Iraqi Journal of Biotechnology, 2024, Vol. 23, No. 2, 119-127

Study the Association of mi 423-5p Expression with Coronavirus-19 Infection in Samples of Iraqi Patients

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Received: September 19, 2023 / Accepted: October 29, 2023 / Published: October 30, 2024

Abstract: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the agent responsible for the extremely contagious sickness known as coronavirus disease 2019 (COVID-19). Non-coding RNAs known as microRNAs (miRNAs) have a vital role in controlling the expression of genes. This study aims to evaluate the role of mi423-5p as a biomarker for the detection and monitoring of COVID-19, it was planned to achieve that through the following steps: a collection of 50 nasopharyngeal swabs and 50 blood samples from the COVID-19 Patient group, and control group (health), detection of gene expression of miR-423-5p by using qRT-PCR.The statistical analysis data of mi 423-5p indicates that there were statistically significant differences between the infected patients and control and patients had the vaccination or not, mi 423-5p was significantly upregulated in patients, that the P value =0.012, and there were significant differences whether the patient had the vaccination or not, the p-value was = 0.028. The study was concluded that mi423-5p could be useful as a biomarker for COVID-19 infection and it also helps evaluate the effectiveness of vaccines used against COVID-19.

Keywords*:* SARS coronavirus -2, miRNA Biomarker, mi423-5p.

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Introduction

The members of the Coronaviridae are known to infect a wide variety of hosts, causing various diseases and symptoms, such as SARS, COVID-19, and MERS, CoV was discovered during the 1960s (1). An encapsulated virus called a coronavirus has a positive single-stranded RNA genome. Four genera α, β, γ, and δ, can be distinguished based on the serotype and genomic features (2,3).

Since then, 216 countries and territories have contracted the disease. The World Health Organization (WHO) proclaimed COVID-19 to be a pandemic on January 30, 2020 (4). With a single-stranded positive-sense RNA genome between 26 and 35 kb in size, SARS-CoV-2 codes for about 27 proteins, some of which share similarities with proteins with established activities. In contrast, others are uncertain, unidentified, or presumptive (5,6). MicroRNAs (miRNAs), a class of small non-coding RNAs, regulate gene expression by identifying homologous sections and blocking processes like transcription, translation, or epigenetic regulation(7). MicroRNA (miRNA) is a short, non-coding RNA with a length of 21–22 nucleotides known to regulate crucial biological functions in plants and

mammals. Exosomes allow cells to leak miRNAs into bodily fluids such as blood, urine, breast milk, and saliva (8). Genes are first translated into big primary transcripts (pri-miRNA) with a 3′ polyadenylated structure and a 5′ cap. Although certain pre-miRNAs are produced by RNA polymerase III, RNA polymerase II normally mediates the transcription. (9). The microprocessor complex comprises the pri-miRNAs that are then broken down into precursor miRNA (pre-miRNA), an 85-nucleotide stem-loop structure, by the RNA-binding protein DGCR8 and type III RNase Drosha. After being transported from the nucleus to the cytoplasm by the Ran/GTP/Exportin 5 complex, the pre-miRNAs are processed by another RNase III enzyme Dicer to a 20–22 nucleotide miRNA/miRNA* duplex (note: * indicates the passenger strand, while the other complementary stand is referred to as the mature or guide strand). Once the duplex has been unraveled, the mature miRNA is integrated into the RNA-induced silencing complex (RISC), a protein complex, and it leads RISC to the target mRNA (10).

MiR-423-5p has been identified as an oncogenic factor in various malignancies, including glioblastoma, gastric cancer, and prostate cancer. It is found inside the first intron of the nuclear speckle splicing regulatory protein 1 gene (NSRP1) on chromosome 17. As a tumor suppressor, however, miR-423-5p may also be used as a diagnostic marker for ovarian cancer and osteosarcoma (11). It is also inappropriately expressed in several malignancies, including osteosarcoma, colon, breast, and ovarian cancer.

