How to Cite:
AL-Masudi, H. S. M., Abid, A. J., & Al-Mammori, R. T. (2022). Evaluation of microRNA and some immunological factors associated with SARS-CoV-2 infection. *International Journal of Health Sciences*, *6*(S6), 2606–2616. https://doi.org/10.53730/ijhs.v6nS6.10559

Evaluation of microRNA and some immunological factors associated with SARS-CoV-2 infection

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***Abstract*---**Coronavirus (CoV) is a family of RNA viruses that cause disease in humans and other animals by entering the body via ACE2 receptors found in various organs such as the heart, lungs, kidneys, and digestive system. Coronaviruses are large particle viruses that are enveloped, spherical, have surface protrusion-forming spikes, and have a large positive- sense single-stranded RNA genome. A total 90 samples were collected from people suspected of being infected with SARSCoV-2, 50 blood samples were collected from patients, and 40 samples were collected from healthy individuals as a control group, who aged 20-80 years and carried out during September (2020) to January (2022) at Al-Hussein Teaching Hospital. All were checked by using real- time quantitative polymerase chain reaction (RTPCR) technology. Evaluations of miRNAs were performed in a group of newly infected patients with the SARSCoV-2 virus and healthy individuals who were cured, the result found MiRNA -192 gene have 84 % positive RT-PCR result in comparison with 16 % in which have negative result , while the other gene MiRNA - 31 have 36% positive result in comparison with 64% as a negative result, as well as 100 % of H.K. gene, The CT value show the high level of MIRNA -31 in comparison with MiRNA - 192 as well as H.K. gene in newly infected patients. The low CT value of MiRNA -31CT value in MiRNA 31 (34.76) refer to high level of such gene in studied patients, increased indicated of lower level in curried patients after infection with SARS-CoV-2 virus, because that inverse relationship between CT value number and MiRNA level. While the CT value of H.K. gene revealed that

(24.39), the optimum control (H.K. gene) CT value should be in between 20-30 CT value as constant index measure.

***Keywords*---**Covid-19, SARS-CoV-2, ACE2, RT-PCR, MiRNA.

Introduction

The word "coronavirus" is derived from the Greek word corn, meaning "crown" or "wreath," and was first used to describe a virus with an appendage like a spike (Tyrrell and Fielder 2020). Coronavirus was not widely recognized until the 2003SARS pandemic (Hung 2003, Huang 2004), prior to the MERS 2012 and recent outbreaks CoV-19. SARS-CoV and MERS-CoV were known to be highly pathogenic and spread from bats to civets or dromedary camels and ultimately to humans (Kim *et al.*; 2017, Sikkema *et al.*; 2019, Rajinikanth *et al.*; 2020). Coronavirus has a single-strand plus- strand RNA genome (+ssRNA) that is larger (27–32 kb) than any other RNA virus. On the outside of the nucleus, the nucleocapsid protein (N) forms the capsid, and the genome is further enveloped by an envelope composed of three structural proteins: membrane protein (M), spike protein (S), and coat protein (E) (Brian *et al.*, 2005). The genome size of SARS-CoV-2, which was recently sequenced as a member of the coronavirus family, is approximately (29.9 kb) (Li *et al.*; 2020). SARS-CoV-2 has 16 non-structural proteins (nsp1-16) and 4 structural proteins (S, E, M, and N).

The miRNAs are short non-coding RNAs, approximately 22 nucleotides in length, that control one-third of all protein-coding human genes that encode proteins (Hammond, 2015). In addition, miRNAs plays an important role in the etiology of various diseases such as viral infections, neurodegenerative diseases, metabolic diseases, chronic pediatric diseases, and parasitosis (Bruscella et al., 2017; Paul et al., 2021a, 2021b, 2020a, 2020b). For viral infection of mammalian cells, miRNA is involved in the regulation of lytic and latent viral replication (Grassmann and Jeang, 2008), especially in the case of coronavirus, in immune response and viral protein expression (Grassmann and Jeang, 2008; Abu-Izneid et al., 2021; Mirzaei et al., 2021). In particular, mirabilsen and RG-101 are the most promising miRNA-based agents that have been widely studied in recent years for thetreatment of hepatitis C (Bonneau et al., 2019), and mirabilsen is a remarkable anti-thoroughness. SARS-CoV-2 effect to be studied (Alam and Lipovich, 2021). Therefore, miRNA-based therapies are described as innovative strategies for alleviating viral diseases, especially COVID-19.

