ORIGINAL ARTICLE

Interleukin 6 Associated with Adrenal Insufficiency in COVID-19 Patient

Nurhasan Agung Prabowo[1,2], Rohmaningtyas Hidayah Setyaningrum[1,2], Hendrastutik Apriningsih[1,2], Hartono[1,2]

[1] Sebelas Maret University Hospital (UNS Hospital), Sukoharjo, Jawa Tengah, Indonesia
[2] Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia

ABSTRACT

Introduction: COVID-19 has been a global problem and many aspects related to the mechanism of COVID-19 in damaging patients' organs have not been known. Interleukin 6 is a severity marker of the cytokine storm in COVID-19. This study aimed to determine the relationship between interleukin 6 with cortisol and adrenaline in COVID-19 patients. **Methods:** Employing a cross-sectional design, the study was conducted at UNS (Sebelas Maret University) hospital from May to September 2020. Examination of interleukin 6, adrenaline and cortisol by taking the patients' blood sample was conducted at 8 AM on the first and seventh day of hospitalization. Statistical test was performed using Pearson correlation. P is significant if it is less than 0.05. **Results:** This study shows that in COVID-19 patients, there is adrenal insufficiency with an average cortisol level of 0.91+0.53 ng/mL and an average adrenaline level of 9.20+4.29 pg/mL. The mean interleukin 6 in this study is 8.31+13.7 pg / mL. Interleukin 6 will increase the secretion of the hormone cortisol ($p = 0.01$; $r = 0.575$) and is not related to adrenaline ($p = 0.057$; $r = 0.771$). **Conclusion:** The COVID-19 patients experienced adrenal insufficiency, and Interleukin 6 associated with an increased cortisol hormone. The use of steroids may be beneficial in COVID-19 patients with adrenal insufficiency.

Keywords: Adrenaline, Adrenal insufficiency, Cortisol, Interleukin 6, COVID-19

Corresponding Author:
Nurhasan Agung Prabowo, MD
Email: dr.nurhasan21@staff.uns.ac.id
Tel: +6282328010430

INTRODUCTION

COVID-19 (coronavirus disease 2019) has been a global health problem affecting many sectors (1). On Maret 6, 2021, in Indonesia, there were 1.373.836 cases of COVID-19 with 147.172 active patients, and the number of deaths reached 37.154 people. This condition proves that COVID-19 is a serious problem in Indonesia (2).

COVID-19 is a new disease whose spectrum of disease has not been well understood. This disease is a systemic disease that can attack various organs in the body. If there is stress on the body, there will be an increase in stress hormones, such as cortisol and adrenaline hormones. However, several studies have shown that the HPA Axis system is compromised in people with COVID-19, and this has broad clinical implications and potential therapeutic impact (3). There are different results in research of the adrenal glands' response to COVID-19. A study shows an increase in the hormone cortisol in COVID-19 and is related to the disease (4). On the other hand, other studies suggest that secondary adrenal insufficiency occurs in COVID-19 (3).

When physical or psychological stress occurs, stress hormones will increase in the body. The first is an increase in corticotropin-releasing factor (CRF) from the hypothalamus. An increase in CRF causes an increase in adrenocorticotropin hormone (ACTH) by the pituitary gland. ACTH increases adrenal gland secretions, including cortisol and adrenaline. Cortisol circulates in the body and plays a role in the coping mechanism. If the stressor received by the hypothalamus is robust, the CRF secreted will increase so that the stimulation obtained by the pituitary also increases, and the secretion of cortisol by the adrenal glands also increases (5). Adrenaline has a role in the respiratory, cardiovascular, metabolic, and immune systems. Excessive amounts of adrenaline cause pulmonary vasoplegia, hypertension, cardiomyopathy, myocardial infarction, hypercoagulopathy, immune system disorders, and diabetes. It is suspected that adrenaline is the primary mediator in COVID-19 (6).

The pro-inflammatory cytokine as the master of COVID-19 infection is interleukin 6 (IL-6). IL 6 is further considered a biomarker for identifying patients requiring anti-inflammatory therapy such as steroids or the use of monoclonal antibodies. Il 6 can be induced from other pro-inflammatory cytokines, polysaccharide bacteria, or viral infections. The mechanism of IL 6 is through

activation of NF-kb and induction of the Il 6 gene (7). IL 6 has been used as a predictor model of mortality(8) and prognostic (7) in COVID-19 patients.