Material and methods

Samples collection

This study included collected 50 samples of nasopharyngeal swabs and 50 samples of whole blood from patients diagnosed by physicians with COVID-19 and 50 nasopharyngeal swabs and whole blood from people who were not not-infected with SARS-CoV-2 was also provided and considered as a control group (Healthy) which were confirmed that they were not infected with covid-19 by RT-PCR. Severe and moderate patients were collected from Imam Hassan Al-Mujtaba Teaching Hospital Karbala-Iraq from June/2022- August/2022 they were classified according to age, sex, and periods of symptoms, shown in Table (2).

Detection of covid-19

RNA extraction

RNA was extracted from nasopharyngeal swab samples with the AddPrep Viral Nucleic Acid Extraction Kit (Addbio/Korea). Real-time reverse-transcription PCR: 20 μL reaction contained 10 μL of RNA, and 10 μL of 2 × reaction buffer provided with Coronavirus COVID-19 Nucleic Acid Detection Kit (Fluorescence RT-PCR Method (AeHealth/UK), Thermal cycling was performed at 50 °C for 10 min for reverse transcription, followed by 95 °C for 3 min and then 40 cycles of 95 °C for 10 s, 55 °C for 30 s. Participating laboratories used Bio-Rad /USA.

Gene expression analysis of miRNA qRT-PCR

Total RNA was extracted from whole blood in an EDTA tube using a Trizol reagent (Transgen/ China). TransScript® miRNA First-Strand cDNA Synthesis SuperMix (TransGen Biotech/ China) was applied for reverse transcription. We applied SYBR Geen

Realtime PCR Master Mix (Luna Universal qPCR MasterMix (M3003S) In addition, U6 snRNA was applied as internal controls. The source of primers used in gene expression was macrogen® (Korea). The name and sequence primer are given in the (Table 1).

Table (1): qRT-PCR primer sequences.

Name of Primer	Sequence	Sequence Reference
miR 423-5p F RT-primer	5'GCCTGAGGGGCAGAGAGC3'	(12)
RNU6	F 5`CTCGCTTCGGCAGCACA 3` R 5`AACGCTTCACGAATTTGCGT 3`	(13)
***rnu6* RT-primer**	5`GTTGGCTCTGGTGCAGGGTCCGAGGTATT CGCACCAGAGCCAACAATCAG3`	

Statistical analysis

The resulting of this study was analyzed related to objectives and presented under the following headings: a general descriptive of the sample and Statistics Package for Social Science (SPSS), version 25 for Windows software was used for statistics analysis, Microsoft package (Excel & Word), The data are normally distributed and expressed as mean ± standard deviation (SD), Chi-Square between variables were determined by Pearson correlation coefficients (P-value) were performed to analyze the statistical significance of the difference between group (1,2), Significant was considered whenever the p-value was equal or less than (0.05).

Results and discussion

A random sample was taken in the first stage consisting of 100 patients, The patients who showed a positive result with COVID-19 infection numbered (59%) patients, including 34(58%) males and 25(61%) females, and with age group, the age group most affected was between -21 30 with 21(66%) as shown in (Table 2) and (Figure 1). As for the period of onset of symptoms, it was the highest value in 3 days for 27(79%) patients and 2 days for 21(43%) patients, as shown in (Figure 2).

Table (2): The distribution of positive and negative patients with their gender, age, and period of symptoms.

Variables		Positive		Negative		Total	
		n	%	n	%	n	%
Sex	**Male**	34	58%	25	42.4%	59	100 %
	Female	25	61%	16	39.0%	41	100%
Age Group	**≤ 20**	7	54%	6	46.2%	13	100%
	21-30	21	66%	11	34.4%	32	100%
	31-40	16	57%	12	42.9%	28	100%
	41-50	10	67%	5	33.3%	15	100%
	50 >	5	42%	7	58.3%	12	100%
Period of symptoms	**2 days**	21	43%	28	57.1%	49	100%
	3 days	27	79%	7	20.6%	34	100%
	4 days	7	70%	3	30.0%	10	100%
	4 days >	4	57%	3	42.9%	7	100%
Total		**59**	**59%**	**41**	**41.0%**	**100**	**100%**

