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Research Article

The relationship between miRNAs and clinical data of COVID-19 patients

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Abstract

SARS-CoV-2, the causative agent of the 2019 coronavirus illness (COVID-19) pandemic, has quickly disseminated over the globe. As the number of COVID-19 cases has skyrocketed, numerous nations have implemented emergency measures and set recommendations to stem the spread of the virus. A number of biomarkers for COVID-19 have been established after extensive study; however, there is currently no biomarker that is both specific and accurate for predicting the prognosis of patients with COVID-19 infection. **Purpose of the study;** Therefore, the purpose of this research was to evaluate the potential of miRNAs as predictive biomarkers for COVID-19 by analyzing the correlation between miRNA-106a expression and clinical data from individuals with this condition. **Basic procedures:** Fifty patients with COVID-19 were hospitalized to Minia University with symptoms including fever, dyspnea, cough, anosmia, GIT signs, and loss of taste sense; samples of 6 ml of venous blood were taken from each patient. Serum miRNA-106a was measured using real-time PCR, and clinical data was gathered after total RNA with preserved miRNAs was isolated from 200 µL of serum from each patient. **Main findings:** There is a strong link between the expression level of serum miRNA-106a and the clinical data of COVID-19 patients including fever, cough and congested throat. **Principle conclusion:** The findings supported the feasibility of miRNAs as potential diagnostic biomarkers and early predictors of COVID-19 infection.

Key words: COVID-19, MicroRNAs, Clinical data.

Introduction

Pneumonia, hyperinflammatory reaction, microangiopathy, widespread thrombosis, damage to blood vessels, and angiogenesis are all symptoms of COVID-19, a new coronavirus illness [1]. The first COVID-19 epidemic began in Wuhan, China in December 2019, however it quickly spread to the rest of the globe via human-to-human contact [2]. The World Health Organization (WHO) declared COVID-19 a pandemic after it spread to 114 nations [3]. Infection of the upper respiratory tract is the first stage, followed by dyspnea and pneumonia in stage two, a deteriorating clinical condition in stage three dominated by hyperinflammatory state and cytokine storm, and finally recovery or death in stage four [1]. The vast majority of people are vulnerable to contracting this illness [4]. Fever, coughing, exhaustion, and breathing problems are some of the most common COVID-19 symptoms.

However, some people are asymptomatic or have minimal symptoms yet they may disseminate the virus to other non-infected persons [5]. Currently no medication is extremely efficient in treating the SARS-CoV-2 infection although the groups of

pharmaceuticals that are frequently employed include antiviral agents, low-molecular-weight heparins, inflammation inhibitors, plasma and immunoglobulins. The investigation of viable COVID-19 therapy options is urgently needed. The best approach to protect yourself against COVID-19 is to be vaccinated [6]; however, SARS-CoV-2 is constantly evolving and spreading, which decreases the efficacy of vaccinations and antibody medicines [7].

MicroRNAs are non-coding RNAs that are just 18-25 nucleotides long [8]. Many genes, including those involved in signalling cascades and other cellular physiological processes such as proliferation, differentiation, apoptosis, metabolism, and the regulation of cell cycle, as well as innate immunity, inflammation, and infection, are under the tight control of miRNAs [9]. Because of their central role in so many different biological processes, microRNAs have been suggested as potential biomarkers for the diagnosis, monitoring, therapy, and prognosis of a wide variety of disorders [10]. It is predicted that miRNAs may act as biomarkers of COVID-19 since viral infection can alter host miRNAs expression and dysregulated miRNAs have previously been explored as biomarkers of numerous infectious disorders [11]. Therefore, the purpose of this research was to explore miRNAs as prospective biomarkers for COVID-19 illness diagnosis and progression by identifying a correlation between blood miRNA-106a and the clinical data of COVID-19 patients as this miRNA was measured in many lung diseases like Avian Influenza A virus (H7N9) and non-small cell lung cancer (NSCLC) so this study was aimed to determine its role in COVID-19 disease.

