Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Acute and post-acute phase of COVID-19: Analyzing expression patterns of miRNA-29a-3p, 146a-3p, 155-5p, and let-7b-3p in PBMC

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ABSTRACT

Background: When a new pathogen, such as severe acute respiratory syndrome coronavirus 2, appears all novel information can aid in the process of monitoring and in the diagnosis of the coronavirus disease (COVID-19). The aim of the current study is to elucidate the specific miRNA profile which can act as new biomarkers for distinguishing acute COVID-19 disease from the healthy group and those in the post-acute phase of the COVID-19 disease.

Methods: The expression level of selected miRNAs including let-7b-3p, miR-29a-3p, miR-146a-3p and miR-155-5p were evaluated in peripheral blood mononuclear cells (PBMCs) of COVID-19 patients, in both the acute and post-acute COVID-19 phase of the disease and healthy groups, by real-time PCR assays. Specificity and sensitivity of miRNAs was tested by receiver operating characteristic (ROC) analysis in COVID-19 patients.

Results: The expression level of all miRNAs in COVID-19 patients was significantly higher than in the healthy group. Therefore, the expression pattern of miR-29a-3p, miR-146a-3p and let-7b-3p in the post-acute COVID-19 phase was significantly different from the acute COVID-19 phase. ROC analyses demonstrated that miR-29a-3p, -155-5p and -146a-3p may serve as the novel biomarker for COVID-19 diagnosis with high specificity and sensitivity. In addition, miR-29a-3p, and -146a-3p can maybe act as novel biomarkers for distinguishing acute from post-acute phase of COVID-19 disease.

Discussion: The difference in miRNA expression pattern between COVID-19 patients and those in the healthy group, and between acute COVID-19 with post-acute COVID-19, suggested that cellular miRNAs could be used as promising biomarkers for diagnosis and monitoring of COVID-19.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which belongs to the family *Coronaviridae*, subfamily *Coronavirinae*, genus *Betacoronavirus* is the causative agent of COVID-19 disease. This virus was known as the cause of pneumonia cases in late 2019 in Wuhan, China [1–3]. The pandemic of SARS-CoV-2 is spread to more than 200 countries in the world and has been the causative agent for the death of over 2 million people. An additional over 95 million coronavirus cases have been detected since composing this article [4]. Novel theories, in-depth, and extensive studies are needed to promote novel strategies for the management of this viral infection and pandemic outbreaks.

Based on clinical data, symptomatic cases of COVID-19 are classified into three categories: severe, moderate, and mild, however, scientists believe that the interpretation of COVID-19 disease conditions and progression based on blood biomarkers will be more accurate [5–8]. Due

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to the limited information available, it is necessary to identify new non-invasive biomarkers to differentiate and prognosticate the COVID-19 disease which is strictly linked to understanding the interaction between virus and host cell, viral pathogenesis mechanism, and cellular/organ damage [9,10]. The ideal biomarker needs to be easily accessible and easily extractable through liquid biopsies from urine, saliva, blood and other bodily fluids. Moreover, biomarkers should have good specificity, high sensitivity, and must also be closely related to the disease progression [11–13]. In the last decade, more and more evidence has suggested that microRNAs (miRNAs) are promising biomarkers due to their high sensitivity and specificity for the diagnosis of various diseases such as viral infections and cancers [14–16]. In addition, the expression pattern of cellular miRNAs, during the acute phase of the disease, is different from the post-acute phase of the disease [17,18]. So, it has the ability to be considered as a prognosis biomarker.

microRNAs are a type of non-coding RNAs with the length 17–25 nucleotides which play a key role in regulating various biological processes such as inflammation, apoptosis, cell proliferation through post-transcriptional gene regulation [19,20]. Moreover, cellular miRNAs can influence the activity and proliferation of immune system cells in response to pathogens [21]. Accumulating evidence has suggested that various viral proteins can potentially, directly or indirectly, deregulate the expression of cellular miRNAs and by doing so escape the host's immune system by affecting cellular pathways [19,22–25]. Viral acute respiratory infections are the most common leading cause of acute respiratory tract illness in humans that are in turn responsible for a significant proportion of morbidity and mortality. Reportedly, the host immune system response against respiratory viruses is associated with altered expression levels of cellular miRNAs which, as a result, can lead to activate antiviral and inflammatory pathways. On the other hand, as mentioned above, viral proteins also escape host antiviral responses through the dysregulation of cellular miRNAs [22,26–28]. For viral acute respiratory infections (e.g., Rhinovirus, human metapneumovirus, Influenza virus, respiratory syncytial virus) miRNAs are involved in both the up and down regulations of the innate immune response [27]. It has been recently suggested that identification of the miRNA(s) expression pattern and their role in viral diseases could lead to the detection of novel diagnostic tools and therapeutic targets for viral acute respiratory diseases [26].

With regard to the interaction between viruses and cellular factors, it is possible that the expression pattern of miRNAs during the acute phase of disease may be different from the post-acute phase of the disease. Since inflammation increases in COVID-19 patients [29–31], it is hypothesized that the expression pattern of miRNA, related to inflammation (including miR-29, miR-146a, miR-155 and Let-7) [32–38], would be different during the acute and post-acute phases of the disease. This in turn may be useful for the identification of novel biomarkers for diagnosis and prognostic monitoring of COVID-19. Therefore, the aim of this study is to investigate the expression pattern of miR-29a-5p, 146a-3p, 155-5p, and Let-7b-3p in PBMC of Covid-19 virus-infected individuals in both the acute and post-acute phases of the disease.

2. Materials and methods

2.1. Subjects

From April 2020 to July 2020, 18 consecutive treatment-naïve Covid-19-infected patients were admitted in Valfajr clinic in Tehran, Iran (related to Iran University of Medical Sciences), were recruited in the current cross-sectional survey. Blood samples were taken from these patients twice (once after diagnosis of Covid-19 virus infection by the molecular method in the acute period of the disease and the next time in the recovery period) and from 15 healthy individuals.

