Original Article

Iranian Journal of Immunology
https://iji.sums.ac.ir

The Relationship between Serum Levels of Interleukin-2 and IL-8 with Circulating microRNA-10b in Patients with COVID-19

Zahra Bagheri-Hosseinabadi[1,2], Hamid Ostad Ebrahimi[3], Fatemeh Bahrehmand[4], Gholamhosein Taghipour[5], Mitra Abbasifard[6,7*]

[1]Department of Clinical Biochemistry, School of Medicine, [2]Pistachio Safety Research Center, [3]Pediatric Department, Medicine Faculty, Non-Communicable Disease Research Center, [4]Department of Internal Medicine, School of Medicine, [5]Department of surgery, Ali-Ibn Abi-Talib Hospital, School of Medicine, [6]Immunology of Infectious Diseases Research Center, Research Institute of Basic Medical Sciences, [7]Department of Internal Medicine, Ali-Ibn Abi-Talib Hospital, School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

ABSTRACT

Background: The role of cytokine storm in the immunopathogenesis of coronavirus disease 2019 (COVID-19) has been implicated. To determine the association of microRNA (miRNA)-10b and serum levels of IL-2 and IL-8 in patients with COVID-19.

Methods: Blood samples were obtained from 33 COVID-19 patients and 29 healthy subjects. After RNA extraction and cDNA synthesis, the transcript level of miR-10b was determined by Real-time PCR. In addition, the serum levels of IL-2 and IL-8 were measured in subjects using ELISA.

Results: The patient group comprised of 33 patients with COVID-19 (62.4 ± 3.7 years old), containing 13 (39%) males and 20 (61%) females. In the control group, 29 subjects (56.6 ± 1.6 years old) containing 9 (31%)males and 20 (69%) females were included. The expression of miR-10b was significantly downregulated in the peripheral blood of COVID-19 patients in comparison to the healthy controls (fold change= 0.12, P< 0.0001). The levels of IL-2 (P< 0.001) and IL-8 (P< 0.001) were significantly increased in the serum samples of COVID-19 patients compared to the healthy subjects. The expression level of miR-10b was correlated significantly with the serum levels of IL-2 and IL-8 as well as with the age of patients, ESR and CRP level.

Conclusions: miR-10b is downregulated in the COVID-19 patients and might result in increased levels of IL-2 and IL-8, hence contributing to cytokine storm.

Keywords: Coronavirus disease 2019, MicroRNA, Cytokine storm, IL-2, IL-18

*Corresponding author:
Mitra Abbasifard, MD;
Department of Internal Medicine, Ali-Ibn Abi-Talib hospital, Mofatteh Blvd, Rafsanjan, Iran
Tel: +98-39-18220001
Email: rh.abbasi70@gmail.com

Cite this article as:
Bagheri-Hosseinabadi Z, Ostad Ebrahimi H, Bahrehmand F, Taghipour G, Abbasifard M. The Relationship between Serum Levels of Interleukin-2 and IL-8 with Circulating microRNA-10b in Patients with COVID-19. *Iran J Immunol.* 2021; 18(1):65-73, doi: 10.22034/iji.2021.88780.1904.

Received: 2020-10-31
Revised: 2021-02-22
Accepted: 2021-03-01

INTRODUCTION

The recently emerging coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become pandemic since late 2019 (1). Based on the declaration of the World Health Organization (WHO), a deadly pandemic of COVID-19 affects all lives (2). SARS-CoV-2 attacks the lungs and other tissues, such as the heart and kidney, that express angiotensin-converting enzyme 2 (ACE2) receptor, and causes damage to these organs (3, 4). While COVID-19 occurs frequently in a mild form, the patients with acute respiratory distress syndrome (ARDS), shock, cardiovascular injury, or multi-organ dysfunction are common that are accompanied by a systemic inflammatory response (5). In this context, the inflammatory cytokines increase and result in cytokine release syndrome (CRS), also known as cytokine storm, which plays a critical function in the severity of COVID-19 clinical symptoms. The produced cytokine profile in COVID-19 cases is usually different. Some studies indicate the role of the innate immune system in cytokine storm development and other studies introduce the cytokines of the adaptive immune system as the main cause of the disease (6). Nevertheless, it was not distinct that which cytokines are the major cause of severe symptoms in the patients with COVID-19 (7).

