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SARS-CoV2 infection induce miR-155 expression and skewed Th17/Treg balance by changing SOCS1 level: A clinical study

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ABSTRACT

Background: One of the regulators in severe acute respiratory syndrome coronavirus2 (SARS-CoV2) infection is miRNAs. In COVID-19 patients, immunological responses to SARS-CoV2 infection may be impacted by miR-155, a miRNA associated to inflammation.

Materials and methods: Peripheral blood mononuclear cells (PBMCs) of 50 confirmed COVID-19 patients /Healthy Controls (HCs) was isolated by Ficoll. The frequency of T helper 17 and regulatory T cells was analyzed by flowcytometry. The RNA was extracted from each sample and after synthesis of c-DNA, the relative expression of miR-155, suppressor of cytokine signaling (SOCS-1), Signal transducer and activator of transcription 3 (STAT3), and Fork Head Box Protein 3 (FoxP3) was evaluated by real-time PCR. The protein level of STAT3, FoxP3 and RORγT in the isolated PBMCs measured by western blotting. The serum level of IL-10, TGF-β, IL-17 and IL21 was assessed by ELISA method.

Results: The population of Th17 cells showed a significant rise, whereas Treg cells reduced in COVID-19 cases. The master transcription factor of Treg (FoxP3) and Th17 (RORγT) relative expression showed the same pattern as flowcytometry. STAT3 level of expression at RNA and protein level increased in COVID-19 cases. FOXP3 and SOCS-1 proteins were down-regulated. The relative expression of miR-155, up-regulated in PBMC of COVID-19

Abbreviations: SARS-CoV2, Severe acute respiratory syndrome coronavirus 2; PBMCs, Peripheral blood mononuclear cells; STAT3, Signal transducer and activator of transcription 3; Th, T helper; SOCS-1, Suppressor of cytokine signaling 1; FoxP3, Fork Head Box Protein 3; RORγT, Retinoic acid-related orphan receptor γt; COVID-19, Corona virus disease-19; miRs, microRNAs; PBS, Phosphate Buffer Saline; FBS, Fetal Bovine Serum; PMA, Phorbol myristate acetate; cDNA, Complementary DNA; PVDF, Polyvinylidene fluoride; BSA, Bovine serum albumin; ECL, Electrochemiluminescence; ELISA, Enzyme-Linked Immunosorbent Assay; BMI, Body mass index; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; HDL, High-density lipoprotein; GFR, Glomerular filtration rate; FBS, Fasting blood sugar; TG, Triglyceride; LDL, Low-density protein; Tregs, Regulatory T lymphocytes; TGF-β, Transforming growth factor- β; IL, interleukin.

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patients and revealed a negative correlation with SOCS-1. The serum cytokine profile showed a reduction in TGF-β, on the other hand an increase was seen in IL-17, IL-21 and IL-10 in COVID-19 cases toward control group.
Conclusion: Based on the studies conducted in this field, it can be suggested that Th17/Treg in covid-19 patients can be affected by miR-155 and it can be considered a valuable diagnostic and prognostic factor in this disease.

1. Introduction:

From the beginning of severe acute respiratory syndrome coronavirus2 (SARS-CoV2) pandemic, a large set of experiments were applied to understand different angles of the virus effect on immune responses [1–3]. As a concept, the virus over takes the upper respiratory tract for massive replication, thus, clinically, corona virus disease-19 (COVID-19) severity largely depend on the expression rate of its receptors on the epithelial cells of lung. From immunological perspective, generally, an elevation in inflammatory based responses and cytokine storm are detectable due to high accumulation of inflammatory cytokines and chemokines in lungs and blood circulation [4–7]. Therefore, an effort to find molecular moderators of the field, either at entry level or immunologic responses are highly valuable.

Within recent years, microRNAs (miRs) have grabbed great attention based on their dual role in possible suppression of viral entrance/replication and modulating local/systemic, immune/inflammatory responses [8]. These small molecules play their role by RNA degradation of target genes or pulling the break of translation process of any related proteins. An imbalance in miRs related to immunity in different infections including viral types have been reported in different studies. Referring to this knowledge that miRs could target myriad genes and manage their expression level, their importance as managers of the immune response becomes more important [9].

Among the miRs, miR-155, has been shown to have serious regulatory effects in immunologic and inflammatory response up on virus challenge [10]. During passed years, various clinical [11] and preclinical [12] studies have shown that after viral infection miR-155 rise which also accompanied with the degree of inflammation in lungs, disease severity and ultimately increased mortality rate. On the other hand, inhibition of this miR provided a calmness in lung inflammation leading to lower mortality rate of preclinical models of respiratory viral infections. Taken together, miR-155 is a remarkable asset of viral immune response.

In this research we aimed to understand how changes in miR-155 level in circulation could affect the cellular immune response with a closer comparative look at Th17/ Regulatory T cells in PBMCs of COVID-19 patients.

Table 1
Primer sequences of evaluated genes.

Gene	Primer	Sequence (5′ → 3′)
RORγT	Forward	GAGGAAGTGACTGGCTACCAGA
	Reverse	GCACAATCTGGTCATTCTGGCAG
STAT3	Forward	CTTTGAGACCGAGGTGTATCACC
	Reverse	GGTCAGCATGTTGTACCACAGG
FoxP3	Forward	GGCACAATGTCTCCTCCAGAGA
	Reverse	CAGATGAAGCCTTGGTCAGTGC
SOCS1	Forward	TTCGCCCTTAGCGTGAAGATGG
	Reverse	TAGTGCTCCAGCAGCTCGAAGA
miR-155	Forward	TGCTAATCGTGATAGGGG
	Reverse	GAACATGTCTGCGTATCTC
GAPDH	Forward	GTCTCCTCTGACTTCAACAGCG
	Reverse	ACCACCCTGTTGCTGTAGCCAA
U6	Forward	CTCGCTTCGGCAGCACAT
	Reverse	TTTGCGTGTCATCCTTGCG

Abbreviations: *RORγT: Retinoic acid-related orphan receptor γt;* STAT3: Signal transducer and activator of transcription 3; FoxP3: Forkhead box P3; SOCS1: Silencing of the suppressor of the cytokine signaling-1 miR-155: MicroRNA-155.

2. Material & methods

2.1. Patients and sample collection

A total number of 50 confirmed COVID-19 patients (32 men and 18 women) with severe condition (with a positive PCR test and chest x-ray) that were admitted to Imam Reza hospital, Tabriz, Iran from November of 2021 to March of 2022 was enrolled in this study. Equal number of healthy individuals (28 men and 22 women) were entered in the study as control subjects. The age spectrum of studied population was 20–60 years old. At the time of study, delta and omicron variants were dominant in the country. The patients with any history of inflammatory based or immune related disease including heart disease, cancers, auto immunities and immune deficiencies were excluded from the study. A signed written consent letter was received from each of the subjects prior to sampling. Tabriz university of medical sciences ethics committee approved the study procedures (IR.TBZMED.REC.1400.865).