The miR-31 has been characterized as a tumor suppressor miRNA with its levels varying in breast cancer cell according to the metastatic state of the tumor (Doy et al., 2010). From its typical abundance in healthy tissue is a moderate decrease in non-metastatic breast cancer cell lines and levels are almost completely absent in mouse and human metastatic breast cancer cell lines (Valastyan et al.; 2009). mir-31-5p has also been observed up regulated in zinc deficient rats compared to normal in ESCC (Esophageal Squamous Cell Carcinoma) and in other types of cancers when using this animal model (Fong et al., 2016). There has also been observed a strong encapsulation of tumor cells expressing miR-31, as well as a reduced cell survival rate (Valastyan et al., 2011). The miR-31 anti-metastatic

effects therefore make it a potential therapeutic target for breast cancer. However these two papers were formally retracted by the authors in 2015.

Many studies have investigated the role of miR-192-5p, however, the functionality of miR-192-3p has hardly been considered. A study by Mysore and his colleagues highlighted the important role of miR-192-3p in adipocyte differentiation and lipid homeostasis. First, they discoversd an interesting phenomenon that miR-192-3p was negatively correlated with serum triglyceride (TG) levels in visceral adipose tissue (VAT) in obese patients. Further mechanistic studies have shown that miR-192-3p targets adipocyte differentiation of human Simpson–Golaby–Bemel syndrome (SGBS) adipocytes by targeting aldehyde dehydrogenase 3 family A2 (ALDH3A2) members and sterol coenzyme A desaturase 1 (SCD) shows to suppress. Significantly reducedTG content and the reduced expression of the adipocyte marker proteins a P2 and perilipin 1 (Mysore *et al.*, 2016; Gharban and Al-Shaeli, 2021). In addition, miR-192-5p and miR-192-3p play a synergistic role in down regulating the expression of farnesoid X receptors (FXRs) and the FXR target genes in the liver and colon cancer-derived cell lines. However, miR-192 has different effects on FXR expression in Huh7 and Caco-2 cells (Krattinger *et al.*, 2016).