Previous research show conflicting results regarding adrenal insufficiency in COVID-19 patients. Therefore, we are interested in researching this and comparing the prognostic marker for COVID-19, namely IL-6. This study aimed to determine the relationship between IL-6 and cortisol and adrenaline in COVID-19 patients at Universitas Sebelas Maret (UNS) hospital and determine the frequency of adrenal insufficiency in the COVID-19 patients.

MATERIALS AND METHODS

Study design

This study's population included patients at the UNS Hospital from May-September 2020 with the following inclusion criteria: aged 18-64 years old, positive COVID-19 swab with mild and moderate clinical symptoms, and willing to participate in the research. The exclusion criteria are as follows: having a history of comorbidities disease (diabetes, chronic lung disease, cardiovascular disease, chronic renal disease) and pregnant women. This study has received ethical approval from the Health Research Ethics Committee based on letter number 1/01 / HREC / 2021. All patients signed informed consent before the examination.

Sample preparation

The Epinephrine ELISA Kit is available from ABNOVA, Catalog Number KA1882. Cortisol assay used USA R&D Systems, Inc kit, Catalog Number KGE008B. The IL 6 assay used USA R&D Systems, Inc, Catalog Number D6050. Blood was obtained from patients two times, on the first day of treatment and the 10th day of treatment. The examination of IL-6 and cortisol used the patients' blood serum, while adrenaline used the patients' blood plasma. The inspection used the ELISA method according to the manufacturer's instructions (9). Examination of interleukin 6, adrenaline and cortisol by taking the patients' blood sample was conducted at 8 AM on the first and seventh day of hospitalization.

Statistical analysis

Demographic data are presented as means and standard deviation. Statistical test was conducted using Pearson correlation. P is significant if it is less than 0.05. Data analysis used SPSS 25 for mac.

RESULTS

This study involved 28 research subjects. Table I show that the adrenaline and cortisol levels of all patients examined either on the first day of the 10th day or the average between the two examinations showed below typical values, or it could be called adrenal insufficiency. Low adrenaline and cortisol concentration in COVID-19 patients follow previous studies that show central adrenal insufficiency in COVID-19 patients (3).

The results showed a graph of the relationship between IL 6 and adrenaline and cortisol (Fig.1). IL 6 has a positive correlation with cortisol with a correlation coefficient or r = 0.575 or has a strong correlation with p = 0.001, while IL 6 with adrenaline does not have a statistically significant relationship.

Table I: Demographic description of research subjects.

Variable	Total	Normal Value
Age (years old)	36.86±12.96	
Sex -Male -Female	28 12 16	
IL 6 on first day (pg/mL)	7.1±10.49	3.13-12.5
IL 6 on 10th day (pg/mL)	10.87±22.75	
Mean IL 6 (pg/mL)	8.32±13.7	
Cortisol on first day (ng/mL)	0.94±0.69	38.2-213
Cortisol on 10th day (ng/mL)	0.89±0.79	
Mean Cortisol (ng/mL)	0.91±0.34	
Adrenaline on first day (pg/mL)	9.08±5.49	
Adrenaline on 10th day (pg/mL)	9.34±4.77	18 – 6667
Mean Adrenaline (pg/mL)	9.2±4.3	