10 ml of anti-coagulated blood sample was taken from each selected control/patient. Peripheral blood mononuclear cells (PBMCs) were isolated by gradient density centrifugation after adding Ficoll-Paque Plus solution as previously described [13].

2.2. Th17 & Treg flowcytometric evaluation

The isolated PBMCs were first resuspended in cell staining buffer (Phosphate Buffer Saline (PBS) with 2 % Fetal Bovine Serum (FBS) and then subjected to staining by designated panel of commercial antibodies according to each cell type. For Th17, FITC-Mouse Anti-Human CD4 (Clone: L200 (RUO)) and intracellular PE-Mouse Anti-Human IL-17 (Clone: N49-653 (RUO)) were applied, respectively. Briefly, after lymphocytes gating, CD4+ lymphocytes were gated. Finally, Th17 cells were gated based on the IL-17 expression of CD4+ lymphocytes. To stain Treg population, an order of FITC-Mouse Anti-Human CD4, PE-Mouse

Table 2
Clinical and biological characteristics of the COVID-19 patients and healthy controls.

Target	Healthy controls (Mean ± SD) N = 50	COVID-19 patients (Mean ± SD) N = 50
Age	42.7 ± 9.44	46.12 ± 11.23
BMI (kg/m^2)	26.83 ± 3.92	27.31 ± 4.59
Systolic blood pressure (mmHg)	110.1 ± 8.62	132.8 ± 18.33**
Diastolic blood pressure (mmHg)	74.5 ± 8.16	79.67 ± 12.35
Fasting Blood Sugar (mg/dl)	101.9 ± 12.73	118.41 ± 20.17*
Triglyceride(mg/dl)	129.86 ± 22.49	179.22 ± 41.39***
Cholesterol(mg/dl)	151.35 ± 38.92	189.7 ± 44.88*
Vitamin D3 (ng/ml)	41.13 ± 15.73	26.8 ± 13.64***
HDL- Cholesterol(mg/dl)	53.6 ± 5.48	48.98 ± 7.23
LDL-Cholesterol(mg/dl)	117.45 ± 29.84	136.91 ± 33.2*
Albumin (g/dL)	3.721 ± 0.244	3.104 ± 0.342*
Creatinine (mg/dL)	1.355 ± 0.711	1.189 ± 0.756
GFR	76.18 ± 25.41	71.29 ± 22.67
Clinically Positive Tests Subjects	0	50

BMI: body mass index; HDL: high density lipoprotein; LDL: low density lipoprotein; GFR: glomerular filtration rate. Data are presented as mean ± standard division. * $P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ was considered as statistically significant.

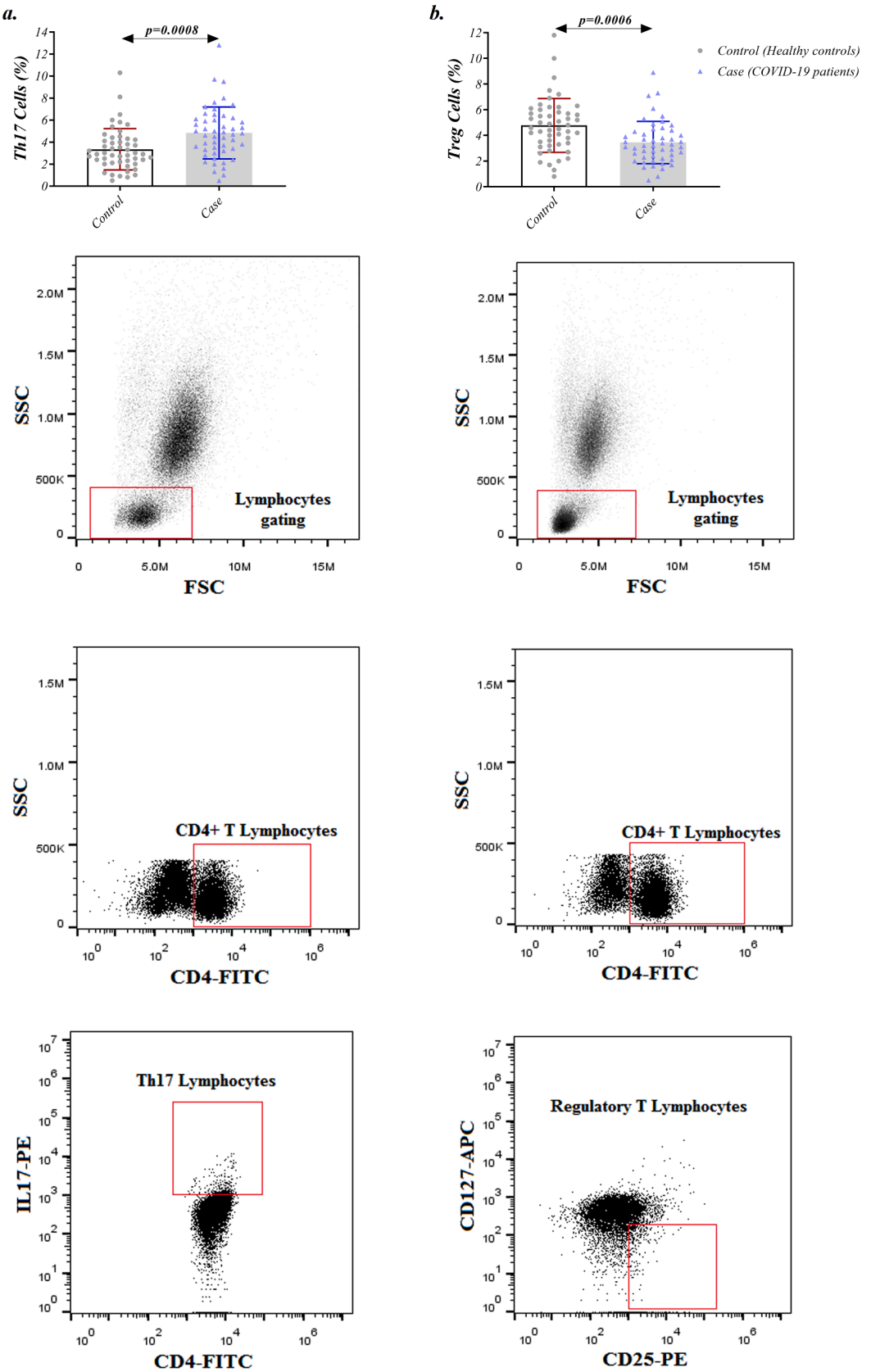

Fig. 1. **a.** Frequency of Th17 cells in cases and HCs and the gating strategy that was used for Th17 verification. As you can see in the figure, the frequency of Th17 lymphocytes is higher in COVID-19 patients compared to healthy controls ($p = 0.0008$). **b.** Frequency of Treg cells in COVID-19 cases compared to HCs and the related gating strategy that was applied for defining Treg cells. As you can see in the figure, the frequency of regulatory T cells is lower in COVID-19 patients compared to healthy group ($p = 0.0006$). The data was shown by Un paired *t*-test analysis and mean ± SD, $p < 0.05$ was considered for being significant.