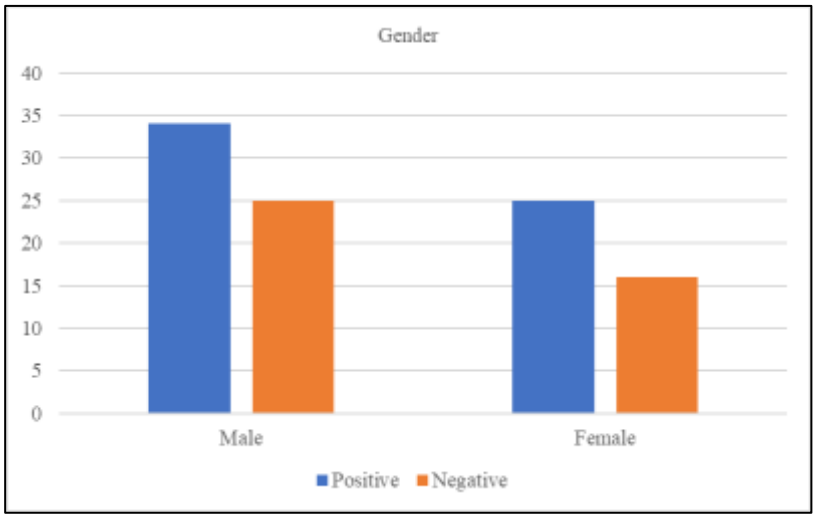

Figure (1): The number of patient samples used in this research with their gender.

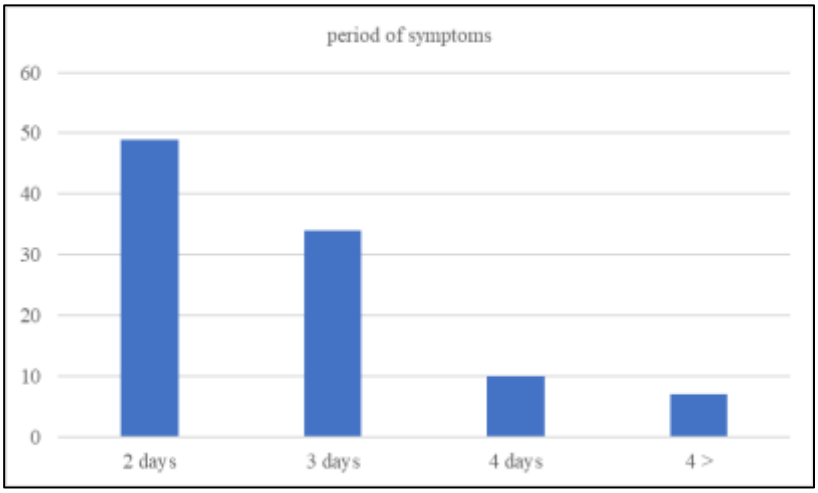

Figure (2): The number of patient samples used in this study with their period of infection symptoms.

Males have an increased risk for infectivity compared with females. Genetic predispositions, sex hormones, immune system responses, and non-biological causes all contribute to the disparity in COVID-19 responses between the sexes. This study agrees with Fabião *et al*. (14), who found men had a relative risk of 1.36 (95%CI: 1.17 to 1.59; I^2 63%, P for heterogeneity <0.01) compared to women. Age was not a significant covariate in meta-analysis heterogeneity (P=0.393) or subgroup analysis. For disease severity, being male was associated with a relative risk of 1.29 (95%CI: 1.19 to 1.40; I^2 48%, P for heterogeneity <0.01) compared to the relative risk of women. Again, age did not influence the meta-regression outcomes (P=0.914) or subgroup analysis. Men had a higher risk of COVID-19 mortality and severity regardless of age. When inquired about whether they had taken the vaccine, it turned out that only 16 (100%) patients took the vaccine, 12 (75%) of them Pfizer, 2 (12.5%) of them AstraZeneca, and 2 (12.5%) of them Sinopharm. As shown in (Table 3).