2.2. Ethical issues

This study was conducted with the ethical principles and the standards as well as national norms for performing medical studies in Iran. Survey ethical approval was received from Iran University of Medical Sciences (IUMS), Tehran, Iran ethics' committee (ethical code: IR.IUMS.REC.1399.223). All individuals provided informed consent to participate in the study and this survey was performed according to the Second Declaration of Helsinki.

2.3. SARS-CoV-2 real-time RT-PCR

Total RNA extraction from the nasal, nasopharyngeal, and oropharyngeal swab specimens of all individuals was carried out by using the QIAamp Viral RNA Mini Kit (Qiagen, Germany) in accordance with the manufacturer's directions. As well, the real-time RT-PCR assay for the qualitative detection of nucleic acid from SARS-CoV-2 (N, and RdRP gene detection) was performed according to the Manufacturer's Instructions of Sansure COVID-19 Nucleic Acid Test Kit (Sansure Biotech Inc.). In addition, to examine the expression levels of selected miRNAs after the negative nasopharyngeal RT-PCR test, the RT-PCR test was repeated every 15 days for 45 days.

2.4. Collection of specimens and isolation of peripheral blood mononuclear cells (PBMCs)

Five mL of peripheral blood was taken from each participant into a sterile EDTA-containing vacutainer tube and PBMCs were isolated by the Ficoll Hypaque density gradient centrifugation (Lympholyte-H, Cedarlane, Hornby, Canada) technique. Next, Separated PBMCs were washed three times with phosphate-buffered saline (PBS) solution (pH $= 7.3 \pm 0.1$) and pleat of PBMCs re-suspended in 250 μL of RNA preservative solution (RNALater, Ambion, Inc., Austin, TX).

2.5. Total RNA isolation and miRNA expression analysis

Total cellular RNA was isolated from PBMC specimens using the miRNeasy Mini (reference 217004, Qiagen, CA) Kit in accordance with the manufacturer's guidelines. The quantity and quality of the isolated RNA were determined on a NanoDrop (Thermo Scientific, Wilmington, MA) spectrophotometer and were kept at −20 °C until use. Synthesis of cDNA was carried out on 5 μg of total RNA by the miScript® II RT Kit (Qiagen, Germany). It is noteworthy that miR-29a-3p, 146a, 155-5p, and Let-7b-3p were selected to determine their expression in the two stages of acute phase and recovery in PBMC samples of patients with SARA-CoV-2 infection as well as healthy individuals based on available information.

The real time PCR assay was performed using the miScript SYBR Green PCR Kit (Qiagen, Valencia, CA; #218073), in accordance with the manufacturer's protocols. This assay was carried out at 95 °C for 3 min, followed by 40 cycles of 95 °C for 20 sec, 60 °C for 25 sec, and 70 °C for 30 sec, using the Rotor-Gene® real time PCR instrument Q (Qiagen, Hilden, Germany). The expression levels of let-7b-3p, miR-29a-3p, miR-146a-3p, and miR-155-5p were normalized to SNORD47 RNA as reference RNA and the fold change was calculated using the Livak [39] method. All the reactions were performed in triplicate.

2.6. Statistical analysis

All data were analyzed with SPSS version 16.0 and GraphPad Prism version 6.0 software. After pre-processing the qPCR data, expression level differences of miRNA between the acute COVID-19 group with the healthy control group and between the post-acute COVID-19 group with the healthy control group were analyzed using the independent-samples *t*-test or the Mann-Whitney *U* test. In addition, paired sample *T*-test was carried out to examine the statistical difference of the expression level of

each miRNA between acute COVID-19 and post-acute phase of COVID-19 disease. Receiver-operator characteristic (ROC) curve analysis was performed for each miRNA to identify the sensitivity and specificity of miRNAs as biomarkers of COVID-19 disease. Also, the Spearman's correlation coefficient was applied to analysis the association among expression level of miRNAs, viral load, N and RdRp genes. In addition, the false discovery rate was controlled according to the Benjamini and Hochberg procedure, and adjusted P-values were calculated.

3. Results

3.1. Characteristics of participants

Eighteen consecutive COVID-19 infected individuals (in both acute and post-acute phases of the disease) and 15 healthy people (as control) were enrolled in this cross-sectional survey. Our results show that both the study group and the healthy group were matched well for age and gender. The mean age of the SARS CoV-2 infected patients was 38.2 ± 11.8 (ranging between 22 and 65 years) and for healthy people was 36.6 ± 12.2 (ranging between 23 and 65 years). Among patients and controls, 9/18 (50%) and 8/15 (53.3%) were male, respectively (Table 1). The clinical and epidemiological characteristics of 18 patients with confirmed COVID-19 are described in Table 2. As well, according to the result of real-time PCR, mean (±SD) duration of SARS-CoV-2 was 24.17 ± 13.75 days.

It should be noted that none of the studied COVID-19 virus infected patients and healthy people were infected with human immunodeficiency virus-1 (HIV-1 Ag/Ab and HIV-RNA negative), hepatitis C virus (HCV Ab and HCV-RNA negative), and hepatitis B virus (HBsAg and HBV-DNA negative).

3.2. Differential miRNA expression in COVID-19 patients and healthy subjects

As shown in Fig. 1, the expression level of miR-29a-3p, 146a-3p, 155-5p, and let-7b-3p in acute and post-acute samples was significantly higher than in healthy samples (P-value < 0.001). The largest expression difference between acute COVID-19 with control groups and between post-acute COVID-19 with control groups were observed for miR-155-5p (3.75-fold change, P-value < 0.0001) and miR-29a-3p (5.3-fold change, P-value < 0.0001), respectively. The expression of miR-29a-3p, miR-146a-3p, and let-7b-3p were 3.5, 2.8, and 1.7-fold higher in acute COVID-19 patients than in the control group. The comparison of expression level of cellular miRNAs between acute and post-acute COVID-19 groups with healthy control group was shown in Table 3.