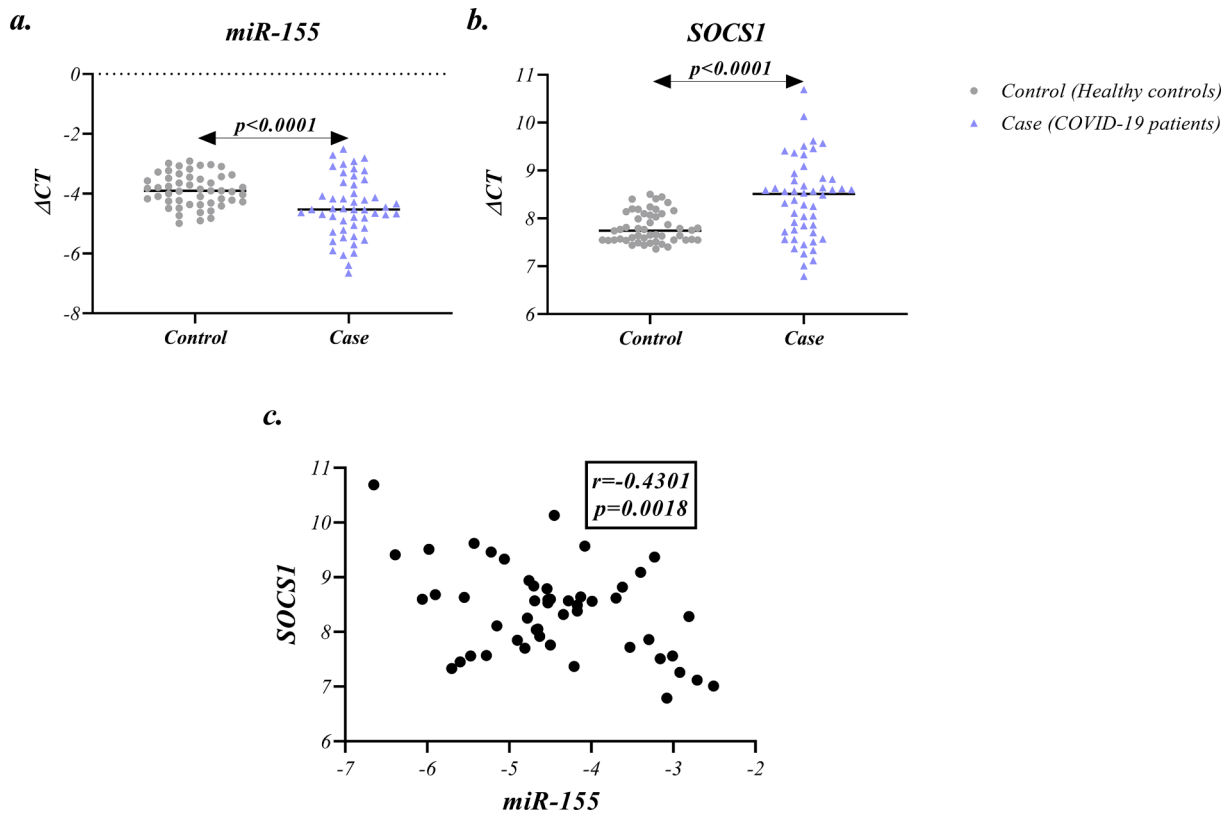

Fig. 2. Gene expression of **a.** *miR-155* and **b.** *SOCS1* in COVID-19 individuals and HCs. As you can see in the figure *miR-155* and *SOCS1* have lower ($p < 0.0001$) and higher ($p < 0.0001$) ΔCT and therefore have higher and lower expression in COVID-19 patients compared to healthy controls, respectively. Un paired *t*-test analysis and mean ± SD were carried out for evaluation, $p < 0.05$ was considered for being significant. **c.** *miR-155* and *SOCS1* negative correlation in COVID-19 cases.

Anti-human CD25 (Clone: M-A251 (RUO)) and APC- Mouse Anti-Human CD127 (Clone: HIL-7R-M21 (RUO)) were used. Briefly, after lymphocytes gating, CD4+ lymphocytes were gated. Subsequently, among the gated CD4+ lymphocytes, CD127-CD25+ lymphocytes were chosen as Treg cells. After staining the cells were washed and resuspended in cell staining buffer prior being read by flowcytometry instrument (FACS Canto II, BD Biosciences). At least a concentration of 1×10^6 cells were used for flowcytometry procedure. All fluorescent labeled antibodies purchased from BD Biosciences company (BD Biosciences, USA).

2.3. Real-time quantitative PCR assay

To evaluate the relative expression level of miR-155 and its targets including suppressor of cytokine signaling (SOCS-1), retinoic acid-related orphan receptor γt (RORγT), signal transducer and activator of transcription 3 (STAT3) and fork head box protein 3 (FoxP3) total RNA was extracted from PBMCs of COVID-19 infected and healthy volunteers by TRIzol. Specific cDNA synthesis kit for miRNA and total RNA (QIAGEN, USA) was used according to manufacturers' instructions. The relative expression of each aforementioned genes was investigated with applying SYBR Green (Takara, Kyoto, Japan) method of Real-Time PCR (Rotor gene, QIAGEN, USA). The sequence of primers that were used for this purpose were summarized in Table1. The small nuclear RNA U6 and GAPDH were considered as housekeeping genes for miR-155 and its target genes relative expressions, respectively. The $2^{-\Delta\Delta ct}$ method was used for analysis of genes fold changes.

2.4. Western blotting

To clarify the STAT3, FOXP3 and SOCS1 differences at protein level in COVID19 patients and healthy controls, we lysed isolated PBMCs with RIPA buffer (Santa Cruz Biotechnology, USA). The concentration of protein was quantified by Bradford assay (Sigma-Aldrich, USA). After wards the samples were treated as following, first subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis, then a polyvinylidene fluoride (PVDF) membrane was used for transferring and ultimately for blocking, samples exposed to 3 % BSA solution of tris buffer saline-tween 20 (TBS-Tween 20) for two hours at room temperature (RT). To continue, anti-GAPDH, anti- STAT3, anti-FOXP3 and anti-SOCS1 antibodies (diluted 1:5000) were applied (2 h at room temperature). After washing, the PVDF membrane was incubated with horseradish peroxidase (HRP)-conjugated antibody (diluted 1:5000) for one hour at room temperature and followed by washing again. In the end, an electrochemiluminescence (ECL) kit (Thermo-Fisher Scientific, USA) and imaging system (Sabz.co, Tehran, IRAN) were used to visualize desired protein bands. All antibodies were purchased from Abcam company (Abcam, USA).

2.5. Enzyme-linked immunosorbent assay (ELISA)

To investigate the IL-17, IL-10, TGF-β and IL-21 concentration levels in the serum of COVID-19 cases and healthy controls an ELISA test was performed for each cytokine based on manufacturers' instructions (MyBioSource, USA).

2.6. Statistical analysis

Graph pad Prism version 8 was used for statistical evaluation. After checking the normality of the data, un-paired *t*-test was carried out for comparison between different groups. Pearson r assay was used to analyze correlation between two variables. A p value < 0.05 was considered as statistically significant.