Interleukin (IL)-2 is one of the most important cytokines of the adaptive immune system and was first defined as a T cell growth factor (8). *IL2* gene is located on chromosome 4 (4q27) (9), and its product (i.e. IL-2 cytokine) is a pleiotropic cytokine. IL-2 induces the proliferation and differentiation of both pro and anti-inflammatory T cells by binding with high affinity (Kd ≈ 10−11 M) to the trimeric IL-2 receptor (IL-2R) (8). Some studies have reported that patients with severe COVID-19 have higher plasma levels of IL-2 and IL-2R than patients with non-severe forms of the disease (10-13).

Known as C-X-C motif chemokine ligand 8 (CXCL8) chemokine, IL-8 is a major mediator of the inflammatory response in the innate immune system (14). In humans, the *CXCL8* gene (that encodes the IL-8 protein) is harbored on chromosome 4 (4q13.3) (15). IL-8, also known as a neutrophil chemotactic factor, is produced by various cells like epithelial cells, macrophages, and airway smooth muscle cells (16). This molecule induces chemotaxis of the target cells, mainly neutrophils as well as other granulocytes, which causes them to migrate to the infection site (17). Severe symptoms in some patients with COVID-19 are accompanied by promoted levels of inflammatory cytokines, such as IL-8 (10, 18).

Nevertheless, the exact roles of IL-2 and IL-8 in the development of cytokine storms in patients with COVID-19 and progression of the disease severity are not completely understood. Additionally, the main mechanism of cytokine storm development and altered expression of these molecules in severe COVID-19 patients is unclear. Therefore, the study of the causes of cytokine storm etiology in patients with COVID-19 is valuable. In this context, regulating factors of transcription and translation are the most important research goals in these patients.

MicroRNAs (abbreviated miRNAs) are small non-coding RNA molecules that contain approximately 22 nucleotides and are involved in the modulation of gene expression level (19). These molecules act through base-pairing with complementary sequences within target mRNAs (20), which mostly interact with the nucleotides of 3′ untranslated region (3′-UTR) to trigger the mRNA degradation and suppression of translation (21). However, it has been reported that miRNAs can interact with other regions of target mRNAs, including the gene's promoter, coding sequence, and 5′-UTR (22). Moreover, it has been shown that miRNAs can activate gene expression under certain conditions (23). miRNAs dynamically interact with their target mRNAs and this interplay relies on numerous factors, such

as the affinity of miRNA-target, the copy number of miRNAs and target mRNAs, and subcellular location of miRNAs (24). miRNAs play pivotal roles in multiple physiological processes and improper expression of them is involved in the occurrence of multiple diseases (25). Furthermore, miRNAs are released in extracellular body fluids and are engaged in cell–cell communications (26). A bulk of evidence has established that extracellular miRNAs are potential biomarkers for various diseases (27, 28). According to the role of miRNAs in regulating other genes, they might be involved in the modulation of cytokine production in COVID-19 patients.

miR-10b can be upregulated by pro-inflammatory cytokines, such as IL-6 and tumor necrosis factor (TNF)-α (29); hence, the cytokine storm in COVID-19 patients might impress the expression of miR-10b. On the other side, miR-10b can regulate the production of cytokines (29, 30). This miRNA regulates the levels of CXCL-8, which in turn modulates the infiltration of inflammatory immune cells to the site of inflammation (31). miR-10b has been implicated in multiple immune system-related disorders as well as cancers (29, 30, 32-34), but its role in COVID-19 is still obscure. Therefore, the current investigation aims at evaluating the potential regulatory role of miR-10b on the plasma levels of IL-2, IL-8, and investigate the relationship between these molecules in emerging clinical presentations in patients with COVID-19.