The results of the Spearman Correlation coefficient analysis between miRNAs with SARS-CoV-2 (RdRP and N) genes and demographic and clinical characteristics are given in Table 4. According to the result of the spearman correlation analysis, a significant negative correlation was found between the delta Ct of miR-155-5p and both delta Ct of N (r = −0.63, P-value < 0.01) and RdRP (r = −0.71, P-value < 0.001) genes. While, there were no significant correlation between expression level of miR-29a-3p, -146a-3p and let-7b-3p with viral factors.

Table 1
The demographic features of the studied participants.

Parameters	Male	Female	Total
Patients			
Age /Year ± SD	**40.8 ± 9.7 (29–55)**	**35.6 ± 13.7 (22–65)**	**38.2 ± 11.8 (22–65)**
No. of patients	**9 (50.0%)**	**9 (50.0%)**	**18 (100%)**
Healthy people			
Age /Year ± SD	**37.9 ± 13.4 (23–65)**	**35.1 ± 11.5 (22–58)**	**36.6 ± 12.2 (23–65)**
No. of patients	**8 (53.3%)**	**7 (46.7%)**	**15 (100%)**

Table 2
The epidemiological characteristics of the studied participants.

Parameters		Positive	Negative
Epidemiological Characteristics			
Fever		**9 (50.0%)**	**9 (50.0%)**
Confusion		**4 (22.2%)**	**14 (77.8%)**
Headache		**10 (55.6%)**	**8 (44.4%)**
Chills		**9 (50.0%)**	**9 (50.0%)**
Skeletal pain		**10 (55.6%)**	**8 (44.4%)**
Dry cough		**7 (38.9%)**	**11 (61.1%)**
Sputum cough		**0 (00.0%)**	**18 (100.0%)**
Chest pain		**3 (16.7%)**	**15 (83.3%)**
Shortness of breath		**5 (27.8%)**	**13 (72.2%)**
Runny nose		**4 (22.2%)**	**14 (77.8%)**
Cape of nose		**5 (27.8%)**	**13 (72.2%)**
Deceased smell		**15 (83.3%)**	**3 (16.7%)**
Deceased taste		**10 (55.6%)**	**8 (44.4%)**
Gastrointestinal symptom		**8 (44.4%)**	**10 (55.6%)**
Bleeding stomach		**1 (55.6%)**	**17 (94.4%)**
Menstrual disorder	**Male/9**	**–**	**–**
	Female/9	**3 (33.3%)**	**6 (67.733.3)**

Also, fold change of miR-29a-3p, -146a-3p, -155-5p were positively correlated with Fever (r = 0.51, r = 0.46, and r = 0.56, respectively, with all P-value < 0.05). Similarly, a remarkable positive correlation was also seen between fold change of miR-29a-3p (r = 0.47, p-value < 0.05), miR-146a-3p (r = 0.49, p-value < 0.05), miR-155-5p (r = 0.49, p-value < 0.05) and let-7b-3p (r = 0.73, p-value < 0.0001) with dry cough. In addition, there was a positive correlation between miR-146a-3p and deceased smell (r = 0.53, P-value < 0.05). More details are presented in Table 4.

3.3. Differential miRNA expression between the acute and post-acute phase of COVID-19 disease

In order to understand whether the expression pattern of selected miRNAs after the acute COVID-19 phase will be similar to control levels or not, the expression level of miRNAs in confirmed COVID-19 patients was re-measured 4–5 weeks after the acute phase. While no significant difference in the level of miR-155-5p expression between the acute and post-acute groups (P-value = 0.26) were found, the expression profile of miR-29a-3p, miR-146 and let-7b-3p were significantly up-regulated in acute and post-acute groups (Fig. 2). The results of the study showed that expression profile of miR-29a-3p, miR-146 and let-7b-3p were different between acute and post-acute groups. Furthermore, mean fold change of miR-146 and let-7b-3p were respectively 2.96 and 2.72-fold higher in the post-acute group than in the acute group. Furthermore, miR-29a-3p was the most upregulated miRNA, with a 3.7-fold change compared to that of the acute group (P-value = 0.001).

3.4. Circulating miRNAs as potential biomarkers for COVID-19 disease

ROC curve analysis revealed that miR-29a-3p (AUC = 0.91; 95% CI: 0.82 to 1.0; P < 0.0001), miR-146a-3p (AUC = 0.87; 95% CI: 0.75 to 0.98; P = 0.0003) and miR-155-5p (AUC = 0.9; 95% CI: 0.78 to 1.0; P < 0.0001) were the useful marker for discriminating between control and acute COVID-19 patients (Fig. 3***A***). Besides, the average fold change of miR-146a-3p, miR-155-5p, and let-7b-3p in PBMC of the post-acute COVID-19 group compared with the healthy group were 4.45, 4.4, and 3.1-fold. According to ROC curve analyses, miR-29a-3p, miR-146a-3p, and let-7b-3p can differentiate post-acute COVID-19 patients from healthy controls, the AUC were 1 (95% CI: 1 to 1; P < 0.0001), 0.98 (95% CI: 0.96 to 1.0; P < 0.0001) and 0.93 (95% CI: 0.86 to 1.0; P < 0.0001), respectively (Fig. 3***B***). In addition, miR-29a-3p and miR-146a-3p presented good value in distinguishing acute COVID-19 patients from post-acute COVID-9 patients with AUC of 0.82 (95% CI: 0.68 to 0.95; p = 0.001) and 0.801 (95% CI: 0.65 to 0.95; p = 0.001), respectively, (Fig. 3***C***).