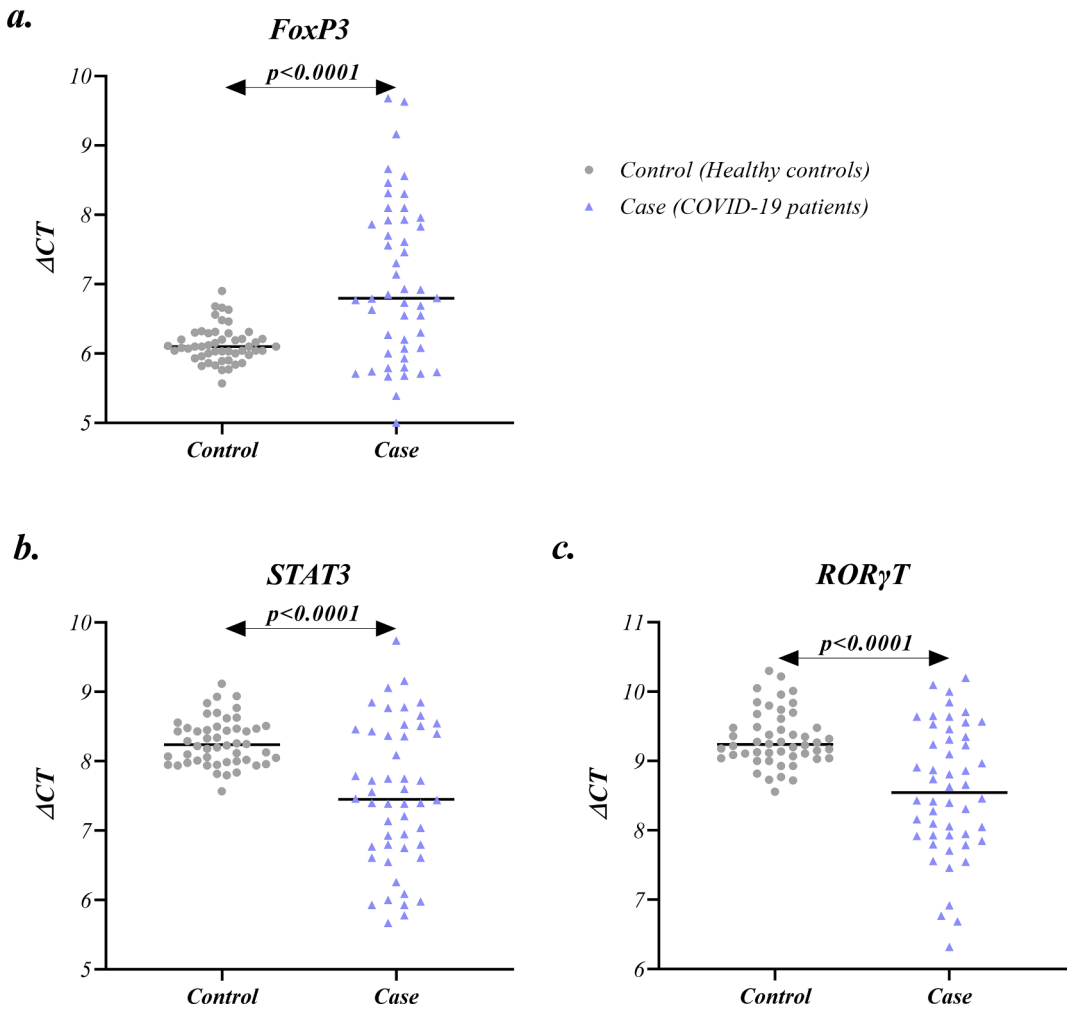

Fig. 3. Gene expression of Th17 and Treg master transcription factors plus STAT3 in COVID-19 cases and HCs. As you can see in the figure **a.** *FoxP3* has increased ($p < 0.0001$) and **b.** *STAT3* ($p < 0.0001$) and **c.** *RORγT* ($p < 0.0001$) have decreased ΔCT and therefore have lower and higher expression in COVID-19 patients compared to healthy controls, respectively. To analyze un paired *t*-test and mean ± SD were assigned and $p < 0.05$ was considered for being significant.

3. Results

3.1. Patient's characteristics

The total features of evaluated subjects were summarized in Table 2. Among the clinical parameters blood pressure and lipid related index were upregulated in infected cases. The level of vitamin D3 and albumin reduced toward HCs.

3.2. T cell frequency skewed to Th17 in COVID-19 patients

As represented in Fig. 1, the population of Th17 increased in COVID-19 individuals compared to normal cases ($p = 0.0008$) (Fig. 1a), in parallel we detected a pattern of decrease in Treg subpopulation of evaluated patients ($p = 0.0006$) (Fig. 1b). This comparison reflected an imbalance of Th17/Treg ratio in patients struggled with COVID-19.

3.3. MiR-155 levels showed negative correlation toward SOCS1 expression in PBMCs of COVID-19 cases

A rise was detected in miR-155 expression of COVID-19 cases, where as a decline was seen in the SOCS1 at transcriptional level ($p < 0.0001$) (Fig. 2a&b). The correlation data revealed a medium negative correlation ($p = 0.0018$) between these two indices (Fig. 2c).

3.4. STAT3 and RORγT expression increased in COVID-19 patients

Following to the aforementioned results, gene expression results of RORγT ($p < 0.0001$) and FoxP3 ($p=<0.0001$) as master transcription factors of Th17 and Treg confirmed the pattern detected in flowcytometry analysis. The STAT3 level of expression also exhibited an increase in COVID-19 infected ones ($p < 0.0001$) (Fig. 3a, b&c).

3.5. Changes in FOXP3, SOCS1 and STAT3 was detected at protein level in COVID-19 cases

The protein level of STAT3 increased in COVID-19 suffering individuals in comparison to control group ($p = 0.0029$). On the other hand, a decreasing status was seen in protein expression of SOCS1 ($p = 0.0074$) and FOXP3 ($p < 0.0001$) in COVID19 patients. In total the western blotting results mirrored the transcriptional results at protein level (Fig. 4 a, b, c&d).