MATERIALS AND METHODS

Study Participants

The local ethics committee by Rafsanjan University of Medical Sciences endorsed the protocol of this study and all individuals voluntarily signed a written consent to participate in the study. Our study included 33 patients who were hospitalized in Ali-Ebn Abi-Taleb Hospital of Rafsanjan city, Kerman, Iran due to the diagnosis of COVID-19. For all COVID-19 patients, diagnosis of the disease was determined by a positive Real-Time PCR test, radiological data confirming COVID-19 infection, and the approval of an infectious disease specialist. Moreover, 29 age- and sex-matched healthy controls were selected. None of the healthy controls were infected with other viruses and they did not have immune-related diseases such as autoimmunity, allergy ,and cancer, or liver diseases. C-reactive protein (CRP) concentration and erythrocyte sedimentation rate (ESR) of participants were also evaluated. The Baseline demographic and clinical specifications of COVID-19 patients and healthy individuals are shown in Table 1.

Sampling

All tested plasma samples of the patients were collected immediately after hospital admission. Approximately 5 ml of peripheral blood was taken using an EDTA-containing

Table 1. Baseline and demographic characteristics of COVID-19 patients and healthy controls.

	Characteristic	COVID-19 patients (*N*=33)	Healthy subjects (*N*=29)	*P* value
Sex	Male, N (%)	13 (39%)	9 (31%)	>0.05
	Female, N (%)	20 (61%)	20 (69%)	
	Age (year)	62.4 ± 3.7	56.6 ± 1.6	>0.05
	ESR (mm/hr)	27.6 ± 16.3	11 ± 2.9	<0.001
	CRP (mg/L)	34.6 ± 13.6	3.8 ± 2.1	<0.001
Comorbidities	Hypertension, N (%)	6 (18%)	NA	NA
	Cardiovascular disease, N (%)	2 (6%)	NA	NA
	Diabetes, N (%)	4 (12%)	NA	NA

COVID-19; Coronavirus disease 2019, ESR; Erythrocyte sedimentation rate, CRP; C-reactive protein, NA; not available.

tube from each patient and healthy control. Plasma samples were isolated by 2500 rpm/10 min centrifugation and stored at −80°C until further experiments.

Cytokines Analysis

Plasma levels of IL-2 and IL-8 were examined exerting the commercial enzyme-linked immunosorbent assay (ELISA) kits (Karmania Pars Gene, Iran) following manufactures' instructions. The sensitivity of the assay was 4 pg/ml for IL-2 and 2 pg/ml for IL-8. The absorbance (optical density (OD)) was measured at 450 nm wavelength.

RNA Isolation and Quantitative mRNA Expression

RNA was isolated from plasma using miRNeasy Serum/Plasma Kit (Cat No. 217184, QIAGEN, USA) complying with the manufactures' guidelines. The purity and concentration of the isolated RNA were confirmed using the relative absorbance ratio at A260/A280 and A260/A230 by a spectrophotometer (Nano Drop 2000, Thermo Scientific, USA). The purified RNA was stored at -80°C for later analysis. For miR-10b quantification via Real-Time PCR in all samples, template RNA was reverse-transcribed by miScript II RT Kit (Cat No. 218161, QIAGEN, USA) following the company's guidelines, in Thermal Cycler instrument (Eppendorf, Germany). Then, cDNA was used in each of the qPCR assays by ABI StepOnePlus real-time PCR System and miScript SYBR® Green PCR Kit (Cat No. 218073, QIAGEN, USA). The qPCR analyses were conducted in triplicate. The expression levels of miR-10b were normalized using U6 small nuclear RNA (U6 snRNA) as reference RNA. Also, the qPCR results were evaluated using the comparative threshold cycle method ($2^{-\Delta\Delta ct}$), as described by Schmittgen and Livak (35).