Materials and Methods

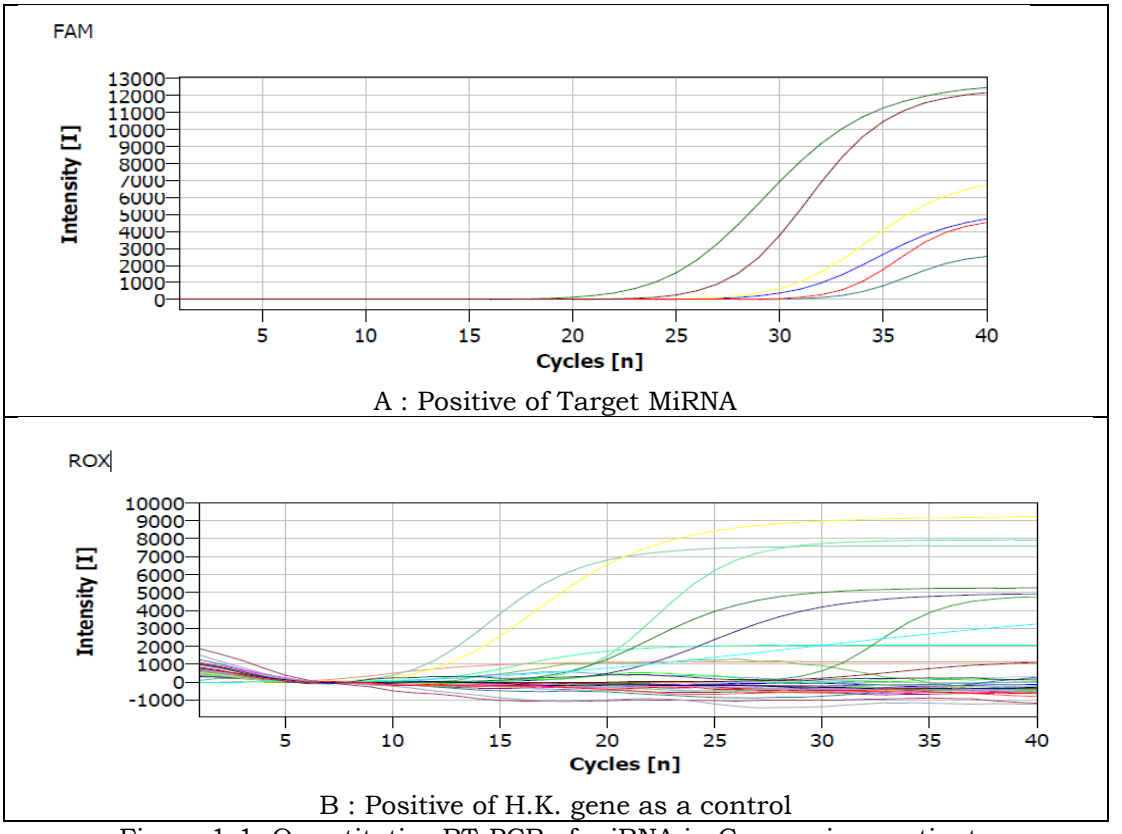
Totally, 90 samples were collected from people that probably patients and healthy with SARS-CoV-2 and 50 blood samples were collected from patients, and 40 samples from healthy as control group. Aged (20-80 years) during 1/September/2020 to 20/January/2022, in AL-Hussein Teaching hospital.The blood samples were placed in an EDTA tube and kept in the refrigerator at 30°C until use in the study.Extracting RNA from a blood sample using human microRNA extraction kits (Favrogen). The extracted RNA Store at -70C. All total RNA samples were checked by real- time quantitative Polymerase Chain reaction (RT-PCR) technique with theGoTaq®1-Step RT-qPCR system using the Kit (promega). Used in microRNA gene 31 and the microRNA gene 192 according to the primers in the following table.

Gene	Nitrogen bases 5'→3'	Company	Reference
miR- 192	F:CTGACCTATGAATTGACAGCCA	Humanizing Genomics Macrogen	NCBI
	R:GCTGTCAACGATACGCTACGT		
miR-31	F:GCCGCAGGCAAGATGCTGGC		
	R:CAGTGCAGGGTCC GAGGT		
H.K gene	F:GTTTTGTAGTTTTTGGAGTTAGTGTTGTGT		NCBI
	R:CTCAACCTACAATCAAAAACAACACAAACA		

First, the extracted RNA sample was taken and Master Mix and other solutions were added, and they used the Real Time method based on Promega Kit. 10µl of Master Mix and 2µlForward and 2µl Reverse primer were added and the solutions were mixed, and entered into the device to estimate RNA values, using a qrtpcR device based on (Promega). Using the followingconditions.denaturation 95°C 10 seconds, annealing and data collection 60°C 30 seconds, extension 72°C 30 seconds.Calculation micro RNA values based on graphs and ct values(kit of valium).

Results and Discussion

By using Quantitative RT- PCR , the assessment of MiRNA were done in a group of patients infected with SARS-CoV-2 virus, with noted that selected of small number of patients, and the result show that the FAM CT value indicated of positive miRNA , in comparison with ROX for H. k. genes as a control miRNA, which are often referred to as housekeeping genes, are frequently used to normalize mRNA levels between different samples or different genes , this genes may change under certain conditions at high to moderate fluorescent intensity as in figure (1-1).