3.6. COVID-19 patients serum cytokine level changed due to changes in Th17/Treg population

A significant change was detected in all evaluated cytokines. As depicted in Fig. 5 (a, b, c, &d), IL-17 ($p = 0.0027$) and IL-21 ($p = 0.006$) concentrations as indicator of active Th17 cells, increased in COVID-19 cases, for IL-10 a slight change recorded for infected cases ($p = 0.0348$)

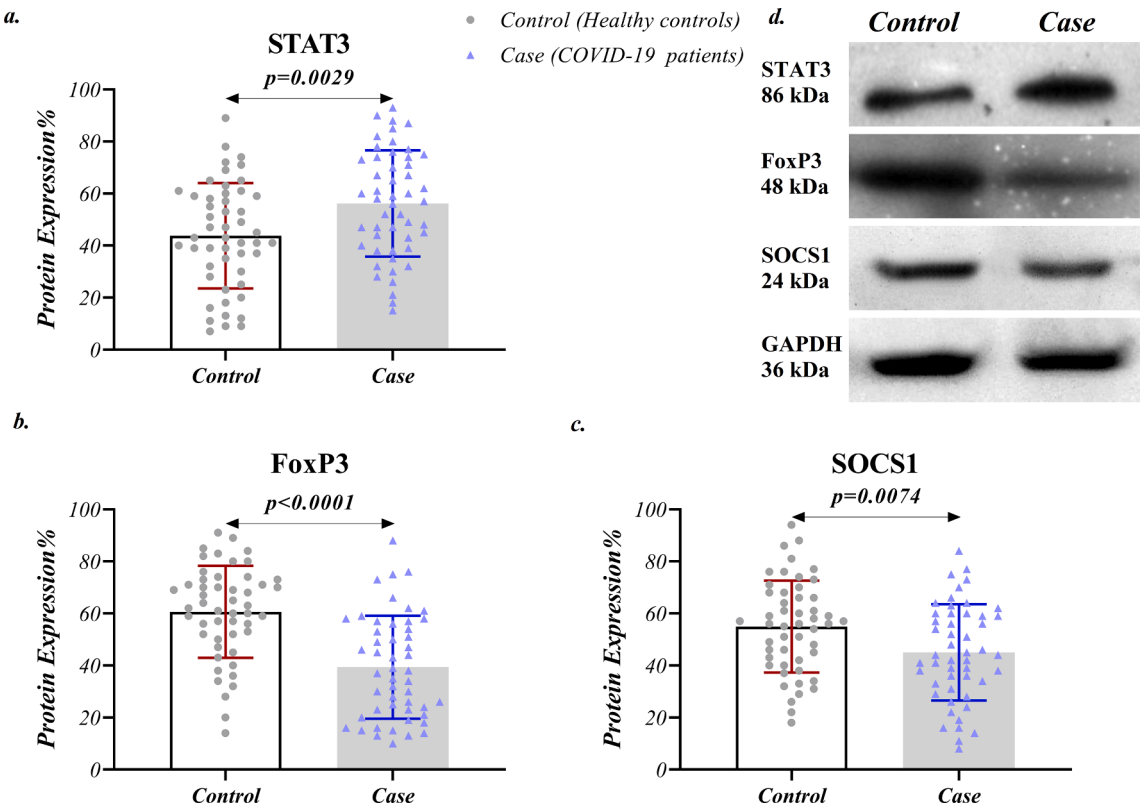

Fig. 4. Percentage of protein expression in STAT3, FOXP3 and SOCS1in PBMCs of COVID-19 cases compared to HCs. As you can see in the figure **a.** STAT3 has increased ($p < 0.0001$) and **b.** Fox3 ($p < 0.0001$) and **c.** SOCS1 ($p = 0.0074$) have decreased protein expression in COVID-19 patients compared to healthy controls, respectively. To analyze un paired *t*-test and mean ± SD were done and $p < 0.05$ was used for statistical changes. **d.** western blotting images of desired protein targets. GAPDH was used as internal control.

whereas for TGF-β ($p = 0.011$) a sharp drop down was observed. The changes in IL-10 and TGF-β reflected the changes in Treg activity based on disease existence.

The total results derived from each experiment was summarized in Table 3 for creation of a full comparative view.

4. Discussion

MiR-155 is a well-known inflammatory based miRNA that affect immune system in various directions [14,15]. During viral infection it may act as a co-director of immune response severity [16]. In this research we have performed a broad and specific evaluation on changes in miR-155 relative expression of COVID-19 PBMCs and the balance of cellular immunity.

As a valid concept that miR-155 is a master regulator of inflammation in different disease [17], as expected we have detected an amplification in miR-155 expression in COVID-19 patients [18]. Our results are in consistence with Abbasi-Kolli et al data, that showed a rise in miR-155 relative expression of acutely infected COVID-19 individuals, it is worth to mention that we have analyzed twice the population that evaluated in Abbasi-kolli et al study. Based on the level of expression of this miR-155, several studies have suggested miR-155 as a potential biomarker for detecting SARS-CoV2 infection and even the severity of the sickness [19–21].

Wang et al have depicted the managing role of miR-155 through SOCS-1 expression on Th17/Treg balance by animal model of acute pancreatitis [22]. We have detected a negative correlation of miR-155 and SOCS-1 in COVID-19 patients. Moreover, we have seen that in parallel, Th17 population and function amplified in COVID-19 cases vs HCs. In a research carried out by Han et al in systemic sclerosis patients, the same pattern was detected [23]. As both disease types are based on chronic inflammation, our results highlight the possible impression of changes in miR-155 on the disease outcome. Soni et al. [24] demonstrated in a different investigation that the lung cytokine storm caused by SORS-CoV2 in ACE2-transgenic mice can be reduced by miR-155 inhibition.

A serious player in the field of inflammation is STAT3. Escobar et al, have illustrated that STAT3 linked directly to miR-155 locus and STAT3-miR-155 axis caused a major proliferative effect on Th17 population in animal model of uveitis [25]. Numerous research on inflammatory illnesses have supported the role of miR-155 in controlling the Th17/Treg balance [22,26–29]. The SOCS1, Jarid2/Wnt/-catenin pathway, and other key molecules in this pathway can be targeted by miR-155 to regulate the Th17/Treg balance [22,26].

Stahl etal have described that in $CD4^+$ T cells of human and mice, over expression of miR-155 caused a state of unresponsiveness in this subpopulation [30]. Based on this study, our results reflected a major reduction in population and function of Treg cells in COVID-19 patients toward HCs. On the other hand, there were studies that showed the expression of miR-155 may not have any effect on FoxP3 [31]. In conflict to these studies, there were evidence on the existence of manager roles for miR-155 on the FOXP3 and STAT5 by SOCS1 expression [32]. Comparing our results to the mentioned studies, it seems that during COVID-19 infection miR-155 over-expression, reduced Treg population and function toward HCs and this might be controlled by the level of SOCS1 that significantly declined in patients. Ultimately, there is a possibility that the protein level of SOCS1 determined the final impact of miR-155 on Treg cells of COVID-19 patients.

