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ORIGINAL PAPERS

Rosuvastatin Administration and Its Effect on the IL-6, IL-1β, and TNF-α Cytokines Levels in Peripheral Blood Mononuclear Cells of Type II Diabetes Mellitus Patients with COVID-19

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Abstract

Objective: Patients with cardiovascular disease (CVDs) have been reported to have the potential to experience severe COVID-19. The inflammatory cytokines such as IL-6, IL-1β, and TNF-α are frequently found in the COVID-19 cytokine storm. Because of the pleiotropic effect Rosuvastatin could be useful as an anti-inflammatory drug to suppress cytokine storms for possible COVID-19 therapy. This study aims to study the effect of rosuvastatin administration on IL-6, IL-1β, and TNF-α on peripheral blood mononuclear cells in type 2 diabetes mellitus patients stimulated with the SARS-CoV-2 spike protein. **Material and methods:** Mononuclear cells were isolated from peripheral venous blood, and then stimulated with the SARS-CoV-2 subunit S1 spike protein and incubated for 24 hours. Cells that had been stimulated by the SARS-CoV-2 spike protein were separated into two groups. Group 1 was the control group, which was not given rosuvastatin. Group 2 was given rosuvastatin at a dose of 20 μM. The expressions of IL-6, IL-1β, and TNF-α were measured from the cell supernatant using the ELISA method. **Results:** Spike protein stimulation significantly increased the expression of IL-6, IL-1β, and TNF-α ($p = 0.37$). Rosuvastatin administered at a dose of 20 μM did not significantly decrease IL-6 ($p = 0.568$) or IL-1β ($p = 0.848$) expression but increased TNF-α expression ($p = 0.792$). **Conclusion:** Rosuvastatin administration did not affect the expression of IL-6, IL-1β, and TNF-α in peripheral blood mononuclear cells of diabetics with stimulation of the SARS-CoV-2 spike protein.

Keywords: peripheral blood mononuclear cells, spike protein SARS-CoV-2, COVID-19, rosuvastatin, cytokines.

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INTRODUCTION

Since being declared a pandemic in March 2020, COVID-19 has caused individual morbidity and mortality, as well as a decline in world economic conditions. The latest data shows that there were 634 million cases recorded globally with 6.6 million deaths worldwide. In the Southeast Asian region, the highest death rates were reported by Indonesia (275 deaths per 100,000 cases), Thailand (64 deaths per 100,000 cases), and India (2638 new cases). This indicates that the COVID-19 pandemic is still a threat throughout the world[1,2]. Health and financial conditions in both developed and developing countries have been severely impacted by COVID-19 to an extent never before seen[3].

The latest research proves that 75% of COVID-19 patients are hospitalized have at least one comorbidity. The most frequently reported comorbidities are hypertension, type 2 diabetes mellitus (type 2 DM), cancer, endothelial dysfunction, and cardiovascular disease. These comorbidities can potentially worsen COVID-19 patients' conditions, prolong their hospitalization, and increase mortality[4-6].

Type 2 DM is a non-communicable disease that affects many of the world's population. Data from the International Diabetes Foundation shows that 537 million people in the world have type 2 DM. This is a three-fold increase over the last 20 years. In Indonesia, type 2 DM has a 10.9% prevalence with a total of 10.3 million afflicted, which places Indonesia in 6th place for type 2 DM patients worldwide. This, coupled with COVID-19, results in worse outcomes compared to the non-type 2 DM population. Patients with type 2 DM have impaired immunity against COVID-19 related to chronic inflammatory conditions, procoagulation, and potential for disruption of angiotensin-receptor receptors converting enzyme-2 (ACE2)[7-9].

ACE2 receptor disruption and cytokine storms are the two main mechanisms that are often cited as causing patients with type 2 DM to be more likely to contract COVID-19 and have a worse outcome. Studies show that COVID-19 patients have increased expression of pro-inflammatory cytokines such as IL-1β, IL-1, IL-2, IL-6, TNF-α, IFN-γ, IP-10, GM-CSF, MCP-1, and IL-10. Currently, cytokine storm prevention is one of the therapeutic targets for COVID-19 patients[10].

One type of drug that is said to have potential in the treatment of COVID-19 patients is the statin group. Statins are one of the most widely consumed drugs among the world's population. Statins have good efficacy and tolerability profiles in lowering cholesterol and for primary and secondary cardiovascular disease prevention.[10] Studies shown that statins have several pleiotropic effects, which have potential benefits when associated with COVID-19 pathogenesis as anti-inflammatories. Statins are also used in type 2 DM patients for primary prevention, which has been shown to reduce atherosclerosis incidence and reduce all mortality related to cardiovascular disease. The aim of this study was to determine the effect of rosuvastatin administration on the expression of IL-6, IL-1β, and TNF-α in peripheral blood mononuclear cells. This phenomenon was observed in people with type-2 diabetes mellitus stimulated with the SARS-CoV-2 spike protein and compared to a control group[12,13]. The IL1β cytokine disrupts insulin communication in hepatocytes and adipocytes, preventing insulin-induced glucose absorption, stifling lipogenesis, and decreasing adiponectin secretion. The cytokine IL-1β acts on the pancreatic islets, causing β-cell death, β-cell replication impairment, and a decrease in insulin output. Moreover, tumour necrosis factor-alpha (TNF-α), a cytokine known to enhance insulin resistance, is promoted by IL-1β1[14].