A : Positive of Target MiRNA

B : Positive of H.K. gene as a control

Figure 1-1. Quantitative RT-PCR of miRNA in Coronavirus patients

The overall results of the studied genes show that miRNA-192 has an 84 percent positive RT-PCR result, compared to 16 percent that have a negative result or no CT value, while miRNA-31 has a 36 percent positive result, compared to 64 percent that have a negative result, and 100 percent of H.K. gene, as shown in table (1-1). This finding might mean that miRNA-192 expression was higher in patients with Covid-19. It was also discovered that miRNA-31 expression was considerably different, and the total survival rate was predicted for a variety of additional medical complications that could be causing low gene expression. The miRNA profiles may reveal biological pathways involved in disease severity, pointing to potential treatment targets (Tribolet et al.; 2020).

In viral infection and replication, host miRNAs play an important role. Viral miRNAs can alter the transcriptome of the host in a reciprocal manner. The relevance of miRNAs in host-pathogn interactions during infection, and the importance of miRNAs in the functionality of numerous immune cell types and host immunological homeostasis, these tiny molecules might be explored as anti-viral therapeutic targets (Iqbal et al.; 2020). In general, targeted delivery and insecurity the primary issues with miRNA-based treatment are toxicity and cost. Targeting a number of important miRNAs might be a powerful and promising strategy in the fight against the virus, but more study is needed to identify individual miRNAs before clinical trials can begin (Nihad et al.; 2021).

Table 1-1
The RT-PCR result of miRNA genes (192 and 31)

Gene types	RT –PCR	No.	Percentage
MiRNA 31	Positive	9	36 %
	Negative	16	64 %
MiRNA 192	Positive	21	84 %
	Negative	4	16 %
H.K. Gene Control	Positive	18	100 %
	Negative	0	0 %

The CT value of MiRNA 31, 192 and House Keeping gene

Because there is an inverse relationship between CT value number and MiRNA level, the high level of miRNA-31 in comparison to MiRNA-192 and H.K. gene in newly infected patients with COVID-19, the low CT value of miRNA-31 (31.04) indicates a high level of such gene in studied patients, and the increased CT value of miRNA-31 (34.76) indicates a lower level in curried patients after infection with SARS-CoV-2 virus. While the CT value of the H.K. gene was (24.39), as a constant index measure, the ideal control (H.K. gene) CT value should be in the range of 20-30 CT. The CT value of the examined miRNA is shown in table 1-2. The miRNA panel's ability to predict death is being investigated.And it's linked to two significant findings: (1) severe COVID-19 causes distinctive molecular alterations in the circulating miRNA profile; and (2) miRNAs, specifically a signature made up of miRNA-192-5p and miRNA-323a-3p, are useful predictors of patient prognosis in the clinically severe phase (Bonneau et al.; 2021).

Table 1-2
The result CT value of studied miRNA

CT value of RT – PCR		No.	Ct Mean	Std. Deviation	P. Value
miRNA-31	Newly infected	5	31.04	1.77	0.000
	Cured (+ve control)	2	34.76	2.94	
	Healthy (-ve Control)	2	33,12	1.30	
miRNA-192	Newly infected	16	33.42	2.37	0.000
	Cured (+ve control)	3	31.90	1.30	
	Healthy (-ve Control)	2	32.21	2.94	
H.K. Gene		18	24.39	4.16	

The expression of selected miRNAs and their mRNA targets restored to normal in COVID-19 patients who responded to therapy. The expression of hsa-miR-31-3p showed a negative significant connection. A better knowledge of the expression of up- and down-regulated inflammatory miRNAs in COVID-19 patients' blood. The information gathered may aid in the diagnosis and prognosis of COVID-19 (Reza et al.; 2021).