Recent studies on serum levels of IL-17 [33,34] and IL-21 [35] represented their effect as an amplifier and boosting factor for the COVID-19 progression. We have seen that in COVID-19 individuals the level of these mentioned cytokines raised above the control patients' level which

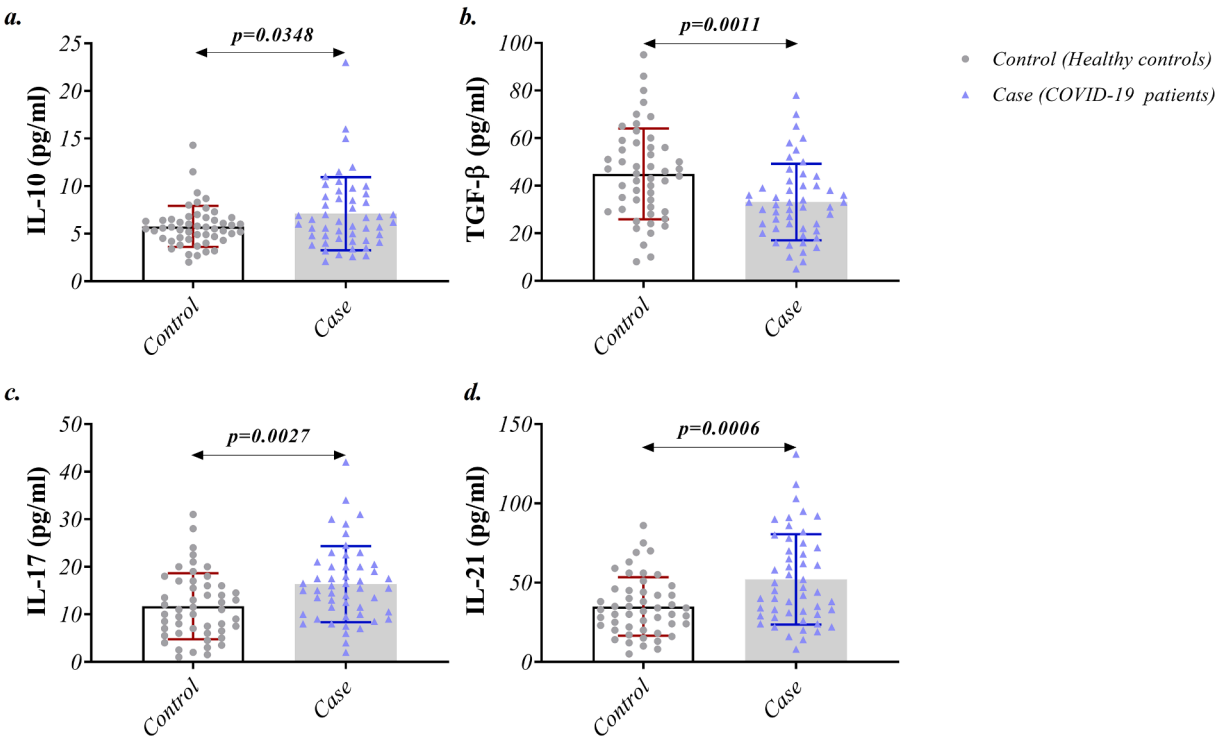

Fig. 5. Concentration of Th17 and Treg related cytokines in serum of patients and HCs. As you can see in the figure increased and decreased concentration of **a.** IL-10 ($p = 0.0348$) and **b.** TGF-β ($p = 0.0011$) as Treg dependent cytokines were seen in COVID-19 patients compared to healthy controls, respectively. In the other hand increased concentration of c. IL-17 ($p = 0.0027$) and d. IL-21 ($p = 0.0006$) as Th17 dependent cytokines were seen in COVID-19 patients compared to healthy controls. The concentrations were reported by ELISA method sand in pg/ml. The comparisons were done by un-paired *t*-test and mean ± SD indicator. $p < 0.05$ was considered for being significant.

Table 3
Molecular and cellular changes in COVID-19 patients vs healthy controls.

Target	Healthy control (Mean ± SD) N = 50	COVID-19 patients (Mean ± SD) N = 50	*p* value
Flow cytometry			
Total Lymphocytes (/μl)	1681 ± 388.4	1023.3 ± 322.8	**<0.0001**
CD4+ T Lymphocytes (/μl)	881.6 ± 254.5	562.5 ± 232.9	**<0.0001**
Th17 Lymphocytes (%)	3.342 ± 1.880	4.826 ± 2.360	**0.0008**
Regulatory T Lymphocytes (%)	4.780 ± 2.093	3.446 ± 1.654	**0.0006**
ΔCT			
RORγT	9.320 ± 0.3945	8.568 ± 0.9378	<0.0001
STAT3	8.279 ± 0.3371	7.515 ± 1.024	<0.0001
FoxP3	6.130 ± 0.2617	6.985 ± 1.182	<0.0001
SOCS1	7.809 ± 0.3184	8.388 ± 0.8336	<0.0001
miR-155	−3.885 ± 0.5467	−4.469 ± 0.9982	<0.0001
ELISA (Serum)			
IL-17 (pg/ml)	11.72 ± 6.939	16.34 ± 7.999	**0.0027**
IL-21 (pg/ml)	34.98 ± 18.40	52.04 ± 28.52	**0.0006**
IL-10 (pg/ml)	5.770 ± 2.156	7.110 ± 3.846	**0.0348**
TGF-β (pg/ml)	44.94 ± 19.09	33.10 ± 16.08	**0.0011**
Protein Expression (%)			
STAT3	43.78 ± 20.26	56.22 ± 20.40	**0.0029**
FoxP3	60.64 ± 17.69	39.36 ± 19.79	**<0.0001**
SOCS1	54.96 ± 17.70	45.04 ± 18.53	**0.0074**

Data are presented as mean ± standard devision. $P < 0.05$ was considered as statistically significant. IL: interleukin; TGF-β: Transforming growth factor- β; RORγT: Retinoic acid-related orphan receptor γt; STAT3: Signal transducer and activator of transcription 3; FoxP3: Forkhead box P3; SOCS1: Silencing of the suppressor of the cytokine signaling-1.

showed that miR-155 indirectly control them by affecting SOCS-1.

IL-10 mainly recognized by its anti-inflammatory roles [36] but it can also be a pro-inflammatory factor, based on the inflammation state. In a recent study, it has been declared that in COVID-19 patients, IL-10 usually increased based on the severity of the disease and it could be counted as poor prognosis factor [37]. Studies that have found elevated levels of IL-10 in COVID-19 patients with severe conditions are in agreement with our findings [34,38]. The elevated IL-10 that was detected in our study in oppose to the drop down of the Treg population in COVID-19 patients versus HCs could be explained by the above interpretation. It's worth to mention that we have observed a drop in serum level of TGF-β that is in line with the reduction of Treg population in COVID-19 patients.

In contrast to our findings, Arroyo et al have shown that an increase in air way secretion of miR-155 could boost the immune responses toward Th1 polarization in young children [11]. The differences in ours results toward Arroyo et al reports might be related to factors including the studied population (children vs adults), subtype of T helpers (Th1/Th2/ vs Th17/Treg) and the evaluated district in the body (local in airways vs systemic in PBMCs). Thus, the exact influence of miR-155 during COVID-19 disease based on the mentioned parameters needed to be clarified by further studies in order to be able to portray the exact act of miR-155 in COVID-19 immunity.

Another dimension that needs specific attention is that based on different studies [12,39] on miR-155 and viral immunity, miR-155 is a friend in innate immunity and early activation of T cell immune responses [40] but through chronic infection it changed to a foe for the balance of the system [41,42]. Therefore, interpretation of its effect should be considered carefully according to the status of the infection. These are the gaps in our study and previous studies that needs to be clarified in near future.

Our study's shortcomings include a lack of information regarding the amount of specific SARS-CoV2 antibodies and viral load in COVID-19

patients, a limited sample size, and a thorough examination of the miR-155/Th17Treg regulatory pathways. The latter issue was brought on by a lack of funding.