MATERIAL AND METHODS

Data sources, searches, and study selection

This study utilized an experimental pre-test and post-test control group design in the in vitro setting. The experimental work was done between December 2022-January 2023. This study adhered to the Declaration of Helsinki's guiding principles. Approval from the Institutional Ethics Committee of the Faculty of Medicine at Universitas Airlangga has been granted for this study's protocols (approval number: 88/EC/KEPK/FKUA/2022). Informed consent was given by the patients as they were willing to be sampled for PMBCs and take part in research projects.

Human peripheral blood mononuclear cell samples (PBMCs) were taken from the peripheral blood of diabetic patients who agreed to participate in the study. Patients with diabetes who were under 40 years old, had negative COVID-19 swab examination results, had no previous SARS-CoV-2 infection, had never been vaccinated for COVID-19, and did not have other concomitant conditions other than diabetes met the inclusion criteria.

The PBMCs were then exposed to the SARS-CoV-2 spike protein and divided into two treatment groups at random. P0 was the label for the control group that did not receive rosuvastatin, while P1 was the group that received 20 μM of rosuvastatin. Each treatment group (P0-P1) and the negative control group underwent three replications. This resulted in 12 well plates overall. Repetitions on this procedure were conducted for each outcome's measurements, specifically for the expression of IL-6, IL-1β, and TNF-α.

RESEARCH PROCEDURES

SARS-CoV-2 Spike Proteins

This study utilized *Eschericia coli*-derived SARS-CoV-2 subunit S1 (RayBiotech, Cat No. 230-01101) as the SARS-CoV-2 spike proteins. Based on a previous study's protocol (2009), the concentration of S1 subunit spike protein used in this study was 28 nM[15]. The SARS-CoV-2 spike protein was put in storage at -80 degrees Celsius and stabilized at 4 degrees Celsius for two weeks.

PBMC Isolation

A total of 20 millilitres of fresh whole blood (less than two hours after extraction) were collected in tubes containing anticoagulants (EDTA, heparin, and citrate). Blood was collected in a 50-mL centrifuge tube (Corning) and homogenized with an equal amount of 1x phosphate buffer saline (PBS).

A Ficoll solution (Ficoll Histopaque, Sigma) of 6 mL was added to a 15-mL centrifuge tube (Corning). Then, 6 mL of the blood and PBS mixture was steadily put into the Ficoll solution, resulting in the formation of two distinct liquid phases. Next, a 40-minute centrifugation was performed at 400 x g at 20°C for 40 minutes. Plasma was extracted and stored in 1.5-mL tubes at -80°C. Using a Pasteur pipette, the buffy coat containing PBMCs was removed and moved to a new 15-mL centrifuge tube. Then, after two washes using PBS three times, the buffy coat volume was obtained after 10 minutes of centrifugation at 100 x g and 20°C.

The supernatant that formed was discarded. The pellet was suspended in 1 mL of IX RPMI 1640 medium supplemented with 10% foetal bovine serum (FBS), 2 mM 1-glutamine, 100 U/mL penicillin, and 100 ug/mL streptomycin. The PBMC quantity was determined using a cell counter. PBMCs were incubated in a 37% incubator with 5% CO2 for one day.

PBMCs Seeding

BMCs in 1x RPMI 1640 medium was supplemented with 10% fetal bovine serum (FBS), 2 mM 1-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. In 18-well plates, PBMCs were seeded for each treatment and infection duration. Each well plate contained 100,000 PBMCs. Each observation was performed in triplicate.

Stimulation of PBMCs with SARS-CoV-2 Spike Protein

The PMC cell culture was exposed to the spike protein SARS-CoV-2 subunit S1, which was dissolved in RPMI medium. The S1 subunit protein was exposed at a concentration of 28 nM, then incubated on ice for 30 minutes and at 37.5 degrees Celsius for 30 minutes. The culture was then replenished with 5 ml of RPMI 1640 and returned to 37 degrees Celsius. After 24 hours, 0.5 ml of medium supernatant was collected, and the ELISA method was used to analyse the basal cytokine levels. Randomization for statin exposure was initiated when there was a considerable increase in cytokine levels compared to the negative control (without exposure to spike proteins). Samples were divided into two groups: the control group and the group receiving rosuvastatin at 20 μM.