Quantitative CT value measurement of MiRNA in Coronavirus patients

Choosing 25 samples for molecular study by evaluating of miRNA, as miRNA31, miRNA192 and H.K. gene as a control gene, the result of miRNA 31, the positive result of patients (5 patients) in comparison with (13) have negative result or no Ct value, in control samples, 4 person have positive result in counter with 3 as negative. miRNA 192 have more positive number (16 patients), and 3 control samples as listed in table (1-3).The miRNAs that showed significant changes are involved in the regulation of immune and inflammatory pathways at various levels, including cytokine and chemokine synthesis (miR-16-5p, miR-192-5p, miR-451a), T cell development, differentiation, and activation, and B cell development, differentiation, and activation. COVID-19 severity has an effect on the circulating miRNA profile. Plasma miRNA profiling appears to be a valuable approach for risk-based patient classification in COVID-19 patients who are critically sick (David et al.; 2021). With a rise in COVID-19 grade, the relative expression of hsa-miR-31-3p, hsa-miR-29a-3p, and hsa-miR-126-3p was down-regulated, while the relative expression of their mRNA targets was up-regulated. This pattern was observed in COVID-19 patients who did not react to therapy throughout their hospitalization (Baud et al., 2020).

Table 1-3
The result of RT-PCR of miRNA among studied groups

RT – PCR Results		Reaction	N	Mean	Std. Deviation	P. Value
miRNA-31	Patients	+ve	5	31.04	1.77	0.000
	Patients	-ve	13	No Ct	No Ct	
	Control	+ve	4	34.76	2.94	
	Control	-ve	3	No Ct	No Ct	
Total			25	9.13	15.02	
miRNA-192	Patients	+ve	16	31.68	1.76	0.141
	Patients	-ve	4	No Ct	No Ct	
	Control	+ve	3	34.36	2.99	
	Control	-ve	2	No Ct	No Ct	
Total			25	21.26	16.38	
H.K.gene		+ve	18	24.39	4.16	

The result of figure show that a direct correlation between both miRNA -31 and 192, the result might be show that increased expression in miRNA 192 lead to increase in miRNA 31 among selected groups of SARS CoV-2 patients as well as curried patients. This result recorded in figure (1-5).

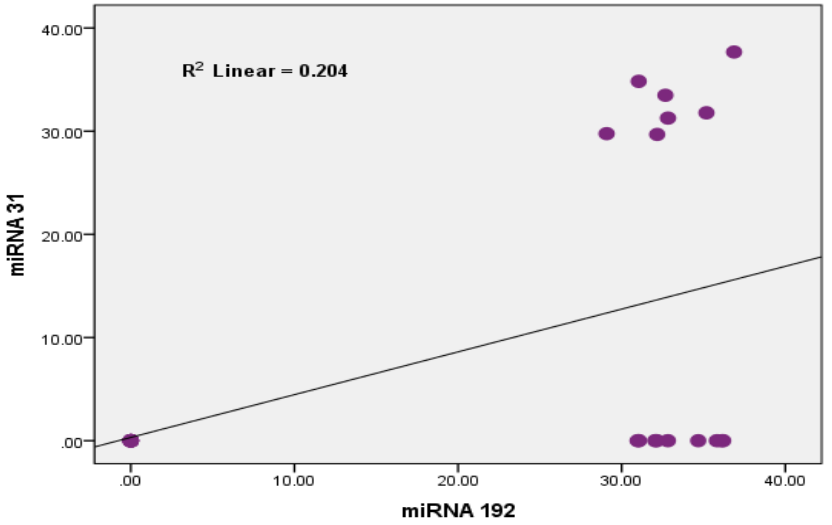


Figure 1-5. Correlation between miRNA 31 and miRNA 192

The results of the picture demonstrate a clear association between miRNA-31 and the H. K. gene, which was utilized as a control human gene. The finding might indicate that higher production of miRNA 31 causes a rise in the H. K. gene in some groups of SARS-CoV-2 patients and curried patients. Figure 1 shows the outcome (1-6).