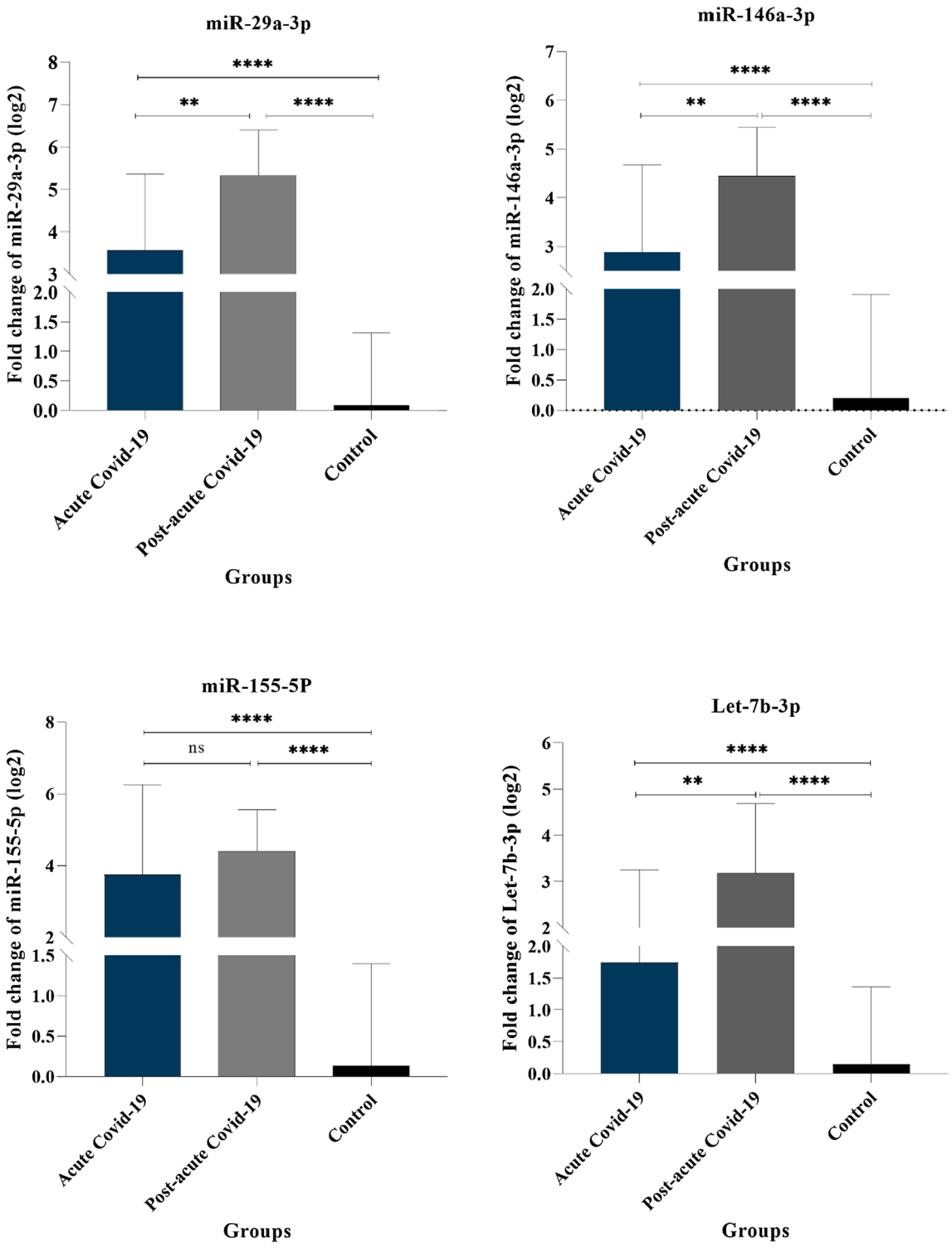

Fig. 1. Comparison of miRNAs expression level between COVID-19 patients with healthy controls.

4. Discussion

The purpose of our study was to investigate expression pattern of cellular miRNAs including Let-7b-3p, miR-29a-3p, miR-146-3p, and miR-155-5p between COVID-19 patients with healthy samples and during acute and post-acute phase of COVID-19 disease, as well as, diagnostic potential of these miRNAs as biomarker.

Immune system cells respond to the SARS-CoV-2 infection-induced damage lung cells through inducing the release of pro-inflammatory cytokines (e.g., TNF, IL-6, and IL-1β) which lead to systemic inflammation [40,41]. However, the exact mechanism of the SARS-CoV-2 invasion and the immune system's aberrant response is not yet well understood. In addition to the cellular proteins involved in regulating inflammation, it has now been shown which miRNAs also play a critical role in regulating the inflammatory process [42]. miR-155 and miR-146 are among the first miRNAs to be stimulated during immune activation, and these miRNAs trigger the production of IFNs and inflammatory cytokines through modulating the TLR-signaling pathways [43].

Table 3
Comparison of miRNAs expression level between acute and post-acute COVID-19 groups with Healthy Control group (Control group as a reference group).

Cellular miRNAs	Acute COVID-19 vs Control group	Post-Acute COVID-19 vs Control group	Acute COVID-19 vs Post-Acute-COVID-19
miR-29a-3p	F: 3.5	F: 5.3	F: 3.4
	T: 6.3	T: 12.8	T: 3.7
	P: <0.0001	P: <0.0001	P: 0.001
miR-155-5p	F: 3.7	F: 4.4	F: 1.3
	T: 5.06	T: 10	T: 1.14
	P: <0.0001	P: <0.0001	P: ns
miR-146a-3p	F: 2.8	F: 4.4	F: 2.9
	T: 4.38	T: 8.9	T: 3.5
	P: 0.0001	P: <0.0001	P: 0.002
Let-7b-3p	F: 1.7	F: 3.1	F:2.7
	T: 3.2	T: 6.3	T: 3.1
	P: 0.002	P: <0.0001	P: 0.006

F: Fold change (log2), T: t-value, P is adjusted P-value based on the marginally adjusted p values by the Benjamini-Hochberg-FDR correction at $\alpha = 0.05$.