Statin Treatment Method

Samples were divided into two groups: the control group and the group receiving rosuvastatin 20 μmol/L. The administration of rosuvastatin was randomized and single-blind. Statin was kept in the medium after being dissolved in 5 mM dimethyl sulfoxide (DMSO). PBMCs dissolved in the medium were incubated with or without statin. The cell culture was added with statins at concentrations of 20 μM. Control cultures were incubated with medium and DMSO at concentrations of 0%, 2%, and 1%, which corresponded to the statin concentration added. Each culture was incubated for 24 hours. At the conclusion of the incubation period, the culture medium was collected, the cells were separated by centrifugation, and the supernatant was stored at -70°C for future cytokine counting assays.

Detection of Cytokines in PBMCs Supernatants Stimulated with SARS-CoV-2 Subunit S1 Spike Protein by the ELISA Method

IL-6, IL-1β, and TNF-α cytokines in PBMCs supernatants stimulated with SARS-CoV-2 spike-protein

were detected using a human cytokine-specific ELISA kit. The workup consisted of the controls and calibrators (triplicate) that were included in the kit. Samples of control and PBMC supernatant were diluted at a ratio of 1:10 with sample diluent. Microwells (12x8 wells) were supplied with 100 µl of negative control, positive control, calibrator, and sample dilutions. Microwells were then sealed with a cover and incubated at 37°C for one hour. Microwells were then washed with lx wash buffer and repeated six times using the Wellwash™ Microplate Washer (Thermo Scientific). After washing, 100 µl of horseradish peroxidase (HRP)-conjugated monoclonal antibody (MAb) was added. Microwells were then sealed with a cover and incubated at 37°C for one hour. After washing each well, 100 µl TMB chromogen was added and incubated at room temperature for 10 minutes. Each well received 100 µl of stop solution. The resulting color intensity was measured at a wavelength of 450 nm using a microplate photometer (Thermo Scientific). Each cytokine level was measured in the two groups, namely the control group (without rosuvastatin administration) and the group where 20 µM of rosuvastatin was administered.

Statistical analysis

The difference was deemed statistically significant if $p<0.05$.

RESULT

The PBMCs were isolated using the density gradient centrifugation technique with a Ficoll solution. The centrifugation process produced a cloudy annulus between the plasma and Ficoll layers containing PBMCs. A mononuclear cell culture was maintained for three days. Then, SARS-CoV-2 subunit S1 spike protein was stimulated and incubated for 24 hours. The mean value of viable cells was 9.45 x 10^5 PBMCs/mL (Figure 1).

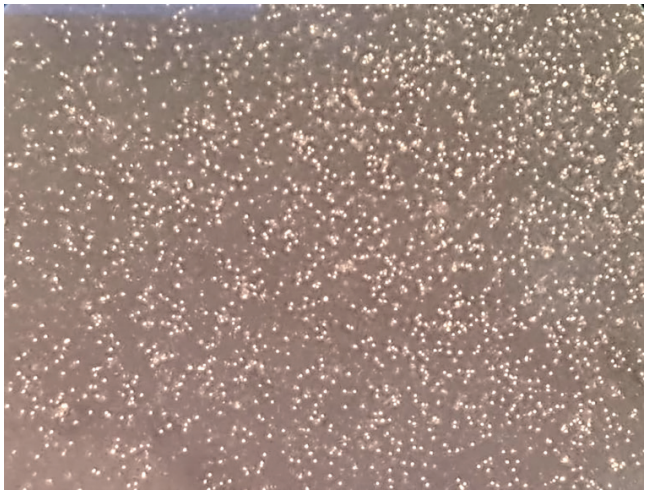

Figure 1. Calculation of cell density using hemacytometer; viable cells appear white with an intact round morphology

After 24 hours of incubation following SARS-CoV-2 spike protein stimulation, cytokine expression levels were assessed. Compared to the PBMCs without spike protein administration group, the TNF-α expression was 1.7 times higher ($p=0.037$) (Figure 2). IL-6 was expressed 4,680 times more than in the PBMCs without spike protein administration group ($p=0.037$). IL-1β expression was 4.9 times greater than that in the group of PBMCs without spike protein administration ($p=0.037$). These findings indicate that SARS-CoV-2 spike protein stimulation increases PBMCs' TNF-α, IL-6, and IL-1β expression.

The T2 test performed on the TNF-α group found no significant differences between groups ($p=0.792$; $p>0.05$). It can be seen that the rosuvastatin administration increased TNF-α expression, but this was not statistically significant. In the IL-6 group, there was no significant difference between groups ($p=0.568$; $p>0.05$). It can be seen that rosuvastatin administration decreased IL-6 expression, but this was not statistically significant. Tests performed on the IL-1β group showed that the data were homogeneous, but there were no significant differences between groups ($p=0.848$; $p>0.05$). It can be seen that rosuvastatin administration decreased IL-1β expression, but this was not statistically significant.