Statistical Analysis

The evaluation of data for normal distribution was done using Kolmogorov–Smirnov test. To compare non-parametric scale values, the Mann–Whitney *U*-test was used. Pearson's and Spearman's correlations, where appropriate, were exerted to measure the correlation between scale variables. For drawing the graphs, the GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla, CA, USA) was used. Data analysis was conducted by SPSS software version 22 for windows (SPSS, Chicago, IL, USA). The study results were presented as mean ± standard error of the mean (SEM), with statistical significance set at a *P* less than 0.05.

RESULTS

Baseline Data of the Patients

Table 1 demonstrates the specifications of the study participants in the current research. The patient group comprised 33 patients with COVID-19, with a mean age of 62.4 ± 3.7 years old, involving 13 (39%) males and 20 (61%) females. In the control groups, 29 subjects, containing 9 (31%) males and 20 (69%) females, with a mean age of 56.6 ± 1.6 years old were included. The patient and control groups were age- and sex-matched. The ESR level in the patients and controls were 27.6 ± 16.3 and 11 ± 2.9 mm/hr, respectively ($P< 0.001$). There were also increased levels ($P< 0.001$) of CRP in the COVID-19 patients compared to the controls (34.6 ± 13.6 *vs.* 3.8 ± 2.1 mg/L). The most causes of comorbidities in the patients were 6 (18%) subjects with hypertension, 4 (12%) cases with diabetes, and 2 (6%) subjects with cardiovascular disease.

miRNA expression and serum level of cytokines

The expression of miR-10b was observed to be significantly downregulated in the blood samples of COVID-19 patients in comparison to the healthy controls (fold change= 0.12, $P< 0.0001$; Figure 1.A). However, the cytokine level of IL-2 was significantly higher in the

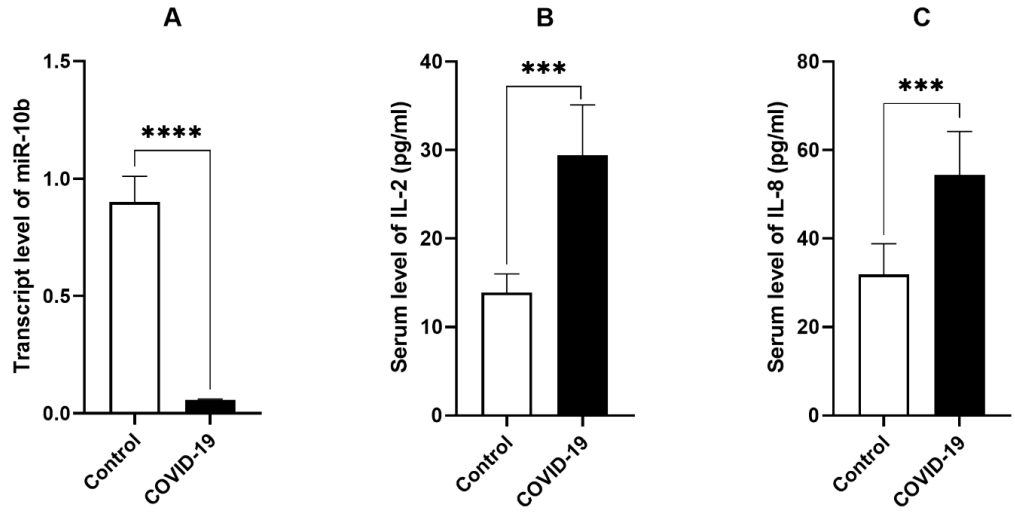

Figure 1. Expression level of miR-10b in the peripheral blood and serum levels of IL-2 and IL-8 in the COVID-19 patients and healthy controls.