Table 4
Spearman's correlation coefficient between the miRNA's expression level with expression level of viral genes (N and RdRp genes), demographic and clinical characteristics.

	miR-29a-3p	miR-146a-3p	miR-155-5p	Let-7b-3p
N gene	0.27 ^{ns}	−0.28 ^{ns}	−0.63**	−0.12 ^{ns}
RdRp	−0.3 ^{ns}	−0.32 ^{ns}	−0.71***	−0.13 ^{ns}
Sex	0.18 ^{ns}	0.21 ^{ns}	0.29^{ns}	0.07 ^{ns}
Age	0.18	0.04	−0.108	0.07 ^{ns}
Fever	0.51*	0.46*	0.56*	0.36 ^{ns}
Confusion	0.05 ^{ns}	0.07 ^{ns}	−0.04 ^{ns}	0.07 ^{ns}
Headache	−0.1 ^{ns}	−0.17 ^{ns}	−0.08 ^{ns}	−0.25 ^{ns}
Chills	0.04 ^{ns}	−0.16 ^{ns}	0.04 ^{ns}	−0.01 ^{ns}
Skeletal pain	−0.05 ^{ns}	−0.19 ^{ns}	−0.03 ^{ns}	−0.19 ^{ns}
Dry cough	0.47*	0.49*	0.49*	0.73***
Sputum cough	−0.01 ^{ns}	0.05 ^{ns}	0.004 ^{ns}	0.071 ^{ns}
Chest pain	−0.09 ^{ns}	−0.13 ^{ns}	0.07 ^{ns}	0.11 ^{ns}
Shortness of breath	−0.27 ^{ns}	−0.28 ^{ns}	−0.15 ^{ns}	0.01 ^{ns}
Runny nose	−0.02 ^{ns}	−0.13 ^{ns}	−0.05 ^{ns}	0.17 ^{ns}
Cape of nose	−0.17 ^{ns}	−0.206 ^{ns}	−0.06 ^{ns}	−0.16 ^{ns}
Deceased smell	0.39 ^{ns}	0.53*	0.44 ^{ns}	0.42 ^{ns}
Deceased taste	0.09 ^{ns}	0.15 ^{ns}	0.3 ^{ns}	0.35 ^{ns}
Gastrointestinal symptom	0.15 ^{ns}	0.19 ^{ns}	0.17 ^{ns}	−0.35 ^{ns}
Bleeding stomach	0.01 ^{ns}	−0.21 ^{ns}	−0.005 ^{ns}	−0.35 ^{ns}

ns: not significant.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Reportedly, the expression level of miR-146a was significantly upregulated in A549 cells infected with the influenza virus and contributes to the replication of influenza virus by inhibiting the production of interferon type I [44]. Similarly, the inhibition of miR-146a expressions led to a decrease in the expression of matrix and nucleoprotein proteins of the influenza virus [45]. Additionally, it has been observed that increasing the expression level of miR-146a reduces lung cell damage by suppressing inflammatory responses [46]. However, the role of this miRNA in the COVID-19 disease has not been investigated. In the current study, we found that the expression level of miR-146a-5p was statistically significantly higher in the COVID-19 patient group than in the healthy group (2.8-fold change, P-value < 0.0001, Fig. 1, Table 3).

Soni et al. [47] investigated the role of miR-155 in SARS-CoV-2-mediated pathogenesis. According to the result of this research, miR-155-5p level PBMC in COVID-19 patients was higher than in individuals from healthy individuals. This study also examined the effect of anti-miR-155 on the lungs of SARS-CoV-2- infected hACE2-transgenic mice and reported that the inhibition of miR-155 cause promotes survival and attenuates inflammation and lung cytokine storm induced by the virus. The overall results of this study suggested that suppression of miR-155 a new therapeutic strategy against decreasing COVID-19-induced lung cytokine storm [47]. Additionally, Chow et al. predicted that upon infected lung epithelial cells with SARS-CoV-2, the miR-155 expression level is significantly increased [48]. In another study, Emanuel and colleagues [48] found that the mean expression level of miR-155 in Calu-3 cell line infected with SARS-CoV-2 significantly higher than the control cell line. Reportedly, miR-155 plays a critical role in respiratory viral diseases by the modulation of anti-viral responses, including inflammatory and immune responses. For example, in vivo inhibition of miR-155 lead to a rapid recovery in influenza virus-infected mice [49,50]. In the current study, in line with these studies, the expression level of miR-155-5p was significantly upregulated in the COVID-19 group compared to the control group (3.75-fold change, P-value < 0.0001, Fig. 1). Also, the results of spearman correlation analysis revealed that a significant inverse correlation was found between miR-155-5p with the SARS-CoV-2 N-gene ($r = -0.63$, p-value < 0.001), and the SARS-CoV-2 RdRp-gene ($r = -0.71$, $p < 0.0001$) (Table 4). According to the results, it is possible that the expression of miR-155 is increased due to an immune response to fight with SARS-COV-2.

Recently, Li et al. [51] suggested that cellular miRNAs maybe can regulates host immune response and replication of SARS-CoV-2 during viral infection. For this purpose, they investigated the profiling of cellular miRNAs in PBMC of COVID-19 patients by high-throughput sequencing assay, and correlation of miRNAs and their target genes. The results of this study showed that expression level of 35 miRNAs (e.g., miR-16-2-3p, -6501-5p and -618) were upregulated and 38 miRNAs (e.g., miR-183-5p, -627-5p and -144-3p) were downregulated in COVID-19 patients compared to healthy control. In addition, cluster analysis of this study revealed that miR-618 may act as a promising novel target to treatment and diagnosis of COVID-19 disease [51].