Table (3):The types and number of vaccination patients used in this study.

Type of vaccines	n	%
Pfizer	12	75
AstraZeneca	2	12.5
Sinopharm	2	12.5
Total	16	100

In the current study, the percentage of use of the Pfizer vaccine was the highest, followed by similar percentages for the AstraZeneca and Sinopharm vaccines, respectively. This is consistent with Shareef *et al*. (15) who did a questionnaire for 1221 eligible participants from various regions in Iraq and found The Pfizer-BioNTech vaccine received 39.6% preference and participants confidence, followed by the Oxford/AstraZeneca vaccine at 18.1% and the Sinopharm vaccine at 14.6%. In the second stage and for organizational purposes, a random sample of 50 people was selected (40 people who did not take the vaccine and 10 who were vaccinated) out of 59 people who had positive responses to the virus, as there was a selection bias. The selection was taken according to gender, and age groups, Table (4**)** below shows the distribution of the samples according to the two stages of the people who took the vaccine and according to the social sex, age groups, and incubation period.

Table (4):The distribution of positive patients that were taken vaccine or not used in this study with their gender, age, and period of symptoms.

Variables		Stage1		Stage 2	
		vaccine	No vaccine	vaccine	No vaccine
Sex	**Male**	11	23	7	22
	Female	5	20	3	18
Age groups	**≤ 20**	1	6	0	6
	21-30	3	18	1	15
	31-40	8	8	6	8
	41-50	4	6	3	6
	50<	0	5	0	5
period of symptoms	**2 days**	3	18	3	15
	3 days	8	19	4	19
	4 days	4	3	3	3
	4 days >	1	3	0	3
Total		16	43	10	40

The molecular experiment of mi423-5p expression was performed to detect the amplification plots and melting curve of the target mi 423-5p and *rnu6* (reference gene) to find the threshold cycle (Ct) value for each one of them. As shown in figures (3,4). The Ct values are used to quantify real-time RT-PCR data that are inversely associated with the amount of starting template and calculate the melting temperature curve.

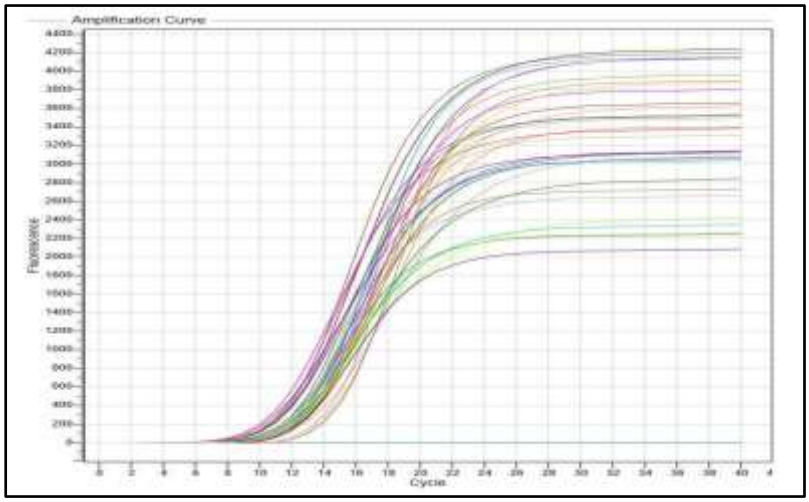

Figure (3): Amplification plots for mi423-5p Expression Obtained by Real-Time PCR.