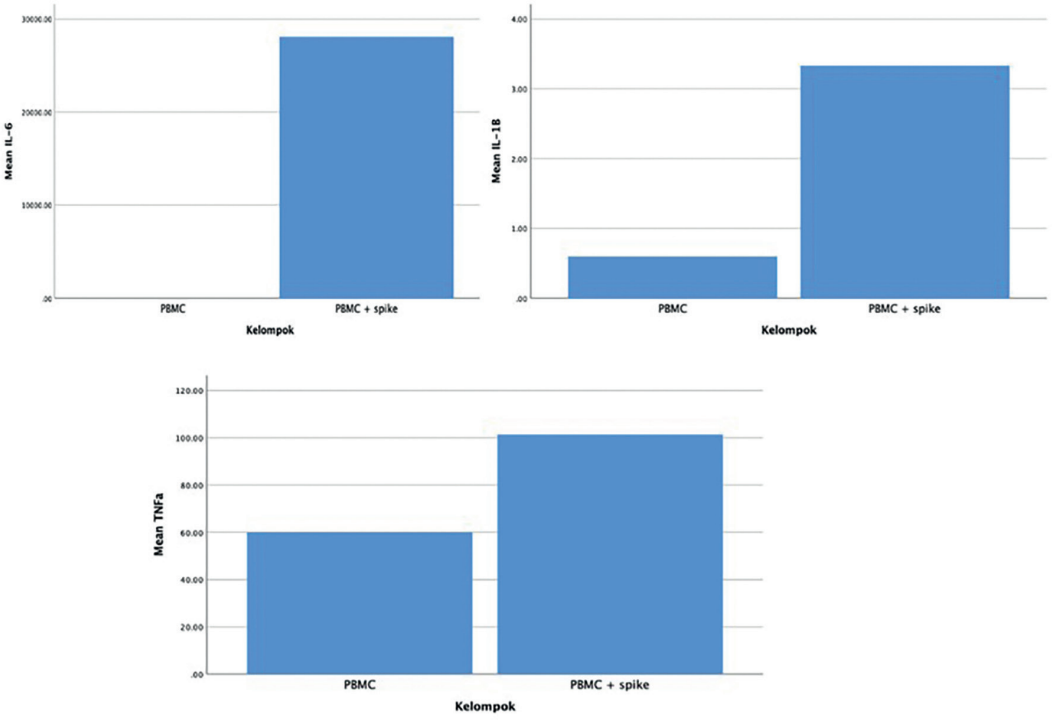

Figure 2. Differences in levels of TNF-α, IL-6, and IL-1β between PBMCs without spike protein and SARS-CoV-2 spike protein stimulation

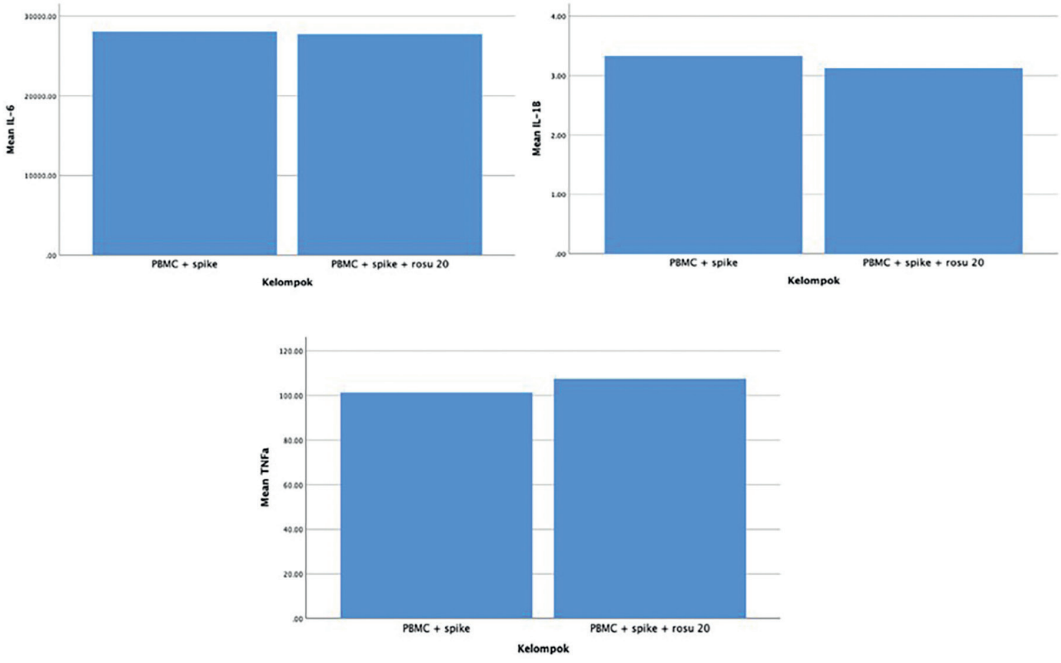

Figure 2. Differences in levels of TNF-α, IL-6, and IL-1β between all doses of atorvastatin and control groups