MiRNA target-prediction software is a useful tool for predicting targets of miRNAs and identifying cellular miRNAs, due to its ability to bind to the virus genome and its ability to act as anti-viral miRNAs [48]. Recently, in silico analyses of the interaction between cellular miRNAs and the SARS-CoV-2 genome were performed by Jafarinejad-Farsang et al. [52]. In this study it was predicted that the sequences of miR-29 family members have the ability to bind to the SARS-COV-2 genome and are bound to the coding sequence for SARS-CoV-2 spike. Furthermore, miR-146a, let-7b, let-7e, miR-21 and miR-16 were identified as miRNAs targeting differentially expressed genes (DEGs) in SARS-CoV-2 infected lung cells [52]. Ma and colleagues [53] found that let-7c was significantly overexpressed in influenza-infected A549 cells and that it can act as an anti-influenza miRNA through reduction of M1 protein of HINI influenza A in A549 cells [53]. Moreover, Inchley et al. [54] investigated the expression pattern of cellular miRNAs in the nasal mucosa of respiratory syncytial virus (RSV)-infected infants. They observed that expression levels of let-7d, miR-155, -203a, -31 and -16 in the RSV-infected infants' group were higher than in the control group. However, the expression level of miR-29c, -27b, -34b, -34c, -125b, 125a and -429 were found to be significantly downregulated in the RSV-infected infant group compared to the control group [54]. However, changes in the expression of miR-29a-3p and let-7 during the COVID-19 disease and its role in SARS-CoV-2 viral infection have not yet been reported. In the present study, for the first time, it was observed which the expression patterns of these miRNAs in the COVID-19 group (3.5-fold for miR-29a-3p) were significantly higher than in the control group (1.7-fold for let-7b-3p) (Fig. 1 and Table 3).

When a new pathogen or new strains of a pathogen appear, all novel information can help the process of monitoring, treatment, and diagnosis of the disease. One of the new candidates for diagnosis and therapy is miRNAs, which may serve as a useful tool in laboratory medicine [55].

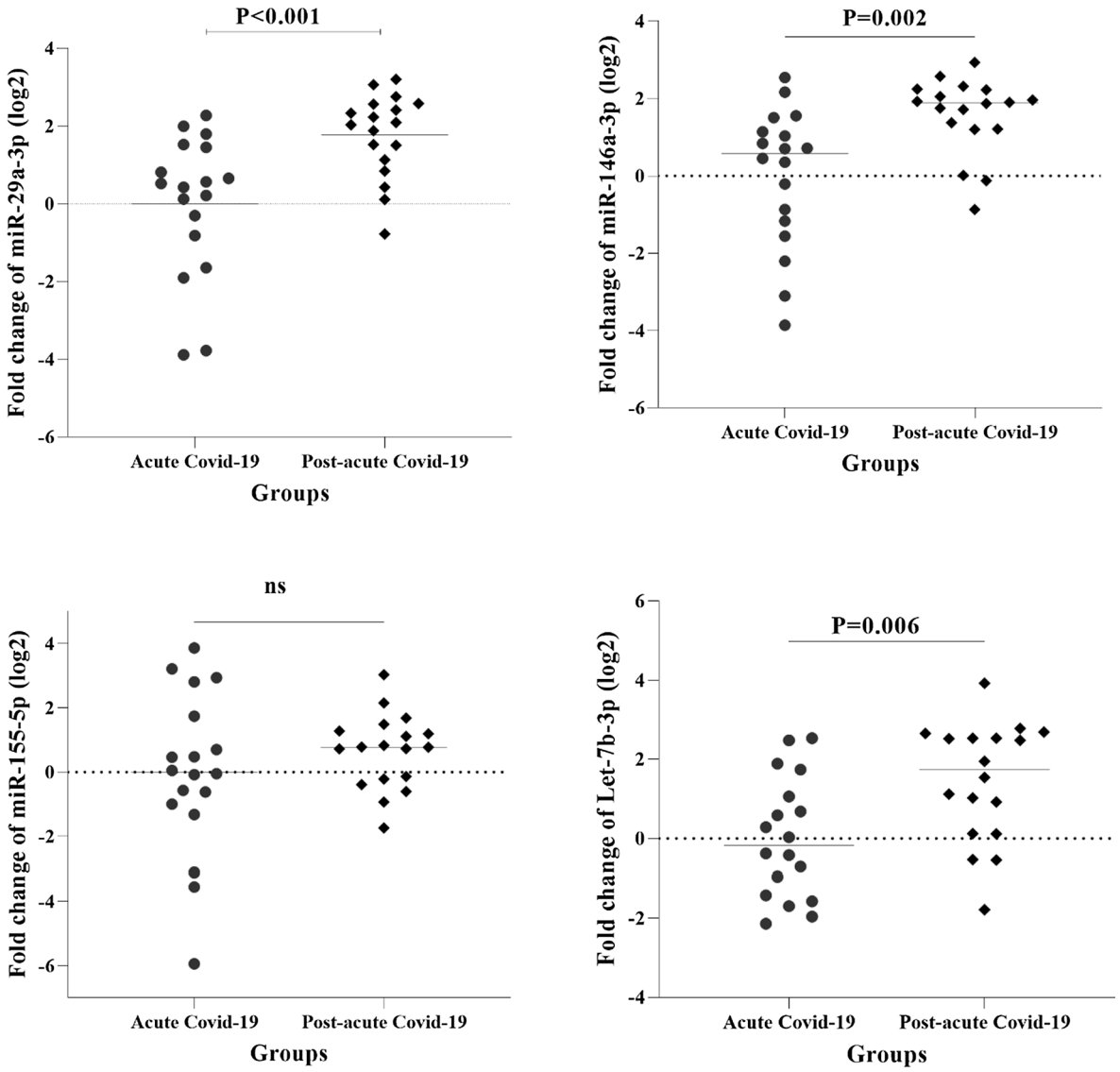

Fig. 2. Comparison of the expression pattern of miRNAs in the COVID-19 patients during Acute and Post-acute COVID-19 disease.

Conceptually, to identify candidate miRNAs there are two main ways: 1) evaluation of the pathogenic agent effect on the expression level of cellular miRNAs, 2) checking the role of miRNA on the pathogen life cycle by the knock-outing or enforcing the expression of miRNA [56]. In the current study, the expression level of selected miRNAs is significantly different between the COVID-19 group and the healthy group (Fig. 1). It is also found to be different between acute and post-acute COVID-19 (Fig. 2). Additionally, the result of ROC analysis shows that miR-29a-3p (AUC: 0.91, P-value < 0.0001), miR-155-5p (AUC: 0.9, P-value < 0.0001), and miR-146a-5p (AUC: 0.87, P-value: 0.0003) may be used as a potential diagnostic marker for COVID-19 vs healthy subjects (Fig. 3).