5. Conclusion

To our knowledge this is the most comprehensive work for analysis of changes in miR-155 and cellular immunity in COVID-19 patients. The equilibrium of Th17/Treg in COVID-19 patients could be affected by miR-155 at different levels and its manipulation or screening in PBMCs of infected individuals could be a great therapeutic/ diagnostic factor.

Ethics approval and consent to participate

This study was approved by Ethics Committee at Tabriz University of Medical Sciences, Tabriz, Iran (Code: IR.TBZMED.REC.1400.865). Written informed consent was obtained from all participants after receiving an explanation of the study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data cannot be shared in public because of ethics and individual privacy restrictions but are limitedly available by contacting the corresponding author of this study, privately.

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Author contributions

M.Y. contributed to the conception and design of the study. M.S.S-Z., M.H., G.S, M.Z., L.A-M., S.O., and N.N. performed the laboratory assays. M.S.S-Z. and A.M. performed the statistical analysis. D.D. and S.G. contributed in revision of the manuscript. M.S.S-Z. and M.H. wrote the manuscript. H.G.M., and A.M. contributed to the acquisition of data. M. Y. and J.A.H. contributed to editing the final version of the manuscript. All authors read and approved the final manuscript.

References

[1] C. Moon, Fighting COVID-19 exhausts T cells, Nat. Rev. Immunol. 20 (5) (2020) 277.
[2] L. Swadling, M.K. Maini, T cells in COVID-19—united in diversity, Nat. Immunol. 21 (11) (2020) 1307–1308.
[3] M.S. Soltani-Zangbar, L. Aghebati-Maleki, M. Hajivalili, M. Haji-Fatahaliha, R. Motavalli, A. Mahmoodpoor, H.S. Kafil, S. Farhang, R. Pourakbari, F. Jadidi-Niaragh, Application of newly developed SARS-CoV2 serology test along with real-time PCR for early detection in health care workers and on-time plasma donation, Gene Reports 23 (2021), 101140.
[4] M. Hajivalili, M. Hosseini, M. Haji-Fatahaliha, Gaining insights on immune responses to the novel coronavirus, COVID-19 and therapeutic challenges, Life Sci. 257 (2020), 118058.
[5] J. Etemadi, S. Bordbar, M.S. Soltani-Zangbar, M. Hajivalili, L. Aghebati-Maleki, R. Motavalli, A. Mahmoodpoor, S. Shahmohammadi-Farid, S. Abedi Azar, B. Niknafs, Prevalence of SARS-CoV-2 specific antibodies in asymptomatic hemodialysis patients, Immunol. Invest. 51 (4) (2022) 993–1004.
[6] M.S. Soltani-Zangbar, A. Mahmoodpoor, S. Dolati, A. Shamekh, S. Valizadeh, M. Yousefi, S. Sanaie, Serum levels of vitamin D and immune system function in patients with COVID-19 admitted to intensive care unit, Gene Reports 26 (2022), 101509.
[7] M.S. Soltani-Zangbar, F. Parhizkar, M. Abdollahi, N. Shomali, L. Aghebati-Maleki, S. Shahmohammadi Farid, L. Roshangar, A. Mahmoodpoor, M. Yousefi, Immune system-related soluble mediators and COVID-19: basic mechanisms and clinical perspectives, Cell Commun. Signal. 20 (1) (2022) 131.
[8] R.M. O'connell, D.S. Rao, A.A. Chaudhuri, D. Baltimore, Physiological and pathological roles for microRNAs in the immune system, Nat. Rev. Immunol. 10 (2) (2010) 111–122.
[9] H.F. Lodish, B. Zhou, G. Liu, C.-Z. Chen, Micromanagement of the immune system by microRNAs, Nat. Rev. Immunol. 8 (2) (2008) 120–130.
[10] E.F. Lind, P.S. Ohashi, Mir-155, a central modulator of T-cell responses, Eur. J. Immunol. 44 (1) (2014) 11–15.
[11] M. Arroyo, K. Salka, E. Chorvinsky, X. Xuchen, K. Abutaleb, G.F. Perez, J. Weinstock, S. Gaviria, M.J. Gutierrez, G. Nino, Airway mir-155 responses are associated with TH1 cytokine polarization in young children with viral respiratory infections, PLoS One 15 (5) (2020) e0233352.
[12] J.P. Natekar, H.A. Rothan, K. Arora, P.G. Strate, M. Kumar, Cellular microRNA-155 regulates virus-induced inflammatory response and protects against lethal West Nile virus infection, Viruses 12 (1) (2019) 9.
[13] M. Hosseini, M. Haji Fatahaliha, L. Aghebati-Maleki, A. Movassagh Pour, S. Rafati, M. Seifi-Najmi, V. Younesi, F. Jadidi-Niaragh, M. Yousefi, Recombinant Leishmania major lipophosphoglycan 3 activates human T-lymphocytes via TLR2-independent pathway, J. Immunotoxicol. 13 (2) (2016) 263–269.
[14] E. Vigorito, S. Kohlhaas, D. Lu, R. Leyland, miR-155: an ancient regulator of the immune system, Immunol. Rev. 253 (1) (2013) 146–157.
[15] R. Motavalli, J. Etemadi, M.S. Soltani-Zangbar, M.-R. Ardalan, H. Kahroba, L. Roshangar, M. Nouri, L. Aghebati-Maleki, F.M. Khiavi, S. Abediazar, Altered Th17/Treg ratio as a possible mechanism in pathogenesis of idiopathic membranous nephropathy, Cytokine 141 (2021), 155452.
[16] I. Faraoni, F.R. Antonetti, J. Cardone, E. Bonmassar, miR-155 gene: a typical multifunctional microRNA, Biochim. Biophys. Acta (BBA)-Mol. Basis Dis. 1792 (6) (2009) 497–505.
[17] G. Mahesh, R. Biswas, MicroRNA-155: a master regulator of inflammation, J. Interferon Cytokine Res. 39 (6) (2019) 321–330.
[18] M. Abbasi-Kolli, J.S. Nahand, S.J. Kiani, K. Khanaliha, A.R. Khatami, M. Taghizadieh, A.R. Torkamani, K. Babakhaniyan, F. Bokharaei-Salim, The expression patterns of MALAT-1, NEAT-1, THRIL, and miR-155-5p in the acute to the post-acute phase of COVID-19 disease, Braz. J. Infect. Dis. 26 (2022).
[19] R.-A.-H. Haroun, W.H. Osman, R.E. Amin, A.K. Hassan, W.S. Abo-Shanab, A. M. Eessa, Circulating plasma miR-155 is a potential biomarker for the detection of SARS-CoV-2 infection, Pathology 54 (1) (2022) 104–110.
[20] X. Li, Y. Wang, Q. Zhou, J. Pan, J. Xu, Potential predictive value of miR-125b-5p, miR-155-5p and their target genes in the course of COVID-19, Infect. Drug Resist. (2022) 4079–4091.
[21] N. Gaytán-Pacheco, A. Ibáñez-Salazar, A.S. Herrera-Van Oostdam, J.J. Oropeza-Valdez, M. Magaña-Aquino, J. Adrián López, J. Monárrez-Espino, Y. López-Hernández, miR-146a, miR-221, and miR-155 are involved in inflammatory immune response in severe COVID-19 patients, Diagnostics 13 (1) (2022) 133.
[22] D. Wang, M. Tang, P. Zong, H. Liu, T. Zhang, Y. Liu, Y. Zhao, MiRNA-155 regulates the Th17/Treg ratio by targeting SOCS1 in severe acute pancreatitis, Front. Physiol. 9 (2018) 686.
[23] L. Han, Q. Lv, K. Guo, L. Li, H. Zhang, H. Bian, Th17 cell-derived miR-155-5p modulates interleukin-17 and suppressor of cytokines signaling 1 expression during the progression of systemic sclerosis, J. Clin. Lab. Anal. (2022) e24489.
[24] D.K. Soni, J. Cabrera-Luque, S. Kar, C. Sen, J. Devaney, R. Biswas, Suppression of miR-155 attenuates lung cytokine storm induced by SARS-CoV-2 infection in human ACE2-transgenic mice, bioRxiv (2020).
[25] T. Escobar, C.-R. Yu, S.A. Muljo, C.E. Egwuagu, STAT3 activates miR-155 in Th17 cells and acts in concert to promote experimental autoimmune uveitis, Invest. Ophthalmol. Vis. Sci. 54 (6) (2013) 4017–4025.
[26] F. Zhu, H. Li, Y. Liu, C. Tan, X. Liu, H. Fan, H. Wu, Y. Dong, T. Yu, S. Chu, miR-155 antagomir protect against DSS-induced colitis in mice through regulating Th17/Treg cell balance by Jarid2/Wnt/β-catenin, Biomed. Pharmacother. 126 (2020), 109909.
[27] H. Yu, M. Shi, J. Sun, H. Yuan, Liposomal nanoparticle-based miR-155 antagonist regulates Th17/Treg signaling pathway and its inhibitory effect against myocarditis, Mater. Express 12 (1) (2022) 49–56.
[28] L. Yan, F. Hu, X. Yan, Y. Wei, W. Ma, Y. Wang, S. Lu, Z. Wang, Inhibition of microRNA-155 ameliorates experimental autoimmune myocarditis by modulating Th17/Treg immune response, J. Mol. Med. 94 (9) (2016) 1063–1079.
[29] L. Li, W. Shan, H. Zhu, F. Xue, Y. Ma, L. Dong, D. Feng, J. Mao, G. Yuan, X. Wang, SJMHE1 peptide from *Schistosoma japonicum* inhibits asthma in mice by regulating Th17/Treg cell balance via miR-155, J. Inflamm. Res. 14 (2021) 5305–5318.
[30] H.F. Stahl, T. Fauti, N. Ullrich, T. Bopp, J. Kubach, W. Rust, P. Labhart, V. Alexiadis, C. Becker, M. Hafner, miR-155 inhibition sensitizes CD4+ Th cells for TREG mediated suppression, PLoS One 4 (9) (2009) e7158.
[31] N. Seddiki, V. Brezar, N. Ruffin, Y. Lévy, S. Swaminathan, Role of mi R-155 in the regulation of lymphocyte immune function and disease, Immunology 142 (1) (2014) 32–38.
[32] L.-F. Lu, T.-H. Thai, D.P. Calado, A. Chaudhry, M. Kubo, K. Tanaka, G.B. Loeb, H. Lee, A. Yoshimura, K. Rajewsky, Foxp3-dependent microRNA155 confers competitive fitness to regulatory T cells by targeting SOCS1 protein, Immunity 30 (1) (2009) 80–91.
[33] F. Maione, G.M. Casillo, F. Raucci, C. Salvatore, G. Ambrosini, L. Costa, R. Scarpa, F. Caso, M. Bucci, Interleukin-17A (IL-17A): A silent amplifier of COVID-19, Biomed. Pharmacother. 142 (2021), 111980.
[34] M.S. Soltani-Zangbar, F. Parhizkar, E. Ghaedi, A. Tarbiat, R. Motavalli, A. Alizadegan, L. Aghebati-Maleki, D. Rostamzadeh, Y. Yousefzadeh, G. Jadideslam, A comprehensive evaluation of the immune system response and