DISCUSSION

SARS-CoV-2 subunit S1 protein may upgrade the inflammation response by increased expression of IL-6, IL-1β, and TNF-α in PBMCs. This observation found a sizeable increment within the IL-6, IL-1β, and TNF-α levels between PBMC businesses with SARS-CoV-2 spike protein incitement and organizations without spike protein incitement. This finding is consistent with that of Gu, who subjected rodents to spike protein and observed a critical increase in TNF-α and IL-6 in their bronchoalveolar lavage liquid (BALF). The pneumonic fiery method appears to have quickened TNF-α and IL-6, and may also incite a cytokine storm in the lung's interior[16]. The preliminary cytokine storm in SARS-CoV-2 infections begin with the release of inflammatory cytokines (IL-6, IL-1, and TNF- α), as well as elevated chemokine levels inside the respiratory epithelium[17]. The disturbance of the respiratory epithelium by TNF-, IL-6, IL-17, and IL-1 is thought to contribute significantly to lung injury in ARDS patients. This is because these cytokines participate in the inflammatory typhoon process, which worsens pulmonary fibrosis, and may lead to abnormal blood flow through the pulmonary capillaries and organ failure[18].

According to previous research, SARS-CoV-2 infects human respiratory epithelial cells via interacting with ACE2 receptors on the glycoprotein S spike[19]. The primary pathophysiology for SARS-CoV-2 is the direct infection of T cells and macrophages[20]. Consequently, SARS-CoV-2 entry into lymphocytes is facilitated by the expression of ACE2 receptors on lymphocytes, particularly T cells[21].

In this experiment, PBMCs from diabetic donors were used. Elevated glucose levels increase SARS-CoV-2 replication directly in monocytes, and glycolysis increases SARS-CoV-2 replication by generating reactive oxygen species in the mitochondria. Due to this, hyperglycaemia may promote viral replication. In addition, COVID-19 patients with concomitant diabetes and uncontrolled hyperglycaemia tend to have a higher risk of death than those with controlled blood sugar[22]. Studies have shown that diabetics have elevated levels of proinflammatory cytokines, such as interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α), and interleukin-1 beta (IL-1β). These cytokines are produced by various cells in the body, including adipose tissue, liver, and immune cells. The increase in inflammatory cytokines in diabetics is thought to be caused by a number of factors, including increased glucose levels, insulin resistance, and oxidative stress. High glucose levels can activate several pathways in the body that lead to inflammatory cytokine production. This leads to higher mortality in diabetics from comorbidities with COVID-19[23]. This study utilized PBMCs from diabetic donors for some of the reasons listed above.

Bessler conducted an earlier in vitro study of human PBMCs exposed to certain types of statins (which did not stimulate protein spikes). IL-6 and IL-1β levels were not affected by statin supplementation at any concentration. However, atorvastatin reduced IL-1ra, IL-2, and IFN-α secretion at high concentrations[24]. Another study was conducted by Loppnow on human smooth muscle cells and monocytes by stimulation with LPS using different statins. Subsequent observation of IL-6 levels revealed that atorvastatin did not inhibit IL-6 production in monocytes. However, it did inhibit IL-6 production in smooth muscle cell isolate cultures or in cocoons of smooth muscle cells with mononuclear cells[25]. Interestingly, in COVID-19 patients, elevated IL-6 levels are associated with a worsening of the condition. Previous studies have shown a direct correlation between the hyperactivity of the humoral immune route and the immunological profile of severely ill Covid-19 patients. Additionally, in addition to IL-6, which is an essential mediator of multiorgan dysfunction, respiratory failure, and shock[26].

The results of this study showed that there were no significant changes in the expression of IL-6, IL-1β, and TNF-α in the PBMC cell group without rosuvastatin compared to the group with rosuvastatin administration. Several retrospective clinical studies and meta-analyses have tested the potential of statins as a treatment for COVID-19 by inhibiting inflammatory cytokines with varying results. A retrospective cohort study involving 290,348 patients showed rosuvastatin's protective effects (aOR 0.91; p=0.00000024) which could reduce the severity of the disease and prevent hospitalization due to COVID-19. Although, this study would have been better if it was followed by clinical trials of rosuvastatin's potency as a prophylaxis. One of the meta analyses conducted by Lao et al. investigated rosuvastatin's protective benefits in COVID-19 patients and demonstrated that statin use was associated with a significant reduction in mortality (OR=0.72, 95% CI: 0.67-0.77; HR=0.74, 95% CI : 0.69, 0.79), ICU admission (OR=0.94, 95% CI: 0.89-0.99;

HR=0.76, 95% CI: 0.60-0.96), and mechanical ventilation (OR=0.84, 95% CI: 0.78-0.92; HR=0.67, 95% CI: 0.47–0.97)[27]. The results of this study indicated that 20 μM of rosuvastatin administration reduced IL-6 and IL-1β expression in PBMC stimulated with the SARS-Cov-2 spike protein, although this was not statistically significant. These results are in line with previous research by Andrianto et al. on subjects taking simvastatin and atorvastatin. That study observed simvastatin administration to PBMC cells of hypertensive patients who were given the SARS-CoV-2 spike protein transfection. The study reported insignificant reductions in IL-6 and IL-1β. Follow-up studies using atorvastatin in three doses (10, 25, and 50 μM) showed no significant changes in IL-6, IL-1β, and TNF-α expression. Atorvastatin administered at a dose of 10 μM resulted in an increase in IL-1β, although it then decreased with increasing doses[28].