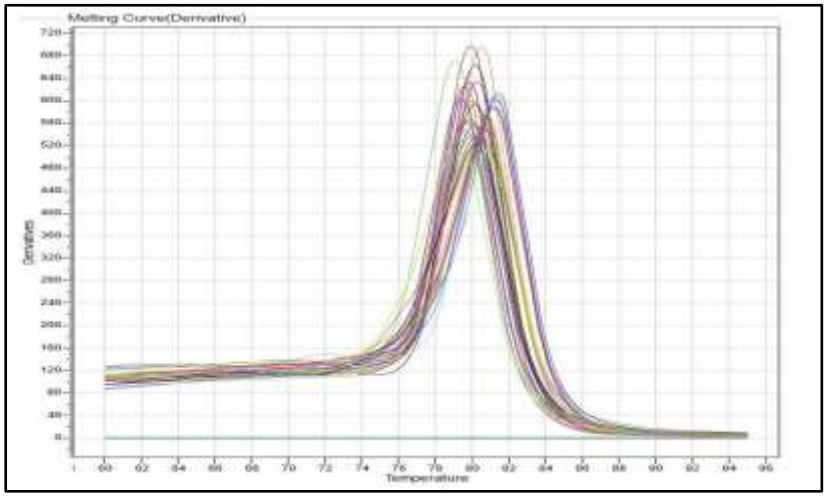

Figure (4): The mi 423-5p expression Melting Curve.

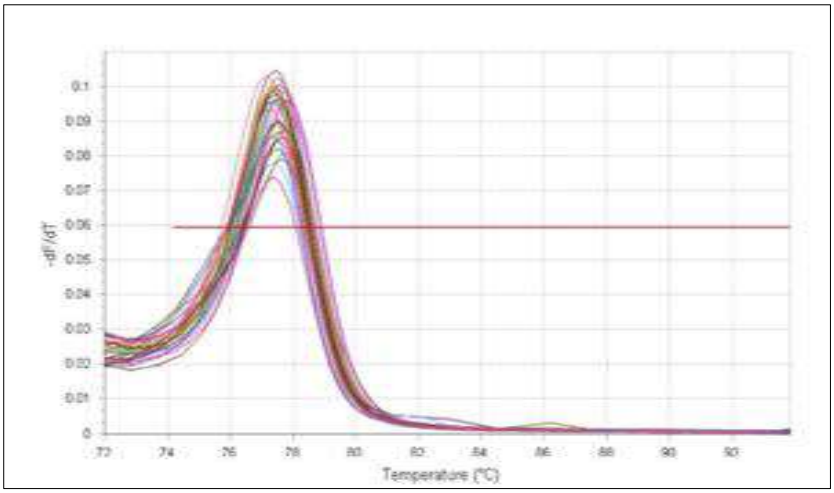

Figure (5): Amplification plot for *RNU6* gene expression.

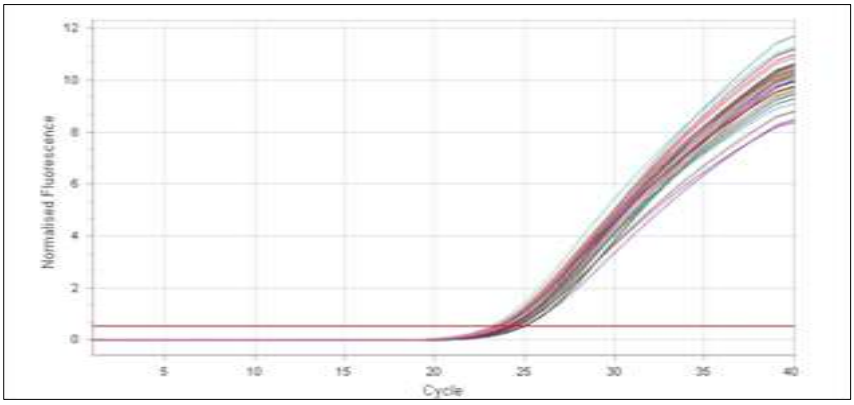

Figure (6): The *RNU6* expression Melting Curve.

