Original Research

Can Plasma microRNA Predict Systemic Inflammatory Response Syndrome In Adult Covid-19 Patients? A Comparative Cross-Sectional Study

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ABSTRACT

Background: Systemic Inflammatory Response Syndrome (SIRS) is one of the acute complications of COVID-19 (Coronavirus infection). MicroRNA (miRNA) complement with 3'untranslated regions of messenger RNA (mRNA) and inhibits the translation of mRNA to proteins. Multiple studies revealed changes in the levels of circulating mi-RNAs in patients with SIRS. Hence analysis of miRNAs could serve as an effective biomarker in patient management by predicting the onset of SIRS in COVID-19 infection.

Methods: In this comparative cross-sectional study, COVID-19 infected patients admitted in wards and Intensive Care Units (ICU) were enrolled. A total of 120 patients were distributed among two study groups with group A containing COVID-19 patients complicated with SIRS and group B containing COVID-19 patients without SIRS.COVID-19 infection was confirmed by RT-PCR (Reverse transcription-polymerase chain reaction). SIRS was diagnosed by validated clinical evaluation method. The quantification of miRNA was done using TB Green on the CFX96 Real-Time PCR detection system. Statistical analysis was performed using SPSS version 22.0 software.

Results: The levels of miRNA-27b were significantly higher in group A compared to group B (28.364 ± 2.967 versus 25.519± 2.790, $p<0.001$). The levels of miRNA 627-5p were significantly lower in group A when compared with group B (30.704± 2.098 versus 31.833± 2.170, $p=0.02$). On ROC (Receiver operator characteristic) curve analysis, a cut-off of >26.91 for miRNA-27b gave 67.8% sensitivity and 75% specificity in predicting SIRS with an AUC (Area under curve) of 0.76. Similarly, for miRNA-627-5p, a cut-off of ≤31.1 gave a sensitivity of 61.4% and a specificity of 69.8% in predicting SIRS with an AUC of 0.68.

Conclusion: Our study established an association between circulating levels of miRNA-27b and miRNA-627-5p in patients affected by COVID-19 infection complicated with SIRS.

Keywords: COVID-19, Systemic Inflammatory Response Syndrome, miRNA-27b, miRNA-627-5p.

INTRODUCTION

In December 2019, many patients presented with pneumonia in Wuhan city of China that rapidly transmitted and became a global pandemic causing higher mortality across countries. Genomic sequencing revealed that the causative pathogen is a novel variant of coronavirus and was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the disease was called Coronavirus disease 2019 (COVID-19) infection.[1-3] SARS-CoV-2 is transmitted between humans through aerosols and droplets by direct or indirect contact with mucous

membranes of the eyes, mouth or nose.[4-6] The Dipeptidyl peptidase-4 (DPP-4) and Angiotensin-converting enzyme 2 (ACE-2) receptors present in the lower respiratory tract acts as binding receptors for the surface spike glycoprotein of SARS-CoV-2.[7-10] The uncontrolled inflammatory response called cytokine storm activated by SARS-CoV-2 could result in irreversible multiple organ dysfunction syndrome (MODS) which is one of the major causes of mortality in COVID-19 infection.[11-13] Systemic inflammatory response syndrome (SIRS) is the clinical manifestation produced due to complex intrinsic mediators of acute phase reaction which can result in end organ dysfunction.[14,15]The diagnosis of SIRS is clinically made when any of the two parameters are present like body temperature above 100.4°F or below 96.8°F, heart rate above 90 beats per minute, respiratory rate above 20 per minute, partial pressure of CO_2 less than 32 mm Hg and white blood cell count more than 12,000 per µL or less than 4000 per µL.[16]A biomarker which could predict the onset of SIRS in COVID-19 infection would be effective in patient management by preventing multiple organ dysfunction and reduction in mortality. MicroRNA (miRNA) are single-stranded small non-coding RNAs which are 20 to 25 nucleotides long. They complement with 3'untranslated regions of messenger RNA (mRNA) and through RNA-induced silencing complex (RISC) cause cleavage of mRNA. This prevents the accessibility of ribosomes and inhibits translation of mRNA to proteins.[17]Through the process of either up regulation or down regulation miRNAs exert their influence on inflammation. There is an increased expression of miRN. As which mediate downregulation by targeting mRNA that code for anti-inflammatory transcriptional activators with increasing severity of COVID-19 infection.[18-20] On the other hand, the decreased expression of certain miRNA relieves mRNA from miRNA mediated repression and causes upregulationleading to uninhibited translation of pro-inflammatory mediators in severe infections.[21]Hence, changes in circulating miRNA in response to infectious diseases could serve as an early investigation tool for predicting impending complications. In this study, we aim to find the effectiveness of a down-regulating miRNA-27b and another up-regulating miRNA-627-5p in predicting SIRS due to COVID-19 infection.