Subjects and methods

1- Study design

The present study included 50 patients, ageing between (22 and 78) years old and of both sexes, all diagnosed clinically by their manifestations (fever, dyspnea, cough, chest pain, fatigue, anosmia, GIT manifestations and loss of taste sensation) and then confirmed by real time PCR as SARS-CoV-2 infection. Thirty healthy volunteers were included in the research as a control group, and they were matched with patients in terms of age and sex. All participants provided their clinical information and signed written permission forms. The research was done "from January 2021 to July 2021 according to the criteria for the use of human subjects' materials according to the "Declaration of Helsinki" and recognised by the Research Ethics Committee (REC) of Minia University Hospital. Egypt.No. 693:11/2020.

2- Samples collection

After collecting 6 ml of blood from each subject by sterile venipuncture, the serum was centrifuged at 1500 g to eliminate all cellular components. Special microtubes were used for isolating serum, which was then frozen at -80°C.

3- Serum miRNA-106a extraction and quantitation

Each patient's 200 µL of serum was used to extract total RNA with preserved miRNAs using a miRNeasy extraction kit (Qiagen, Valencia, USA). Using the miRNeasy serum/plasma reverse transcription kit, we reverse-transcribed 12 µL of total RNA in a final volume of 20 µL (incubated for 60 minutes at 37°C, 5 minutes at 95°C, and then maintained at 4°C) (Qiagen, Valencia, CA, USA). miScript SYBR Green Master Mix was used in miR-106a real-time polymerase chain reaction quantification studies (Qiagen, Valencia, USA). As a reference point, we used the miRNA-U6 gene. All primers were purchased from Qiagen (Qiagen, Valencia, USA). Techne (Cambridge) LTD., United Kingdom Experiments were run on an Applied Biosyst 7500 fast Real-Time PCR System at 95°C for 15 minutes, then 40 cycles at 94°C for 15 seconds, 55°C for 30 seconds, and 70°C for 30 seconds.

The Primer sequence for amplification of miRNA-106a gene [12] as follows:

Sense	5' GAG AAC AGC AGG TCC AGC AT '3
Anti-sense	5' CTT CCT CAG CAC AGA CCG AG '3

The Primer sequence for amplification of miRNA-U6 gene [12] as follows:

Sense	5' CTC GCT TCG GCA GCACA'3
Anti-sense	5' AAC GCT TCA CGA ATT TGC GT'3

After subtracting the ΔCT values of miRNA-U6 from those of the target miRNAs, the resulting ΔCt value was used to report miRNA abundance. The fold change (relative quantity, or "RQ") of miRNA levels, using healthy controls as calibrators, is determined by the following equation: $2^{-\Delta\Delta Ct}$. ΔΔCt = ΔCT of patient – mean of ΔCT of control.

Statistical analysis of data

Means and standard deviations of the data were calculated (SEM). Version 23 of the statistical package SPSS was used for the analysis (SPSS Inc, Chicago). The quantitative information was evaluated using the Student t test. For this reason, we utilised the Chi-square (X2) test for categorical data. Mann-Whitney was employed for comparison between the various groups. Statistical significance was assumed at the.05 level.

Results

1- Demographic data of COVID-19 patients and healthy controls

The study was performed on 50 COVID-19 patients with mean age of 55.9±1.8 years, 28 (56%) were male and 22 (44%) were females and 30 healthy control subjects with mean age of 54.8±1.2 years, 17 (56.7%) were males and 13 (43.3%) were females **(Table 1)**.

2- Relation of clinical data of the COVID-19 patients and miRNA-106a expression

Analyzing serum miRNA-106a profile in COVID-19 patients regarding clinical data revealed that miRNA-106a showed a highly significant relation with fever, cough and congested throat with P value <0.05 **(Table 2)**.