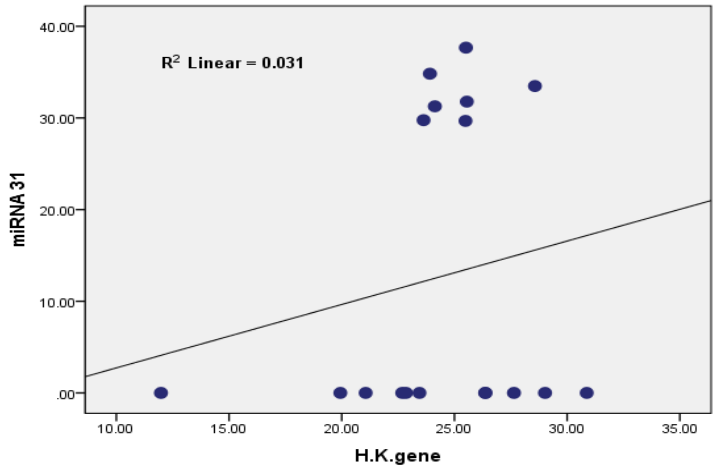


Figure 1-6. Correlation between miRNA 31 and H.K. gene

The results of the picture demonstrate a negative association between miRNA 192 and H. K. gene, which was used as a control human gene. This might mean that higher expression of miRNA 192 leads to a decrease in H. K. gene among specific groups of Sars –Cov2 patients and curried patients. Figure 1 shows the result (1-7).

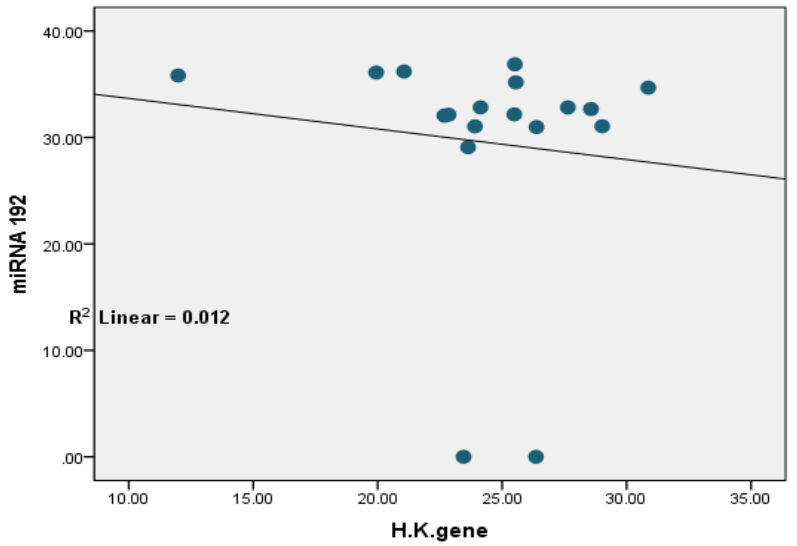


Figure 1-7. Correlation between miRNA 192 and H.K. gene

Discussion

Through our study of the results of patients with corona virus for the microRNA gene 31 and the microRNA gene 192, we found that microRNA 192 is increased by 84%, compared to microRNA 31 is low by 36%. Another study indicated that as illness severity rose, the relative expression of hsa-miR-31-3p, hsa-miR-29a-3p, and hsa-miR-126-3p fell significantly, whereas the relative expression of their mRNA targets (ZMYM5, COL5A3, and CAM- SAP1) increased significantly. In contrast, as illness severity grew, the relative expression of hsa- miR-17-3p increased significantly, whereas its mRNA target (DICER1) declined significantly. This pattern was observed in COVID-19 patients who did not react to therapy throughout their hospitalization. The expression of specific miR- NAs and their mRNA targets recovered to normal in COVID-19 patients who responded to therapy.level. In COVID-19 patients with any grade (P 0.05) and during hospitalization, there was a negative significant association between the expression of (1) hsa-miR-31-3p and ZMYM5, (2) hsa-miR-29a-3p and COL5A3, (3) hsa-miR-126-3p and CAMSAP1, and (4) hsa-miR-17-3p and DICER1. Our findings are in line with those of other scientists. For example, in COVID-19, the expression of miR-31 was changed (Farr et al.,2021). (Centa and colleagues, 2021). Furthermore, miR-192-5p and miR-192-3p work together to suppress the expression of farnesoid X receptor (FXR) and FXR target genes in cell lines originating from liver and colon cancer. However, in Huh7 and Caco-2 cells, miR-192 had differing impacts on FXR expression (Krattinger et al., 2016).

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