OBJECTIVES

1. To compare the circulating levels of miRNA-27b and miRNA-627-5p in adult patients infected with COVID-19 infection complicated with SIRS and without SIRS.
2. To study the effectiveness of circulating mi-RNA as a biomarker for predicting the onset of SIRS in patients affected with COVID-19 infection.

MATERIAL & METHODS

Study design: Comparative cross-sectional study.

Study duration: 12 months: July 2021 to June 2022.

Study population: The study was conducted among patients admitted in COVID-19 wards and Intensive care units (ICU) of Government Medical College Hospital, Kozhikode, Kerala. The study population was divided into two groups. Inclusion criteria for Group I included 60 patients from both sexes and in the age group above 18 years admitted for management of COVID-19 infection with SIRS. Exclusion criteria for patients in group I included age less than 18 years and presenting with SIRS not due to COVID-19 infection. Group II included 60 patients from both sexes in the age group above 18 years and admitted for COVID-19 infection not complicated with SIRS. Exclusion criteria for group II included patients with COVID-19 infection who were below 18 years of age. Informed consent was obtained from all the participants.

Specimen and study parameters: Venous samples were collected in purple vacutainer tubes containing ethylenediaminetetraaceticacid (EDTA) by the principal investigator under the supervision of the COVID nodal officer, following the guidelines to protect against COVID-19 transmission. COVID-19 infection was confirmed by RT-PCR.[22,23]SIRS was diagnosed by validated clinical criteria evaluation tool.[16]

Reagents and instrumentation: The miRNA isolation was done using isopropyl alcohol, exosome precipitation solution supplied by Macherey-Nagel, Germany and Lysis buffer, protein precipitation buffer, columns and collection tubes supplied by Takara Bio, USA. The cDNA synthesis was done using Mir-X^{TM}miRNA first strand synthesis kit and quantification of microRNA was done using TB Green, qRT-PCR kit both supplied by Takara Bio, USA on CFX96 Real-Time PCR detection system.[24]

Statistical analysis: Statistical Package for Social Sciences [SPSS] for Windows version 22.0 was used to perform statistical analyses. Descriptive analysis of all the explanatory and outcome parameters was done using frequency and proportions for categorical variables, whereas in mean & standard deviation (SD) for continuous variables. Chi-square test was used to compare the symptomatology and comorbidity factors between the two groups. Independent student's t-test was used to compare the mean circulating plasma miRNA levels between the two groups. Mann Whitney Test was used to compare the mean duration of symptoms between two groups. ROC Curve analysis was performed for plasma miRNAparameters in SIRS. The level of significance was set at $p<0.05$.

RESULTS

The two study groups differed significantly with respect to the mean age (60.85 ± 15.58) among group