type-I Interferon signaling pathway in hospitalized COVID-19 patients, Cell Commun. Signal. 20 (1) (2022) 1–15.
[35] N.A.A. Öztürk, A. Ursavaş, A.G. Dilektaşli, E. Demirdöğen, N.F. Coşkun, D. Ediger, A.E. Uzaslan, D.Y. Ermiş, M. Karaca, O.E. Terzi, Interleukin-21: a potential biomarker for diagnosis and predicting prognosis in COVID-19 patients, Turk. J. Med. Sci. 51 (5) (2021) 2274–2284.
[36] A.P. Hutchins, D. Diez, D. Miranda-Saavedra, The IL-10/STAT3-mediated anti-inflammatory response: recent developments and future challenges, Brief. Funct. Genomics 12 (6) (2013) 489–498.
[37] H. Islam, T.C. Chamberlain, A.L. Mui, J.P. Little, Elevated interleukin-10 levels in COVID-19: potentiation of pro-inflammatory responses or impaired anti-inflammatory action? Front. Immunol. 2485 (2021).
[38] H. Han, Q. Ma, C. Li, R. Liu, L. Zhao, W. Wang, P. Zhang, X. Liu, G. Gao, F. Liu, Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors, Emerg. Microbes Infect. 9 (1) (2020) 1123–1130.
[39] C. Su, Z. Hou, C. Zhang, Z. Tian, J. Zhang, Ectopic expression of microRNA-155 enhances innate antiviral immunity against HBV infection in human hepatoma cells, Virol. J. 8 (1) (2011) 1–11.
[40] D.T. Gracias, E. Stelekati, J.L. Hope, A.C. Boesteanu, T.A. Doering, J. Norton, Y. M. Mueller, J.A. Fraietta, E.J. Wherry, M. Turner, The microRNA miR-155 controls CD8+ T cell responses by regulating interferon signaling, Nat. Immunol. 14 (6) (2013) 593–602.
[41] E. Stelekati, Z. Chen, S. Manne, M. Kurachi, M.-A. Ali, K. Lewy, Z. Cai, K. Nzingha, L.M. McLane, J.L. Hope, Long-term persistence of exhausted CD8 T cells in chronic infection is regulated by MicroRNA-155, Cell Rep. 23 (7) (2018) 2142–2156.
[42] A. Mahmoodpoor, M. Hosseini, S. Soltani-Zangbar, S. Sanaie, L. Aghebati-Maleki, S.H. Saghaleini, Z. Ostadi, M. Hajivalili, Z. Bayatmakoo, M. Haji-Fatahaliha, Reduction and exhausted features of T lymphocytes under serological changes, and prognostic factors in COVID-19 progression, Mol. Immunol. 138 (2021) 121–127.