Table (1): Mean ± SEM of demographic data of patients and healthy control

Data		Control group N0=30	COVID-19 group N0=50	P value
Age	**Range** **Mean ±SEM**	41-66 54.8±1.2	22-78 55.9±1.8	0.6
Sex	**Male**	17 (56.7%)	28 (56%)	0.9
	Female	13 (43.3%)	22 (44%)	

Table (2): Mean ± SEM of clinical data of COVID-19 patients and miRNA-106a expression

Data		Present Mean±SEM	Absent Mean±SEM	P value
Fever	**miRNA106aRQ**	0.72±0.2	0.33±0.04	0.009*
Cough	**miRNA106aRQ**	0.74±0.08	0.33±0.0.09	0.007*
Wheezy chest	**miRNA106aRQ**	0.39±0.08	0.49±0.1	0.4
Dyspnea	**miRNA106aRQ**	0.44±0.08	0.43±0.1	0.9
Diabetes mellitus	**miRNA106aRQ**	0.45±0.1	0.41±0.08	0.8
Hypertension	**miRNA106aRQ**	0.32±0.07	0.50±0.09	0.1
GIT Symptoms	**miRNA106aRQ**	0.60±0.1	0.36±0.06	0.1
Congested throat	**miRNA106aRQ**	0.72±0.2	0.33±0.04	0.009*

: Significant level at P value < 0.05.

Discussion

Large outbreaks of COVID-19, brought on by SARS-CoV-2 mutations (Delta, Mu, Lambda, and Omicron), are expected to peak around 2022 [13]. Various efforts have been made to learn more about the SARS-CoV-2 infection pattern and identify potential therapies. However, there is no proof that the medications being used are effective or safe [14]. Therefore, it may be possible to effectively block and alleviate COVID-19 infection by using the concepts and molecular players of gene regulatory processes. To better comprehend the mechanics of COVID-19 infection and to develop new treatments against this virus, miRNAs have been proposed as possible targets [15]. Therefore, the purpose of this research was to examine the connection between COVID-19 patient clinical data and the expression of cellular miRNAs like miRNA-106a.

The clinical characteristics of COVID-19 are variable ranging from asymptomatic condition to acute respiratory distress syndrome and multi organ failure. Symptoms including fever, cough, headache, sore throat, myalgia, weariness, conjunctivitis, and difficulty breathing are very typical. So, they are indistinguishable from other respiratory illnesses. Pneumonia, respiratory failure, and mortality may occur in certain people with this condition [16].

These results are consistent with those of Chen N et al.,, 2020, who found that 83% of patients in their study experienced fever, 82% experienced cough, 31% experienced shortness of breath, 11% experienced muscle ache, 9% experienced confusion, 8% experienced headache, 5% experienced sore throat, 4% experienced rhinorrhea, 2% experienced chest pain, and 1% experienced diarrhea 1% [16] and also in accordance with Wang et al.,, 2020 who studied 138 COVID-19 patients and showed that fever (98%), tiredness (70%), dry cough (59%), myalgias (40%), anorexia (40%), dyspnea (31%), and sputum production (27%), were the most prevalent clinical features [17].

One of the most critical uses of miRNAs in modern medicine is in diagnostics. The word "biomarker" refers to a wide variety of diagnostic procedures that may provide light on either healthy or diseased states [18]. MiR-106a, which is located on the X chromosome as part of the miR-106a363 cluster, regulates a large number of genes involved in cell signaling. But before we can completely establish any of these genes, we need to do further research and validation [19]. We predicted that miRNAs found in COVID-19 patients might be exploited as possible diagnostic biomarkers for predicting COVID19 severity, building on the work of Fernández-Pato et al., (2022) [20] and Srivastava et al., (2023) [21]. This is the first investigation on miRNAs and the severity of COVID-19 that we are aware of.

Conclusion

The present study concluded that there is a relationship between serum miRNA-106a expression level and fever, cough and congested throat manifestations of COVID-19 patients so further study on the relationship between miRNAs and other clinical data of COVID-19 patients can be valuable to use miRNAs as a diagnostic marker for COVID-19 infection.

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