A and group B (48.18 ± 19.23) respectively with *p <0.001*. The most predominant symptom reported by our patients was fever (n=78, 65%) followed by breathlessness (n=46, 38.3%) and cough (n=44, 36.7%). The least predominant symptoms were dizziness (n=1, 0.8%), melena (n=1, 0.8%) and hemoptysis (n=1, 0.8%).On comparing both the groups concerning symptomatology, the number of patients with breathlessness was significantly higher in group A compared to group B (n=38, 63.3% versus n=8, 13.3%, *p<0.001*). Number of patients who complained of cold and headache were significantly higher in group B compared to group A (n=18, 30% vs. n=4, 6.7%, *p = 0.001* and n=16, 26.7% vs. n=6, 10%, *p = 0.02* respectively) (Table 1). With regard to the mean duration of symptoms among both the study groups, it was observed that the mean duration of symptoms among patients was significantly longer in group A as compared to group B (5.53±2.58 vs. 3.98±1.68, *p < 0.001*). (Table 2)While assessing various comorbidities, the most common comorbidity observed among the study population was hypertension (n=46, 38.3%) followed by diabetes mellitus (n=33, 27.5%). Least common comorbidities present among our study groups were asthma (n=1, 0.8%), dyslipidemia (n=1, 0.8%), cardiac arrhythmias (n=1, 0.8%), anaemia (n=1, 0.8%), antiphospholipid antibodies (APLA) (n=1, 0.8%), autoimmune hypothyroidism (n=1, 0.8%), myelofibrosis (n=1, 0.8%) and hyponatremia (n=1, 0.8%). (Table 3).On assessing the vaccination history, it was found that 71 patients enrolled have not received any COVID-19 vaccine (59.2%) whereas 25 patients have received one dose (20.8%) and 24 patients have received both doses (20%). On statistical analysis, we found no significant difference between the study groups with respect to vaccination history (*p = 0.66*).On comparing the mean circulating plasma miRNA levels between the two groups it was found that the levels of miRNA-27b were significantly higher in group A compared to group B (28.364± 2.967 vs. 25.519±2.790, *p<0.001*). It was also found that the levels of miRNA-627-5p were significantly lower in group A compared with group B (30.704±2.098 vs. 31.833± 2.170, *p = 0.02*).(Table 4)A receiver operating characteristic curve (ROC) curve analysis was done for COVID-19 with SIRS patients with respect to the test miRNA.(Table 5). The results suggest a cut-off of >26.91 for miRNA-27b which gave 67.8% sensitivity and 75% specificity in predicting SIRS with an area under curve (AUC) of 0.76 (Figure 1). Similarly for miRNA-627-5p, a cut-off of≤31.1 gave a sensitivity of 61.4% and a specificity of 69.8% in predicting SIRS with an AUC of 0.68 (Figure 2).

Variable	Category	Group A		Group B		p-value
		n	%	n	%	
Symptoms	Fever	41	68.3%	37	61.7%	0.44
	Breathlessness	38	63.3%	8	13.3%	<0.001*
	Cough	25	41.7%	19	31.7%	0.26
	Cold	4	6.7%	18	30.0%	0.001*
	Headache	6	10.0%	16	26.7%	0.02*
	Sore throat	2	3.3%	4	6.7%	0.40
	Vomiting	2	3.3%	2	3.3%	1.00
	Tiredness	1	1.7%	2	3.3%	0.56
	Chest pain	2	3.3%	1	1.7%	0.56
	Malaise	2	3.3%	0	0.0%	0.15
	Neck pain	1	1.7%	1	1.7%	1.00
	Abdominal pain	2	3.3%	0	0.0%	0.15
	Altered sensorium	2	3.3%	0	0.0%	0.15
	Dizziness	1	1.7%	0	0.0%	0.32
	Melena	1	1.7%	0	0.0%	0.32
	Hemoptysis	1	1.7%	0	0.0%	0.32

Table1. Comparison of symptoms between two groups (Group A: COVID-19 patients complicated by SIRS and Group B: COVID-19 patients without SIRS) using Chi-square test (COVID-19:Corona virus disease 2019; SIRS: Systemic inflammatory response syndrome)

Variable	Category	Group A		Group B		p-value
		Mean	SD	Mean	SD	
Duration	Mean	5.53	2.58	3.98	1.68	<0.001*[a]
	Range	01 - 14		01 – 09		

Table 2. Comparison of mean duration in days of symptoms between two groups (Group A: COVID-19 patients with SIRS and Group B: COVID-19 patients without SIRS) (COVID-19:Corona virus disease 2019; SIRS: Systemic inflammatory response syndrome; SD:Standard deviation)

Variable	Category	Group A		Group B		p value
		n	%	n	%	
Comorbidity	Hypertension	33	55.0%	13	21.7%	<0.001*
	Diabetes mellitus	23	38.3%	10	16.7%	0.008*
	CAD	12	20.0%	3	5.0%	0.01*
	CKD	8	13.3%	2	3.3%	0.04*
	CVA	8	13.3%	0	0.0%	0.003*
	COPD	6	10.0%	1	1.7%	0.04*
	Parkinsonism	4	6.7%	0	0.0%	0.04*
	Rheumatoid Arthritis	1	1.7%	1	1.7%	1.00
	Pulmonary tuberculosis	2	3.3%	0	0.0%	0.15
	Bronchial asthma	1	1.7%	0	0.0%	0.32
	Dyslipidemia	1	1.7%	0	0.0%	0.32
	Cardiac arrhythmias	0	0.0%	1	1.7%	0.32
	Anemia	0	0.0%	1	1.7%	0.32
	APLA	1	1.7%	0	0.0%	0.32
	Autoimmune hypothyroidism	1	1.7%	0	0.0%	0.32
	Myelofibrosis	1	1.7%	0	0.0%	0.32
	Hyponatremia	1	1.7%	0	0.0%	0.32
	Nil	9	15.0%	36	60.0%	<0.001*