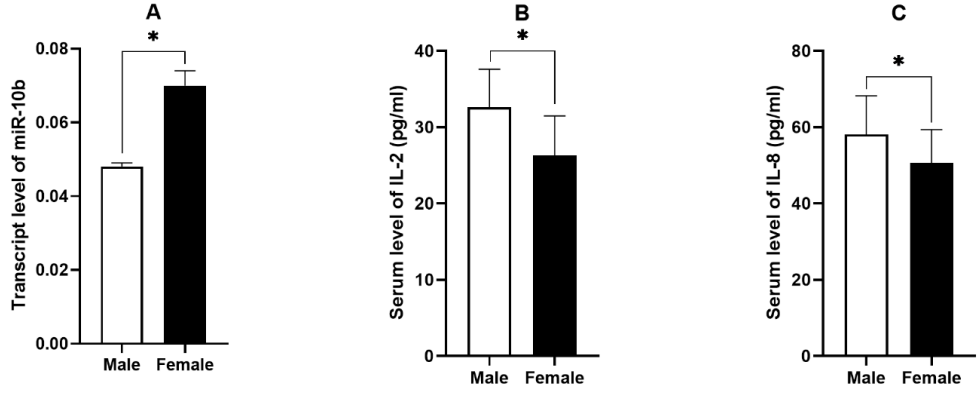

Figure 2. Expression level of miR-10b in the peripheral blood and serum levels of IL-2 and IL-8 in the male and female subjects with COVID-19.

serum of COVID-19 subjects compared to the healthy control group ($P< 0.001$; Figure 1.B). There was a higher level of IL-8 in the serum of the patient group than the healthy control group ($P< 0.001$; Figure 1.C).

The expression level of miR-10b was significantly downregulated in the blood samples of male COVID-19 cases in comparison to female COVID-19 subjects (fold change= 0.63, $P= 0.032$; Figure 2.A). It was detected that serum level of IL-2 was higher in the male COVID-19 cases compared with female COVID-19 patients ($P= 0.015$, Figure 2.B). Furthermore, the serum level of IL-8 was higher significantly in the male COVID-19 subjects compared with female COVID-19 cases ($P= 0.028$, Figure 2.C).

Correlation analysis

The expression level of miR-10b was correlated significantly with the serum levels of IL-2 ($r= -0.66$, $P= 0.017$) and IL-8 ($r= -0.58$, $P= 0.024$) in the COVID-19 patients. The expression level of miR-10b in serum was correlated significantly with the age of patients ($r= -0.53$, $P= 0.037$). However, the serum levels of IL-2 and IL-8 were not correlated with the patient's age. A correlation

Table 2. Correlation of miRNA, IL-2, and IL-8 with demographic and para-clinical characteristics of the COVID-19 subjects.

Item	IL-2	IL-8	Age	ESR	CRP
miR-10b	*r*= -0.66, *P*= 0.017	*r*= -0.58, *P*= 0.024	*r*= -0.53, *P*= 0.037	*r*= -0.64, *P*= 0.011	*r*= -0.71, *P*= 0.008
IL-2	-	*r*= 0.12, *P*= 0.166	*r*= 0.18, *P*= 0.245	*r*= 0.41, *P*= 0.074	*r*= 0.68, *P*= 0.019
IL-8	-	-	*r*= 0.23, *P*= 0.371	*r*= 0.72, *P*= 0.004	*r*= 0.39, *P*= 0.088

IL; Interleukin, ESR; Erythrocyte sedimentation rate, CRP; C-reactive protein

was detected between the transcript level of miR-10b and ESR (*r*= -0.64, *P*= 0.011) and CRP level (*r*= -0.71, *P*= 0.008) in the COVID-19 subjects. The serum level of IL-2 was correlated significantly with CRP level in the subjects (*r*= 0.68, *P*= 0.019). However, the IL-18 level indicated significant correlation (*r*= 0.72, *P*= 0.004) with ESR in the patients (Table 2).

