasma therapy were significantly different. The mean value obtained in the study for the controll group was 8.86 pg/mL, this result was higher than the studies (13), which showed IL-10 levels before administration. convalescent plasma therapy of 3.1 pg/mL. In COVID-19 patients who have been given convalescent plasma therapy, the value is 17.73 pg/mL, this value is higher than the study (12) which have values ranging from 2.3 to 4.1 pg/mL. The value of IL-10 levels appeared to be lower in the study with the mean value of the controll group of 17.33 pg/mL and treatment groups of 21.82 pg/mL.

The average value of IL-12 levels in this study was still within normal levels (18), which was in the range of 19.6-56.3 pg/mL. The mean value of IL-12 levels in the control group was 26.06 pg/mL, which was higher than the maximum value for COVID-19 patients in the mild group (8). Patients who had received convalescent plasma therapy or treatment group showed a mean of 40.46 pg/mL. There was no difference in IL-12 levels in this study between the control and treatment groups in accordance with the statement (14) that convalescent plasma therapy had no effect on the inflammatory response in COVID-19 patients.

In the pathogenesis of COVID-19 there are a number of cytokines in high levels such as IL-1, IL-2, IL-4, IL-7, IL-10, IL-12, IL-13, IL-17, macrophage colony-stimulating factor (M-CSF), monocyte chemoattractant protein-1 (MCP-1), hepatocyte growth factor (HGF), cytokines such as IFN-γ and TNF-α so that lung injury in COVID-19 patients with severe symptoms due to inflammatory reactions which causes a cytokine storm (19). administration of convalescent plasma therapy during the early stages of COVID-19 infection can prevent the transfer of innate immune cells and prevent lung injury. This is due to the role of macrophages as anti-inflammatory, when macrophages are given intravenous immunoglobulin, they produce more IL-10 and less IL-12 (20). Interleukin 1-beta (IL-1), IL-13, IL-12p70, interferon gamma (IFN), IL-17A, interferon gamma-induced protein 10 (IP-10), macrophage inflammatory protein-1 (MIP-1), IFN, and IL-1 had positive correlations with donation time. the late donation of convalescent plasma treatment will result in an increase in pro-inflammatory cytokines (21).

The treatment of convalescent plasma therapy improves the clinical condition of COVID-19 patients in this study (22), although there were differences in the results of statistical analysis between IL-10 and IL-12 levels. An increase in IL-10 coincides with an improvement in clinical conditions (13) such as body temperature, respiratory rate, heart rate, PaO2/FIO2 values. Administration of convalescent plasma therapy such as with Intravenous Immunoglobulin in which dendritic cells are inhibited maturation will increase the production of IL-10 and reduce the production of IL-12 (10, 23, 24). Giving IVIg therapy to patients can decrease Th1 cell activity and increase Th2 cells (10). Increased activity of Th2 cells will increase the amount of anti-inflammatory IL-10. Increased levels of IL-12 in this study indicate the occurrence of negative regulators in the immune condition of COVID-19 patients. IL-10 in negative regulators has a significant role in TLR-induced IL-12 production. Both IL12a and IL12b genes are repressed by IL-10 mainly at the transcriptional level, and their induction has different requirements for de novo protein synthesis. At present, it is uncertain how IL-10 decreases Il12a transcription. Nuclear factor Interleukin 3-regulated (NFIL3), a transcription factor B-ZIP, binds to an enhancer 10 kb upstream of the transcriptional starting point of IL12b. Myeloid cells lacking NFIL3 produce lot of IL-12p40 and IL-12p70 resulting in an increase in IL-12 (25).

CONCLUSION

Although there are statistically significant changes in the levels of IL-10 and IL-12, convalescent plasma treatment can ameliorate the clinical symptoms of COVID-19 patients.

ACKNOWLEDGEMENTS

Thank you to Rector of Universitas Airlangga for funding this research.

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