Table 3. Comparison of comorbidities between two groups (Group A: COVID-19 complicated by SIRS and Group B: COVID-19 patients without SIRS) (CAD:Coronary artery disease; CKD:Chronic kidney disease; CVA:Cerbrovascular accident; COPD:Chronic obstructive pulmonary disease; APLA:Antiphospholipid antibody; COVID-19:Corona virus disease 2019; SIRS:Systemic inflammatory response syndrome)

Variable	AUC	Standard error	95% Confidence Interval		p-value	Cut off	Sn (%)	Sp (%)
			Lower	Upper				
miRNA- 27-b	0.76	0.04	0.67	0.83	<0.001*	> 26.91	67.8	75.0
miRNA- 627-5p	0.68	0.06	0.57	0.77	0.002*	≤ 31.1	61.4	69.8

Table 4. Comparison of mean circulating plasma miRNA levels between 2 groups (Group A: COVID-19 complicated by SIRS and Group B: COVID-19 patients without SIRS) using Independent student's t-test(miRNA;microRNA; AUC:Area under curve; Sn: scale measuring typical distances between values: Sp;Sum of products of deviation)

microRNA	Group	N	Mean	SD	Mean Difference	95% CI		p-value
						Lower	Upper	
27-b	Group A	59	28.364	2.967	2.845	1.800	3.890	<0.001*
	Group B	60	25.519	2.790				
627-5p	Group A	44	30.704	2.098	-1.129	-2.038	-0.219	0.02*
	Group B	43	31.833	2.170				

Table 5. ROC curve analysis for plasma miRNA parameters among two groups. (Group A: Covid-19 complicated by SIRS and Group B: Covid-19 patients without SIRS) (ROC: Receiver operating characteristic curve; SD:Standard deviation; CI:Confidence interval)

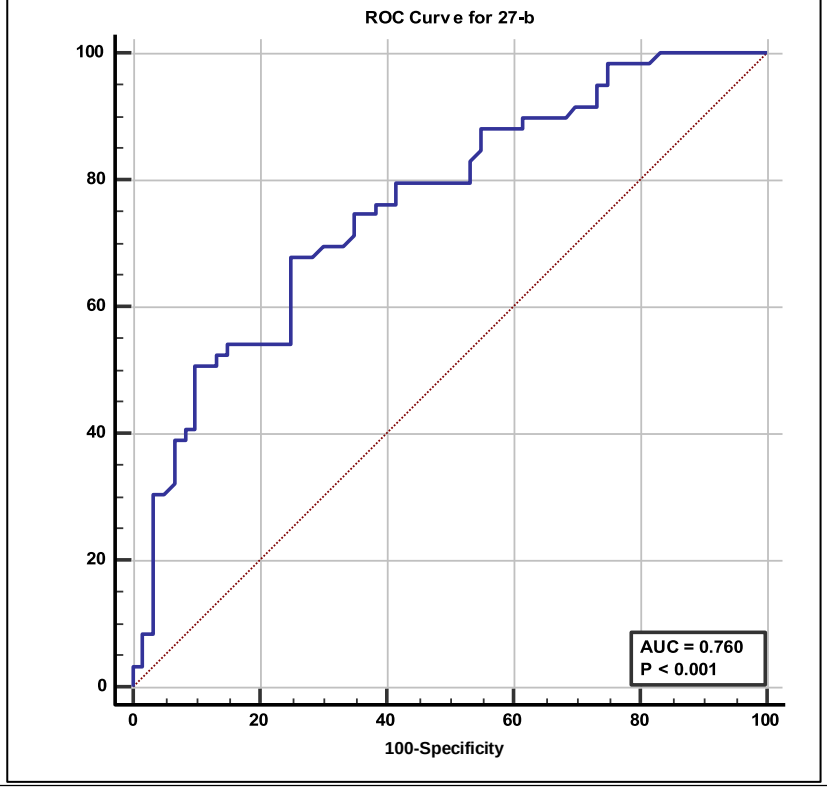

Figure1. ROC curve for miRNA27b (ROC:Receiver operating characteristic curve; miRNA:micro RNA; AUC:Area under curve)