DISCUSSION

Here in this research, we determined, for the first time, the transcript level of miR-10b in the peripheral blood samples of the COVID-19 cases and evaluated its association with the IL-8 and IL-2 as well as pre-clinical presentations of the patients. It was detected that patients with COVID-19 had decreased expression levels miR-10b compared to the healthy controls. Besides, the serum levels of IL-2 and IL-8 were higher in the COVID-19 cases in comparison to the healthy controls.

In a study to profile the transcript level of miRNAs, Li *et al*. (36) examined the peripheral blood of 10 COVID-19 cases and four healthy controls. This study indicated that 35 miRNAs were overexpressed and 38 miRNAs were underexpressed in the peripheral blood of COVID-19 patients in comparison to controls. The top 10 miRNAs with highest reduction were hsa-miR-183-5p, hsa-miR-627-5p, hsa-miR-941, hsa-miR-21-5p, hsa-miR-20a-5p, hsa-miR-146b-5p, hsa-miR-454-3p, hsa-miR-18a-5p, hsa-miR-340-5p, and hsa-miR-17-5p. By performing cluster analysis, it was recognized that all of the differentially expressed miRNAs targeted genes involved in the modulation of molecular functions, cellular components, and biological processes. By performing enrichment analysis, it was detected that protein kinases, peptidases, and the ubiquitin system had the greatest enrichment categories. As a consequence, aberrant expression of miRNAs might play a role in the modulation of the immune responses and viral replication in the COVID-19 (36). Our analysis indicated that miR-10b was downregulated in the peripheral blood samples of the COVID-19 cases in comparison to the controls.

Cytokine storms in the COVID-19 patients associate with the uncontrolled production of proinflammatory cytokines, such as IL-2, IL-6, IL-8, and TNF-α, which leads to aberrant hyper-responses of the immune system. This hyperactivation of the immune system culminates in acute lung injury and ARDS. As a consequence, acknowledging the mechanisms causing cytokine storms and targeting the involved cytokine with different strategies can contribute to ameliorate the COVID-19 patients and achieve better clinical outcomes (37). IL-2 is involved in the stimulation of the proliferation and differentiation of both pro and anti-inflammatory T cells by ligation to IL-2R (8). Several investigations have demonstrated that severe COVID-19 cases have increased plasma levels of IL-2 and IL-2R than patients with non-severe forms of the disease (10-13). Furthermore, IL-8 is involved in the chemotaxis of immune cells, particularly neutrophils, to the site of involvement by an infectious agent (17). Studies have reported that IL-8 is higher in the serum of COVID-19 cases than eventuated in the presentation of

severe manifestations in the patients (10, 18). Our study also indicated that levels of both IL-2 and IL-8 were higher in the serum samples of the COVID-19 cases compared to the controls. In addition, the increased levels of these cytokines were correlated with an inflammatory state in the subjects, as presented by CRP and ESR. Furthermore, we observed that downregulated transcription level of miR-10b was correlated with increased cytokine levels. As a consequence, miR-10b might be involved in the modulation of cytokine storm and inflammation in the COVID-19 cases.

Liu *et al*., by conducting a retrospective investigation of 1190 COVID-19 cases, indicated that males were more frequently infected with the disease. Additionally, increased rates of acute cardiac injury (9.1% vs. 4.3%), acute kidney injury (5.5% vs. 2.9%), and disseminated intravascular coagulation (2.5% vs. 0.7%,) were observed in male subjects compared to the females. Additionally, male COVID-19 subjects had an increased hospital mortality rate (15.7% vs. 10.3%) in comparison to the female COVID-19 subjects. It appears that the male gender is related to a poor prognosis of COVID-19 (38). In line with these pieces evidence, our investigation indicated that cytokine storm-related mediators in the COVID-19 patients, IL-2 and IL-8, were higher in the male patients in comparison to the female patients. On the other side, the miRNA regulating these cytokines (miR-10b) was detected to be lower in the male subjects compared to the female patients. Besides , aging has been associated with higher frequency and severity of the COVID-19 (39). We observed that miR-10b had a negative correlation with the age of COVID-19 patients. These observations suggest that a higher decrease in the miR-10b levels per aging in the COVID-19 patients may be associated with higher inflammation and cytokine storm, leading to higher mortality rate and severity of the diseases. However, it should be noted that none of the IL-2 and IL-8 levels were correlated with the age of the subjects. As a result, miR-10b may regulate other cytokines involved in the cytokine storm phenomenon of the COVID-19 subjects, a hypothesis that requires further examination.