A fold of mi 423-5p expression is shown in (Table 5), The statistical analysis data of mi 423-5p indicates that there were statistically significant differences between the infected patients and control, mi 423-5p was significantly upregulated in patients (1.32) in comparison to the control (1). We also noted that P value =0.012, which is smaller than Sig = 0.05, but when the statistical test was conducted for those infected only and according to the categories, it was found that in most of them, there were no significant differences, as the p-value was greater than sig = 0.05. Except for whether the patient taking the vaccine or not, as it turned out that there were significant differences, so the p-value was = 0.028, which is smaller than sig = 0.05, The gene expression of mi 423-5p was higher in unvaccinated patients than in vaccinated patients, and for all the previously mentioned vaccines, shown in (Table 5).

Table (5): Mean ± standard deviation of the **fold of mi423-5p Expression between control and patient, sex, age group, period of symptoms, Number of previous infections with Coronavirus and vaccines.**

Variables		Mi 423-5p	
		Mean ± Sd	P value
Sample	patient	1.32±0.86	0.012*
	control	1±0	
Sex	Male	1.2±0.94	0.219 NS
	Female	1.49±0.72	
Age group	≤ 20	1.14±0.28	0.503 NS
	21-30	1.27±0.71	
	31-40	1.42±1.03	
	41-50	1.65±1.17	
	50<	0.83±0.53	
period of symptoms	2 days	1.35±0.93	0.051 NS
	3 days	1.53±0.79	
	4 days	0.45±0.35	
	4 days <	1.24±0.88	
Number of previous infections with Coronavirus	no infection	1.26±0.83	0.623 NS
	one time	1.29±0.78	
	two times	1.87±1.42	
	three times	1.37±0.62	
Vaccine	Yes	0.77±0.79	0.028*
	No	1.46±0.83	

*** The mean difference is significant at the 0.05 level**
NS: Not Significant

Exosomal miRNAs, including microRNA-423-5p, control immunological responses to viral infection (11). The interaction between viral RNA and miRNAs is crucial to comprehending the pathogenic mechanisms of viral infections. Because viruses depend on the cellular machinery of their hosts for replication, they are an easy target for host destructors like miRNAs (16). The statistical analysis of 423-5p shows an increase in gene expression compared to control samples, it was upregulated and according to the period of infection symptoms show that the gene expression ratio was almost constant during the first week of infection, this study agrees with Farr *et al*. (17), who found COVID-19 induced differential expression of 55 host-encoded microRNAs, with miR-31, -4742 and -3125 strongly up-regulated and miR-1275, -3617 and -500b down-regulated. Logistic regression analysis revealed the measurement of three miRNAs (miR-423-5p, miR-23a-3p, and miR-195-5p). A three-miRNA signature (miR-423-5p, miR-195-5p, and miR-23a-3p) as independent and accurately diagnosed COVID-19 patients and samples obtained from COVID-19 patients 2–15 days (average 8 days) post-symptomatic disease onset, suggest this response is associated with early-stage COVID-19. The results indicated a difference in gene expression between vaccinated and unvaccinated patients. This helps in evaluating the vaccines used in Iraq, as the study showed a decrease in gene expression for vaccinated infected people compared to unvaccinated infected people and for the three vaccines used for the current study in Iraq. In a comparative study, Lin *et al*. (18) found that the seven miRNAs expressed differently in gravid women who had received vaccines (Moderna) with COVID-19 compared to those who had not. In contrast, miRNA-16-5p, miRNA-486-5p, miRNA-21- 5p, and miRNA-451a were reported to be overexpressed in individuals who had not received vaccines. MiRNA-1972, miRNA-191-5p, and miRNA-423-5p were shown to be overexpressed in those who had received vaccinations.

Conclusion

The mi 423-5p signatures discovered in the current work may provide a unique method for early vital status prediction in COVID-19 patients during the disease Specific miRNA expression data may predict a person's COVID-19 infection severity. The use of these miRNA targets as therapeutic targets may benefit from their validation with a bigger cohort, and microRNA expression may be used as a valuable tool for real-time monitoring of antibody effects.

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