More recently, some studies have reported that after the acute phase of the COVID-19, patients still experience some clinical symptoms of disease such as fatigue, weakness, and headache, low grade fever, and cough [57,58]. These symptoms are believed to be prevalent during post-acute COVID-19 phase and do not depend on prior health status, or the severity of acute COVID-19 illness [58].

Recently, Zheng and colleagues [59] analyzed the transcriptome profiles including mRNA, miRNAs, and long noncoding RNAs (lncRNAs) in PBMC of COVID-19 patients with mild, moderate, and severe symptoms during their treatment, convalescence, and rehabilitation. The result of their study showed that downregulation of humoral immunity and IFN-I response, as well as T cell activation and differentiation, were the main events that occurred during the recovery phase of COVID-19 disease. Also, they reported that expression level of let-7b-5p, miR-103a-2-5p, -200c-3p and -2115-3p was decreased during recovery from COVID-19 disease and these miRNAs are involved in the regulation of T cell differentiation [59]. Further, in the present research, the level of selected miRNAs, after about 4–5 weeks of the acute COVID-19 phase (Post-acute COVID-19), was examined and the expression level of miR-29a-3p, -146a-3p and let7b-3p in the post-acute COVID-19 group were 3.7, 2.9 and 2.7-fold higher respectively than in the acute COVID-19 group (Fig. 2). However, there was no significant difference in the mean expression level of miR-155-5p between the two groups (Fig. 2). By contrast to Zheng and colleagues [59] reports, in our study observed that expression level of let-7b-5p in acute phase of COVID-19 disease significantly higher than that post-acute COVID-19.

Moreover, ROC curve analysis demonstrated that the high sensitivity and specificity for miR-29a-3p and miR-146 offers potential biomarkers for discriminating acute from the post-acute COVID-19 phase (Fig. 3). The difference in the miRNA expression pattern during the acute and post-acute phase of COVID-19 disease proposed which cellular miRNAs can be used as novel biomarkers for monitoring of COVID-19 disease.

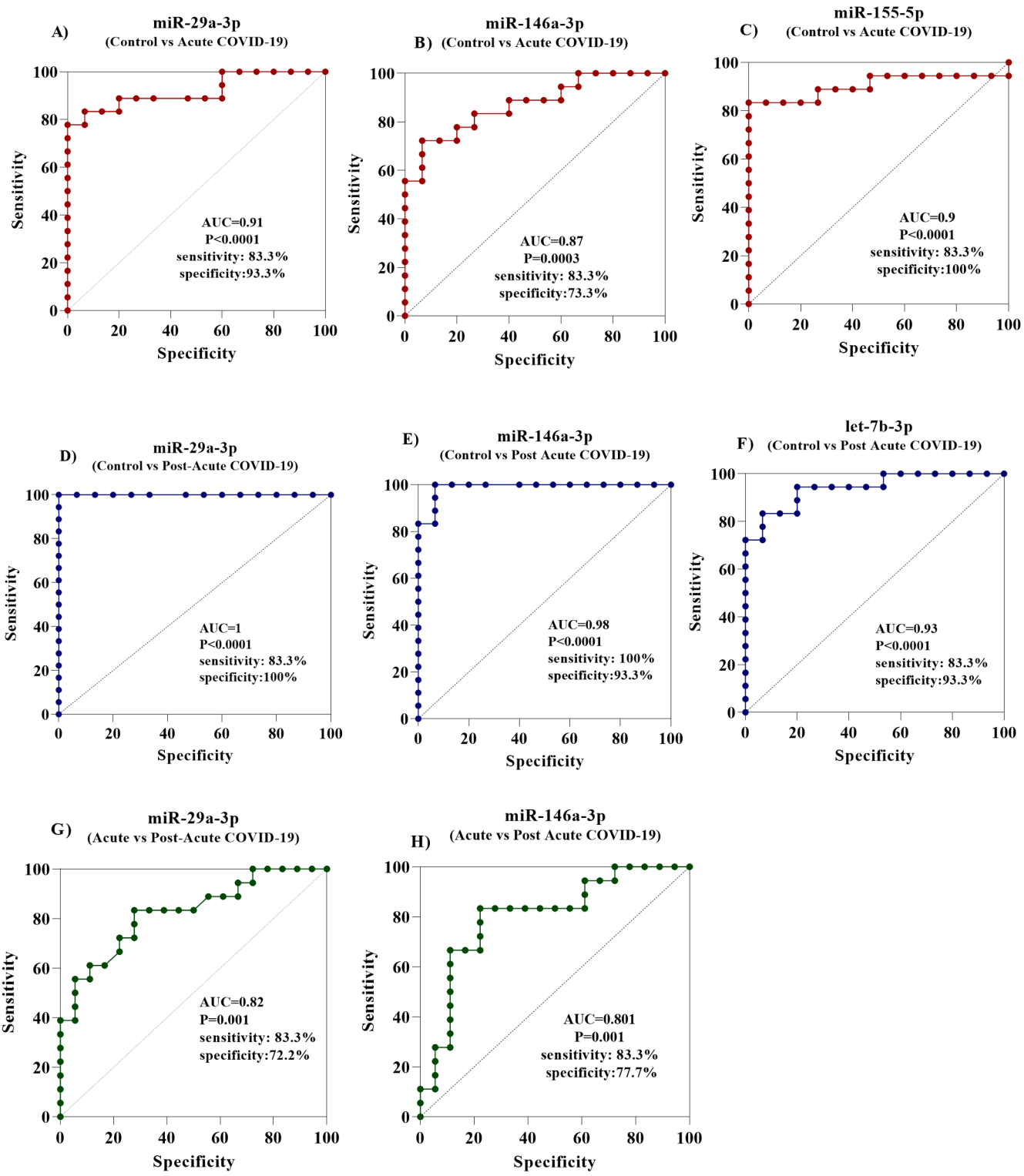

Fig. 3. ROC curve analysis using PBMC miR-29a-3p, miR-146a-3p, miR-155-5p, and let-7b-3p for discriminating control and COVID-19 patients. miR-29a-3p, -146a-3p, and-155-5p were able to diagnose acute COVID-19 disease as compared with healthy controls (A-C). PBMC miR-29a-3p, -146a-3p, and let-7b-3p were useful to diagnose acute COVID-19 disease as compared with healthy control (D-F) as well as, miR-29a-3p and -146a-3p were the good marker for discriminating between acute and Post-acute COVID-19 diseases (G-H).