Unlike IL-6 and IL-1β expressions which decreased after administration of 20μM rosuvastatin, TNF-α expression increased, although this was not statistically significant. Research that specifically examines rosuvastatin's effects on PBMCs exposed to the SARS-Cov-2 spike protein has never been done before. Clinical research by Sadeq et al. looked at the effects of 40mg of atorvastatin and 40mg of rosuvastatin for one month in patients with acute coronary syndrome with and without a history of COVID-19. This study found a decrease in HS-CRP and IL6 ($p < 0.001$) in blood samples in both groups. TNF-α expression did not change significantly in the two groups at the end of the study (p=0.813) and (p=0.553). Statin administration increased TNF-α expression in this study. It increased from the baseline throughout one month of exposure, although this was not statistically significant (p=0.324)[29].

In addition to the exact time of administration, the duration of statin administration is also thought to have an influence on the resulting pleiotropic effect. A meta-analysis by Diaz-Arocutipa et al. found that only patients on long-term statin use had lower mortality independently. This suggests that prolonged exposure to statins is necessary to realize their beneficial effects in COVID-19 patients[30]. An in vitro study by Gasbarrino et al. has also identified pleiotropic properties of oral statins (atorvastatin and rosuvastatin) in modulating the adiponectin-AdipoR pathway in the monocyte-macrophage lineage. However, the same study also found that statin therapy can damage adiponectin and its receptors' expression and functions, thus increasing the release of inflammatory cytokines including TNF-α. The current study isolated PBMC cells and did not specifically isolate macrophage cells, and this could be the cause of the less pronounced statin pleiotropic effects. Cellular-based research such as in vitro studies can show a high degree of inter-laboratory and intra-laboratory heterogeneity due to the complex nature of cell cultures. Biological (cell type, seed density, and media composition) and technical (drug type, time of drug administration, dosage, and conditions and duration of incubation) parameters may contribute to variation. This may, in turn, contribute to the differential effects observed between various investigations of statin effects associated with the inflammatory response in PBMC cells[31].

Cellular doses that are not in line with clinical doses can also be the cause of insignificant results. Based on a study conducted by Bergman et al., a dose of 20 mg of atorvastatin is equivalent to a maximum concentration of 40 nM in human serum. This is much lower than the dose used in previous in vitro studies, which was between 10-20 μMol/L. However, this study did not include cellular rosuvastatin doses[32]. Another study by Dikmen et al. stated that rosuvastatin doses of 100μM began to cause toxicity to cells[33]. This made it necessary to rationalize the use of rosuvastatin doses in in vitro and in vivo studies. Intracellular statin concentrations at the level of endothelial and immunologically active cells is still not widely studied[32].

CONCLUSION

Administration of rosuvastatin did not affect expression of IL-6, IL-1β, and TNF-α in peripheral blood mononuclear cells of diabetics with stimulation of the SARS-CoV-2 spike protein.

Conflict of interests

None to declare. All authors participated in the care of the patients and documentation, writing and corrections of the article.

Ethical Standards

The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all of the patients included in this study.