CONCLUSIONS

Considering all the facts, we indicated that miR-10b is downregulated in the peripheral blood samples of the COVID-19 cases that might be in association with increased levels of IL-2 and IL-8 in these patients. The aberrant levels of these factors were associated with male sex, aging, and inflammatory state in the COVID-19 patients. Further investigations are required to disclose the exact involvement of miR-10b, IL-2, and IL-8 in the immunopathogenesis of COVID-19.

ACKNOWLEDGMENTS

The authors are grateful to the patients for their participation in the study.

Conflict of interest: None declared.

REFERENCES

1. MacIntyre CR. Global spread of COVID-19 and pandemic potential. Global Biosecurity. 2020;1(3).
2. Covid C, COVID C, COVID C, Chow N, Fleming-Dutra K, Gierke R, et al. Preliminary estimates of the prevalence of selected underlying health conditions among patients with coronavirus disease 2019—United States, February 12–March 28, 2020. Morbidity and Mortality Weekly Report. 2020;69(13):382.
3. Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. nature. 2020;579(7798):270-3.
4. Turner AJ, Hiscox JA, Hooper NM. ACE2: from vasopeptidase to SARS virus receptor. Trends in pharmacological sciences. 2004;25(6):291-4.
5. Han S, Mallampalli RK. The acute respiratory distress syndrome: from mechanism to translation.