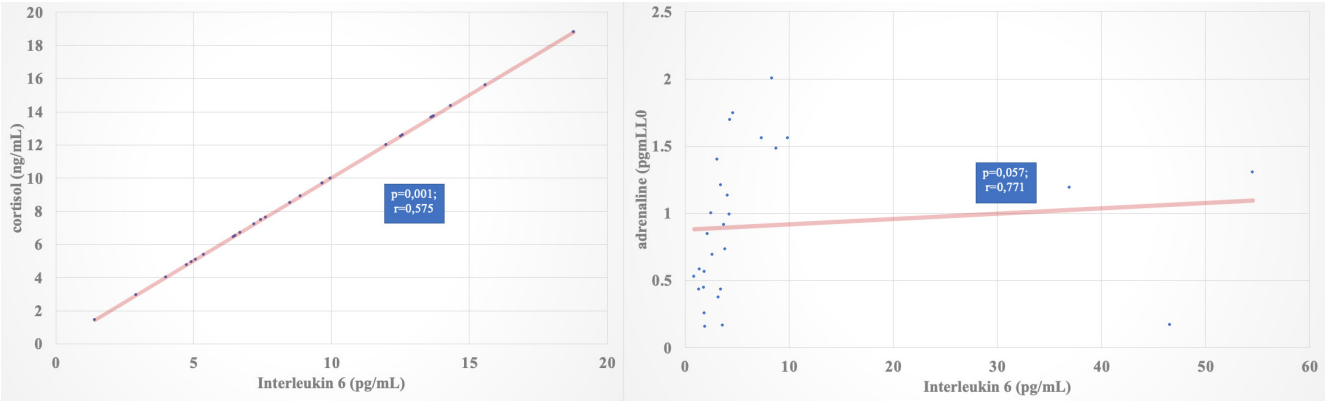

Figure 1: Correlation between Interleukin 6 with cortisol and adrenaline. The left image shows the correlation between IL-6 and cortisol, while the right image shows the correlation between IL-6 and adrenaline. ng/mL=nanogram per mili litres, pg/mL=pico-gram per mili litres.

DISCUSSION

Alzarani et al. state that low cortisol levels are caused by low adrenocorticotropic hormone (ACTH) levels in COVID-19 patients (3). The results of this study indicate that all the COVID-19 patients excperienced adrenal insufficiency with low cortisol and adrenaline levels below-average values (3). IL 6, as a marker of cytokine storms, is associated with cortisol but not with adrenaline. The increase in IL 6 is related to the rise in cortisol levels.

There are several mechanisms for adrenal insufficiency in COVID-19, such as angiotensin-converting enzyme-2 (10). ACE-2 receptor is widely expressed in various cells such as vascular cells, pituitary gland, adrenal gland, and hypothalamus (11). An autopsy on the SARS outbreak in 2003 identified that the virus enters the cortex's adrenal cells, causing necrosis and cell damage and demonstrating the virus' direct cytopathic effect on adrenal cells (3).

Low adrenaline and cortisol levels have been objects of discussions on the pathogenesis of COVID-19. There is interference from the pituitary hypothalamus and adrenal Axis. Impaired adrenocortical response to COVID-19 is caused by a decrease in ACTH (adrenocorticotrophic hormone) secretion (3). The coronavirus can affect the hypothalamus and pituitary gland. A study showed that out of 63 patients infected with SARS, 40% had central adrenal insufficiency three months after recovering (12). The SARS virus also has another mechanism to block the stress response by expressing several amino acids similar to ACTH, resulting in ACTH deficiency and secondary adrenal insufficiency in COVID-19 patients (13).

Another mechanism shows that adrenal hormones will increase in COVID-19. The mechanisms under observation include physiological stress due to critical illness, increasing cortisol concentrations, and increasing bioavailability with the activation of the hypothalamic-pituitary-adrenal axis. Also, there is a decrease in cortisol metabolism and a reduction in the amount of protein binding. These lead to an increase in the amount of cortisol in the body, thereby stimulating changes in cardiovascular metabolism and immune system regulation (14).

The virus can have a direct effect on the hypothalamus or pituitary gland. 40% of 63 SARS patients develop adrenal central insufficiency three months after recovering from the disease. Adrenal central deficiency occurs because the virus causes pituitary and thalamic damage, improving over time (12). Also, the virus has a mechanism to avoid the increase in cortisol by expressing several amino acids similar to ACTH, such as amino acids 26 29 31 33 37 and 39, which are similar to ACTH, causing relative deficiency of ACTH secondary adrenal insufficiency (3). Tan et al. demonstrated that cortisol would increase in COVID-19 patients, and multivariate analysis shows that increased cortisol concentration is associated with mortality in COVID-19 patients (4).

n increase in cytokine storms is associated with mortality and disease severity. Catecholamines will increase IL 6 production and other properties, the mechanism through a self-amplifying feed-forward loop within myeloid cells. It affects alpha adrenoreceptors. The increase in cytokine concentrations associated with a poor outcome in COVID-19 patients (6). In this study, there was no effect of adrenaline on IL 6. One of the causes is the possibility of central adrenal insufficiency, which causes a decrease in the secretion of adrenal hormone so that the adrenal hormone cannot cause systemic effects following the existing theory.

In this study, IL 6 is associated with cortisol. Previous research by Pal et al. indicated that IL 6 would cause an increase in a protein that plays a vital role in steroidogenesis in the adrenal zone, namely protein StAR and 5Se (13). Conversely, the stress hormone or the hormone cortisol will cause an increase in IL 6 production (15).