References

1. World Health Organization, "COVID-19 Weekly Epidemiological Update," Nov. 2022.
2. Jamison DA Jr, Anand Narayanan S, Trovão NS, Guarnieri JW, Topper MJ, Moraes-Vieira PM, Zaksas V, Singh KK, Wurtele ES, Beheshti A. A comprehensive SARS-CoV-2 and COVID-19 review, Part 1: Intracellular overdrive for SARS-CoV-2 infection. Eur J Hum Genet. 2022 Aug;30(8):889-898. doi: 10.1038/s41431-022-01108-8.
3. Bhandari S, Kakkar S, Dube A, Grover M, Bhargava S, et.al. Mucormycosis Infections during the Second Wave of COVID-19: Experience from a Tertiary Care Centre in India. Modern Medicina. 2021, Vol. 28, No. 4. https://doi.org/10.31689/rmm.2021.28.4.425
4. Ahmad Malik J, Ahmed S, Shinde M, Almermesh MHS, Alghamdi S, Hussain A, Anwar S. The Impact of COVID-19 On Comorbidities: A Review Of Recent Updates For Combating It. Saudi J Biol Sci. 2022 May;29(5):3586-3599. doi: 10.1016/j.sjbs.2022.02.006. Epub 2022 Feb 10.
5. Rey-Reñones C, Martinez-Torres S, Martín-Luján FM, Pericas C, Redondo A, Vilaplana-Carnerero C, Dominguez A, Grau M. Type 2 Diabetes Mellitus and COVID-19: A Narrative Review. Biomedicines. 2022 Aug 26;10(9):2089. doi: 10.3390/biomedicines10092089.
6. Nassar M, Daoud A, Nso N, Medina L, Ghernautan V, Bhangoo H, Nyein A, Mohamed M, Alqassieh A, Soliman K, Alfishawy M, Sachmechi I, Misra A. Diabetes Mellitus and COVID-19: Review Article. Diabetes Metab Syndr. 2021 Nov-Dec;15(6):102268. doi: 10.1016/j.dsx.2021.102268.
7. Indonesian Endocrinology Association, "Guidelines for the Management and Prevention of Adult Type 2 Diabetes Mellitus in Indonesia-2021," 2021.
8. Singer ME, Dorrance KA, Oxenreiter MM, Yan KR, Close KL. The type 2 diabetes 'modern preventable pandemic' and replicable lessons from the COVID-19 crisis. Prev Med Rep. 2022 Feb;25:101636. doi: 10.1016/j.pmedr.2021.101636. Epub 2021 Nov 18.
9. Hu B, Huang S, Yin L. The cytokine storm and COVID-19. J Med Virol. 2021 Jan;93(1):250-256. doi: 10.1002/jmv.26232. Epub 2020 Sep 30.
10. Zanza C, Romenskaya T, Manetti AC, Franceschi F, La Russa R, Bertozzi G, Maiese A, Savioli G, Volonnino G, Longhitano Y. Cytokine Storm in COVID-19: Immunopathogenesis and Therapy. Medicina (Kaunas). 2022 Jan 18;58(2):144. doi: 10.3390/medicina58020144.
11. Vitiello A, Ferrara F. Plausible Positive Effects of Statins in COVID-19 Patient. Cardiovasc Toxicol. 2021 Oct;21(10):781-789. doi: 10.1007/s12012-021-09674-x. Epub 2021 Jul 13.
12. Ramos R, Comas-Cufí M, Martí-Lluch R, Balló E, Ponjoan A, Alves-Cabratosa L, Blanch J, Marrugat J, Elosua R, Grau M, Elosua-Bayes M, García-Ortiz L, Garcia-Gil M. Statins for primary prevention of cardiovascular events and mortality in old and very old adults with and without type 2 diabetes: retrospective cohort study. BMJ. 2018 Sep 5;362:k3359. doi: 10.1136/bmj.k3359
13. Kostapanos MS, Milionis HJ, Elisaf MS. An overview of the extra-lipid effects of rosuvastatin. J Cardiovasc Pharmacol Ther. 2008 Sep;13(3):157-74. doi: 10.1177/1074248408318628.
14. Cioti, A. M.; Grajdeanu, I. V.; Simionescu, A. A.; Bejan, C. G.; Stanescu, A. M. A. Metabolic Syndrome. Particularities During SARS-CoV-2 Infection. Medicina Moderna ; 28(2):119-125, 2021. https://doi.org/10.31689/rmm.2021.28.2.119
15. Dosch SF, Mahajan SD, Collins AR. SARS coronavirus spike protein-induced innate immune response occurs via activation of the NF-kappaB pathway in human monocyte macrophages in vitro. Virus Res. 2009 Jun;142(1-2):19-27. doi: 10.1016/j.virusres.2009.01.005. Epub 2009 Jan 29.
16. Guo Y, Hu K, Li Y, Lu C, Ling K, Cai C, Wang W, Ye D. Targeting TNF-α for COVID-19: Recent Advanced and Controversies. Front Public Health. 2022 Feb 11;10:833967. doi: 10.3389/fpubh.2022.833967.
17. Law HK, Cheung CY, Ng HY, Sia SF, Chan YO, Luk W, Nicholls JM, Peiris JS, Lau YL. Chemokine up-regulation in SARS-coronavirus-infected, monocyte-derived human dendritic cells. Blood. 2005 Oct 1;106(7):2366-74. doi: 10.1182/blood-2004-10-4166. Epub 2005 Apr 28.
18. Montazersaheb S, Hosseiniyan Khatibi SM, Hejazi MS, Tarhriz V, Farjami A, Ghasemian Sorbeni F, Farahzadi R, Ghasemnejad T. COVID-19 infection: an overview on cytokine storm and related interventions. Virol J. 2022 May 26;19(1):92. doi: 10.1186/s12985-022-01814-1.
19. Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. The proximal origin of SARS-CoV-2. Nat Med. 2020 Apr;26(4):450-452. doi: 10.1038/s41591-020-0820-9.
20. Perlman S, Dandekar AA. Immunopathogenesis of coronavirus infections: implications for SARS. Nat Rev Immunol. 2005 Dec;5(12):917-27. doi: 10.1038/nri1732.
21. Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, Ji R, Wang H, Wang Y, Zhou Y. Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis. Int J Infect Dis. 2020 May; 94:91-95. doi: 10.1016/j.ijid.2020.03.017. Epub 2020 Mar 12.
22. Cauchois R, Koubi M, Delarbre D, Manet C, Carvelli J, Blasco VB, Jean R, Fouche L, Bornet C, Pauly V, Mazodier K, Pestre V, Jarrot PA, Dinarello CA, Kaplanski G. Early IL-1 receptor blockade in severe inflammatory respiratory failure complicating COVID-19. Proc Natl Acad Sci U S A. 2020 Aug 11;117(32):18951-18953. doi: 10.1073/pnas.2009017117. Epub 2020 Jul 22. Erratum in: Proc Natl Acad Sci U S A. 2020 Sep 8;117(36):22604.
23. Lainampetch J, Panprathip P, Phosat C, Chumpathat N, Prangthip P, Soonthornworasiri N, Puduang S, Wechjakwen N, Kwanbunjan K. Association of Tumor Necrosis Factor Alpha, Interleukin 6, and C-Reactive Protein with the Risk of Developing Type 2 Diabetes: A Retrospective Cohort Study of Rural Thais. J Diabetes Res. 2019 Aug 8;2019:9051929. doi: 10.1155/2019/9051929.
24. Bessler H, Salman H, Bergman M, Straussberg R, Djaldetti M. In vitro effect of statins on cytokine production and mitogen response of human peripheral blood mononuclear cells. Clin Immunol. 2005 Oct;117(1):73-7. doi: 10.1016/j.clim.2005.06.014.
25. Loppnow H, Zhang L, Buerke M, Lautenschläger M, Chen L, Frister A, Schlitt A, Luther T, Song N, Hofmann B, Rose-John S, Silber RE, Müller-Werdan U, Werdan K. Statins potently reduce the cytokine-mediated IL-6 release in SMC/MNC cocultures. J Cell Mol Med. 2011 Apr;15(4):994-1004. doi: 10.1111/j.1582-4934.2010.01036.x.
26. Hussein, Sura Zahim. The Relationship of Some Biochemical Markers with Inflammatory Prognosis in COVID-19 Patients. Modern Medicina. 2022, Vol. 29, No. 3. https://doi.org/10.31689/rmm.2022.29.3.241
27. Lao US, Law CF, Baptista-Hon DT, Tomlinson B. Systematic Review and Meta-Analysis of Statin Use and Mortality, Intensive Care Unit Admission and Requirement for Mechanical Ventilation in COVID-19 Patients. J Clin Med. 2022 Sep 16;11(18):5454. doi: 10.3390/jcm11185454.
28. Andrianto A, Pikir BS, Amalia R, Yusrizal T, Alzahra F, Qurota A'yun M, Jonatan M. The effect of statin administration on IL-6