In conclusion, this study was the first report of differentially expressed miRNA profile including let-7b-3p, miR-29a-3p, miR-146a-3p and miR-155-5p in COVID-19 group and healthy group, and also during the acute and post-acute phase of the COVID-19 disease. As a result, we found that the expression level of miRNAs was upregulated in the PBMC specimens of COVID-19 patients and post-acute COVID-19 phase. A sequence of transcription factors (TFs) plays critical roles in COVID-19 pathogenesis. In these regards, performance of construction and analysis of miRNA-TF regulatory network indicated that the measured miRNAs could be associated with some genes (i.e., NF-kB) that are involved in COVID-19 progression (Fig. 4). In addition, specificity and sensitivity of these miRNAs was tested by ROC analysis in COVID-19

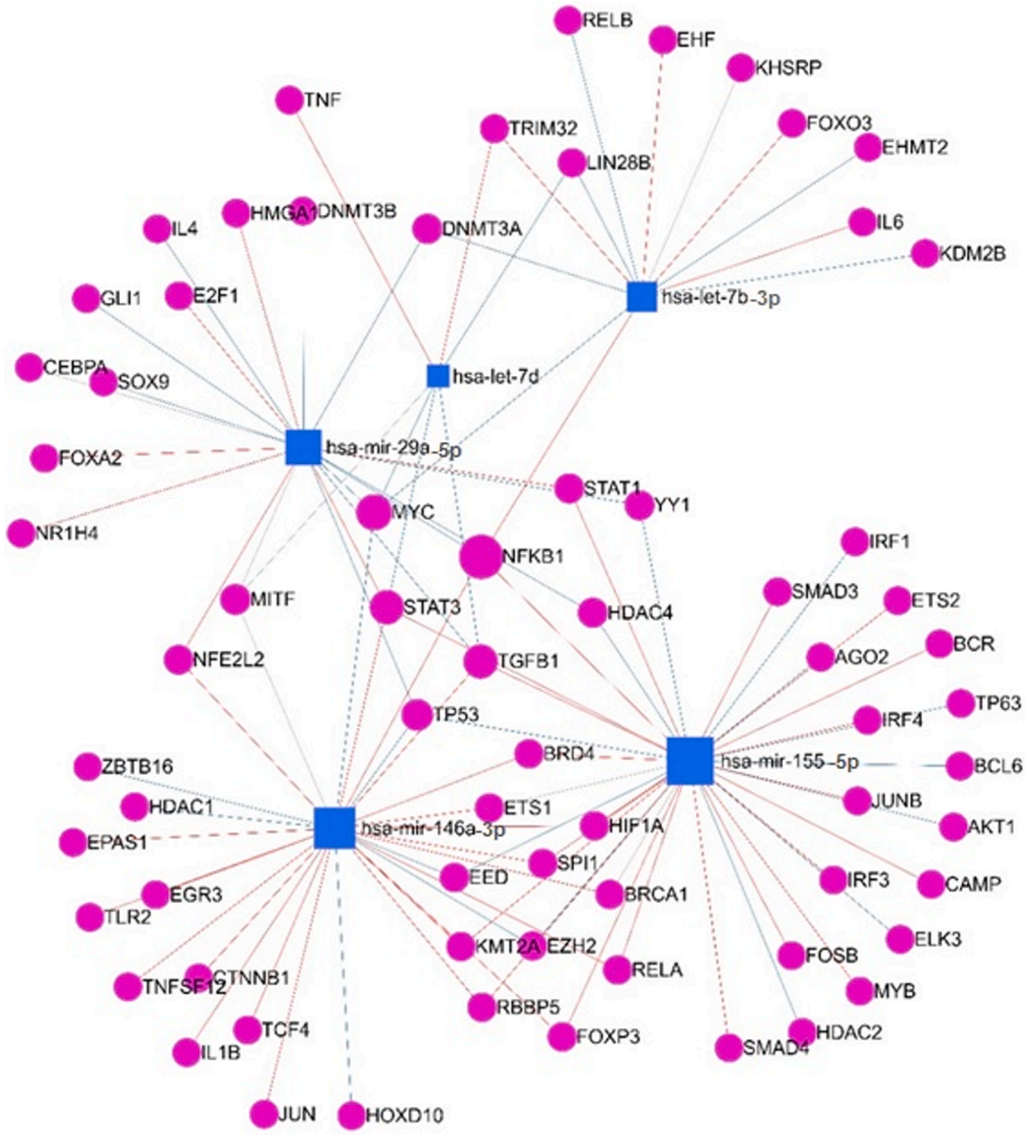

Fig. 4. A schema of miRNA-TF regulatory network of let-7, miR-29a-3pa, -155-5p and -146a-3p and their targets.

patients and it was observed that miR-29a-3p, miR-155-5p and miR-146a-3p may be used as a potential diagnostic marker for diagnosis of acute-COVID-19 disease and also miR-29a-3p, and miR-146a-3p can probably act as novel biomarkers for distinguishing post-acute from acute phase of COVID-19 disease. The small sample size and the lack of other respiratory disease as controls were the limitations of this study, so more studies with larger volunteers are needed to show the diagnostic value of these miRNAs as diagnostic biomarker for COVID-19.

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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.

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