This study has some limitations. The number of patients is still small and it was conducted only at one centre. Therefore, a multicenter follow-up study needs to be carried out with a bigger number of patients. The other limitation is the severity of the disease and the use of steroids for each patient is not included in the study. The other limitation is that adrenaline and cortisol were examined only on two occasions. Everyday test needs to be conducted.

CONCLUSION

The present study showed that Interleukin 6 is associated with the increase of cortisol hormone in COVID-19 patients at UNS hospital. Insufficiency of adrenal was found in all COVID-19 patients at the hospital. Further research with a larger sample size and multicentre approach needs to be done to determine when to treat insufficiency of adrenal in managing COVID-19 patients.

ACKNOWLEDGMENTS

This research was funded by the Indonesian Ministry of Research and Technology and Sebelas Maret University.

REFERENCES

1. Djalante R, Lassa J, Setiamarga D, Sudjatma A, Indrawan M, Haryanto B, et al. Review and analysis of current responses to COVID-19 in Indonesia: Period of January to March 2020. Prog Disaster Sci. 2020 Apr 1;6:100091.
2. Gugus Penanganan COVID-19. Peta Sebaran

[Internet]. Peta Sebaran. 2020. [cited 2021 april 29]. Available from: https://covid19.go.id/peta-sebaran
3. Alzahrani AS, Mukhtar N, Aljomaiah A, Aljamei H, Bakhsh A, Alsudani N, et al. The Impact of Covid-19 Viral Infection on the Hypothalamic-Pituitary-Adrenal Axis. Endocr Pract. 2020 Dec 1;27(2):83–9.
4. Tan T, Khoo B, Mills EG, Phylactou M, Patel B, Eng PC, et al. Association between high serum total cortisol concentrations and mortality from COVID-19. Lancet Diabetes Endocrinol. 2020 Aug; 8(8): 659–660.
5. Lisdiana. Regulasi Kortisol Pada Kondisi Stres Dan Addiction. Biosaintifika: Journal of Biology & Biology Education. 2012 March ;4(1).
6. Gubbi S, Nazari MA, Taieb D, Klubo-Gwiezdzinska J, Pacak K. Catecholamine physiology and its implications in patients with COVID-19. Lancet Diabetes Endocrinol. 2020 Dec;8(12):978-986.
7. McElvaney OJ, Hobbs BD, Qiao D, McElvaney OF, Moll M, McEvoy NL, et al. A linear prognostic score based on the ratio of interleukin-6 to interleukin-10 predicts outcomes in COVID-19. EBioMedicine. 2020 Nov; 61: 103026.
8. Utrero-Rico A, Ruiz-Hornillos J, Gonzбlez-Cuadrado C, Rita CG, Almoguera B, Minguez P, et al. Interleukin-6-based mortality prediction model for COVID-19: validation and update in multicentre and second wave cohorts. J Allergy Clin Immunol. 2021 May;147(5):1652-1661.e1.
9. Reno CM, Skinner A, Bayles J, Chen YS, Daphna-Iken D, Fisher SJ. Severe hypoglycemia-induced sudden death is mediated by both cardiac arrhythmias and seizures. Am J Physiol Endocrinol Metab. 2018 Aug 1;315(2):E240-E249.
10. Li X, Geng M, Peng Y, Meng L, Lu S. Molecular immune pathogenesis and diagnosis of COVID-19. J Pharm Anal. 2020 Apr; 10(2): 102–108.
11. Zemlin AE, Wiese OJ. Coronavirus disease 2019 (COVID-19) and the renin-angiotensin system: A closer look at angiotensin-converting enzyme 2 (ACE2). Ann Clin Biochem. 2020. May;57(5):339–50.
12. Leow MKS, Kwek DSK, Ng AWK, Ong KC, Kaw GJL, Lee LSU. Hypocortisolism in survivors of severe acute respiratory syndrome (SARS). Clin Endocrinol (Oxf). 2005 Aug;63(2):197-202.
13. Pal R. COVID-19, hypothalamo-pituitary-adrenal axis and clinical implications. Endocrine. 2020 May;68(2):251-252.
14. Tйblick A, Peeters B, Langouche L, Van den Berghe G. Adrenal function and dysfunction in critically ill patients. Nat Rev Endocrinol. 2019 Jul;15(7):417-427.
15. Kumar R, Joy KP. Stress hormones modulate lipopolysaccharide stimulation of head kidney interleukin-6 production in the catfish Heteropneustes fossilis: In vivo and in vitro studies. Gen Comp Endocrinol. 2019 Aug 1;279:109-113.