and IL-1b expression in peripheral blood mononuclear cells of a hypertensive patient with SARS-CoV-2 spike protein stimulation. Med Glas (Zenica). 2022 Aug 1;19(2). doi: 10.17392/1481-22.
29. Sadeq A, Al Saffar H and Alabdali S. Inflammatory markers in patients who presented with acute coronary syndrome and history of COVID-19 infection: a cross-sectional study. F1000Research 2022, 11:987 https://doi.org/10.12688/f1000research.123896.1
30. Diaz-Arocutipa C, Melgar-Talavera B, Alvarado-Yarasca Á, Saravia-Bartra MM, Cazorla P, Belzusarri I, Hernandez AV. Statins reduce mortality in patients with COVID-19: an updated meta-analysis of 147 824 patients. Int J Infect Dis. 2021 Sep;110:374-381. doi: 10.1016/j.ijid.2021.08.004.
31. Gasbarrino K, Hafiane A, Zheng H, Daskalopoulou SS. Intensive Statin Therapy Compromises the Adiponectin-AdipoR Pathway in the Human Monocyte-Macrophage Lineage. Stroke. 2019 Dec;50(12):3609-3617. doi: 10.1161/STROKEAHA.119.026280.
32. Björkhem-Bergman L, Lindh JD, Bergman P. What is a relevant statin concentration in cell experiments claiming pleiotropic effects? Br J Clin Pharmacol. 2011 Jul;72(1):164-5. doi: 10.1111/j.1365-2125.2011.03907.x.
33. Dikmen N, Özkan H, Çimen F, Çamdeviren B, Ay E, Ambarcioğlu P, Duran N, Yakin A. Dose-dependent effects of simvastatin, atorvastatin and rosuvastatin on apoptosis and inflammation pathways on cancerous lung cells. Ankara Univ Vet Fak Derg. March 2023;70(2):141-148. doi:10.33988/auvfd.938418