The Journal of Immunology. 2015;194(3):855-60.
6. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. Journal of autoimmunity. 2020:102433.
7. Azkur AK, Akdis M, Azkur D, Sokolowska M, van de Veen W, Brüggen MC, et al. Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. Allergy. 2020;75(7):1564-81.
8. Ross SH, Cantrell DA. Signaling and function of interleukin-2 in T lymphocytes. Annual review of immunology. 2018;36:411-33.
9. Tindall EA, Hoang HN, Southey MC, English DR, Hopper JL, Giles GG, et al. The 4q27 locus and prostate cancer risk. BMC cancer. 2010;10(1):1-6.
10. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The lancet. 2020;395(10223):497-506.
11. Hu B, Huang S, Yin L. The cytokine storm and COVID-19. Journal of medical virology. 2020.
12. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. The Journal of clinical investigation. 2020;130(5).
13. Liu J, Li S, Liu J, Liang B, Wang X, Wang H, et al. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. EBioMedicine. 2020:102763.
14. Liang N, Kitts DD. Chlorogenic acid (CGA) isomers alleviate interleukin 8 (IL-8) production in Caco-2 cells by decreasing phosphorylation of p38 and increasing cell integrity. Int J Mol Sci. 2018;19(12):3873.
15. Zhang S, Gao Y, Huang J. Interleukin-8 Gene−251 A/T (rs4073) Polymorphism and Coronary Artery Disease Risk: A Meta-Analysis. Medical science monitor: international medical journal of experimental and clinical research. 2019;25:1645.
16. Hedges JC, Singer CA, Gerthoffer WT. Mitogen-activated protein kinases regulate cytokine gene expression in human airway myocytes. American journal of respiratory cell and molecular biology. 2000;23(1):86-94.
17. Petri B, Sanz M-J. Neutrophil chemotaxis. Cell and tissue research. 2018;371(3):425-36.
18. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of Immune Response in Patients With Coronavirus 2019 (COVID-19) in Wuhan, China. Clinical Infectious Diseases. 2020;71(15):762-8.
19. Catalanotto C, Cogoni C, Zardo G. MicroRNA in Control of Gene Expression: An Overview of Nuclear Functions. Int J Mol Sci. 2016;17(10):1712.
20. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009;136(2):215-33.
21. Ha M, Kim VN. Regulation of microRNA biogenesis. Nature reviews Molecular cell biology. 2014;15(8):509-24.
22. Broughton JP, Lovci MT, Huang JL, Yeo GW, Pasquinelli AE. Pairing beyond the seed supports microRNA targeting specificity. Molecular cell. 2016;64(2):320-33.
23. Vasudevan S. Posttranscriptional upregulation by microRNAs. Wiley Interdisciplinary Reviews: RNA. 2012;3(3):311-30.
24. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Frontiers in endocrinology. 2018;9:402.
25. Carrington JC, Ambros V. Role of microRNAs in plant and animal development. Science. 2003;301(5631):336-8.
26. Sohel MH. Extracellular/Circulating MicroRNAs: Release Mechanisms, Functions and Challenges. Achievements in the Life Sciences. 2016;10(2):175-86.
27. Wang J, Chen J, Sen S. MicroRNA as biomarkers and diagnostics. Journal of cellular physiology. 2016;231(1):25-30.
28. Huang W. MicroRNAs: biomarkers, diagnostics, and therapeutics. Bioinformatics in MicroRNA Research: Springer; 2017. p. 57-67.
29. Chen L, Al-Mossawi M, Ridley A, Sekine T, Hammitzsch A, de Wit J, et al. miR-10b-5p is a novel Th17 regulator present in Th17 cells from ankylosing spondylitis. Annals of the rheumatic diseases. 2017;76(3):620-5.
30. Han X, Yan S, Weijie Z, Feng W, Liuxing W, Mengquan L, et al. Critical role of miR-10b in transforming growth factor-β1-induced epithelial–mesenchymal transition in breast cancer. Cancer gene therapy. 2014;21(2):60-7.
31. Fang Y, Shi C, Manduchi E, Civelek M, Davies PF. MicroRNA-10a regulation of proinflammatory phenotype in athero-susceptible endothelium in vivo and in vitro. Proceedings of the National Academy of Sciences. 2010;107(30):13450-5.
32. Liu Y, Li M, Zhang G, Pang Z. MicroRNA-10b overexpression promotes non-small cell lung cancer cell proliferation and invasion. European journal of medical research. 2013;18(1):1-8.
33. Varendi K, Kumar A, Härma M-A, Andressoo J-O. miR-1, miR-10b, miR-155, and miR-191 are novel regulators of BDNF. Cellular and Molecular Life Sciences. 2014;71(22):4443-56.
34. Biagioni F, Bossel Ben-Moshe N, Fontemaggi G, Canu V, Mori F, Antoniani B, et al. miR-10b*, a master inhibitor of the cell cycle, is down-regulated in human breast tumours. EMBO molecular medicine. 2012;4(11):1214-29.
35. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C T method. Nature

protocols. 2008;3(6):1101.
36. Li C, Hu X, Li L, Li JH. Differential microRNA expression in the peripheral blood from human patients with COVID-19. Journal of clinical laboratory analysis. 2020:e23590.
37. Mehta Y, Dixit SB, Zirpe KG, Ansari AS. Cytokine Storm in Novel Coronavirus Disease (COVID-19): Expert Management Considerations. Indian journal of critical care medicine : peer-reviewed, official publication of Indian Society of Critical Care Medicine. 2020;24(6):429-34.
38. Liu J, Zhang L, Chen Y, Wu Z, Dong X, Teboul JL, et al. Association of sex with clinical outcomes in COVID-19 patients: A retrospective analysis of 1190 cases. Respiratory medicine. 2020;173:106159.
39. Setiati S, Harimurti K, Safitri ED, Ranakusuma RW, Saldi SRF, Azwar MK, et al. Risk factors and laboratory test results associated with severe illness and mortality in COVID-19 patients: A systematic review. Acta medica Indonesiana. 2020;52(3):227-45.