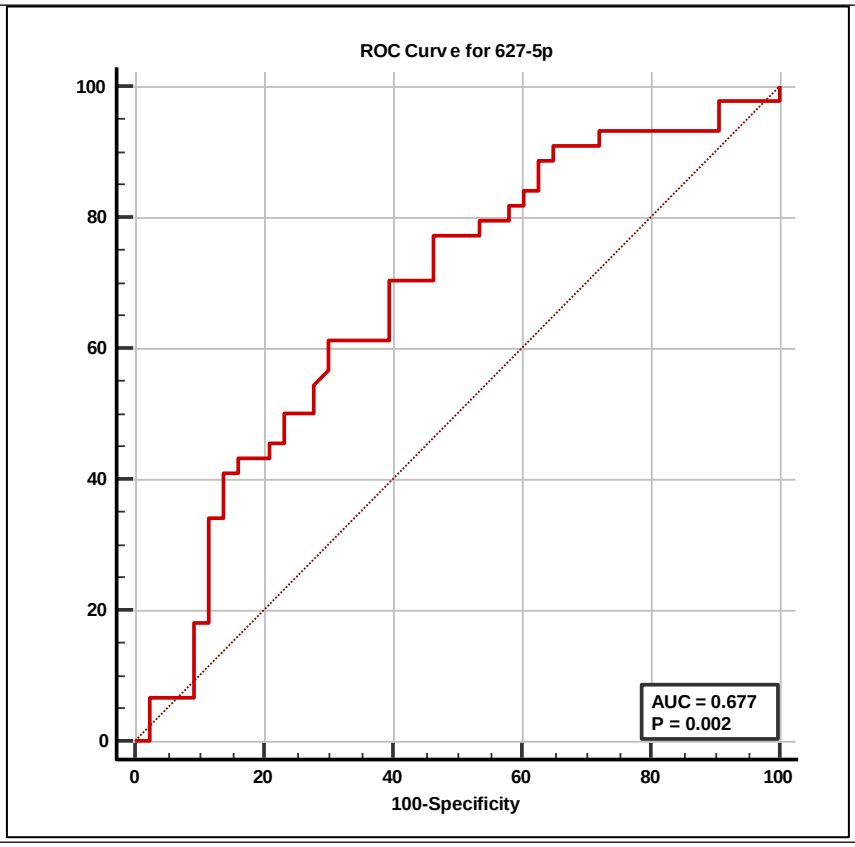

Figure 2: ROC curve for miRNA 627-5p (ROC:Receiver operating characteristic curve; miRNA: micro RNA; AUC:Area under curve)

DISCUSSION

Our study was a cross-sectional comparative study where we compared COVID-19 patients who developed SIRS and COVID-19 patients who did not develop SIRS. In the study conducted by ParasherA,the predominant symptoms of COVID-19 infection were fever, cough, myalgia and breathlessness which was reflected in our study too.[25]Ejaz H et al. identified that the presence of comorbidities like old age, diabetes, cardiovascular disease and chronic respiratory illnesses were results echoed the same results.[26]Keikha R et al. found out that the relative expression of pro-inflammatory miRNAs like miRNA-27b progressively increased with increasing severity of COVID-19.[27] They also analysed the expression of miRNA-27b with respect to one of its target mRNA called PPARS mRNA. The study revealed a progressive increase in the level of miRNA27b with a corresponding decrease in PPARS mRNA with increasing severity of COVID-19. They had included patients without any comorbidities which highlighted the exclusive role of miRNA27b in the pathophysiology of covid-19. Their results correspond with our findings where we got a statistically significant increased expression of miRNA-27b in COVID-19 complicated with SIRS as compared to COVID-19 patients without SIRS. Sardar R et al. have emphasized the role of miRNA 27-b in the upregulation of the ACE-2 receptor, which is identified as the receptor for entry of covid-19 to host cells.[18] This relation is significant as most of the co-morbidities enlisted as risk factors for severe COVID-19 infection like diabetes mellitus, coronary heart disease (CAD) and chronic respiratory illness were associated with ACE-2 receptor dysfunction. The involvement of this receptor also results in an increased rate of complications whenever COVID-19 is associated with co-morbidity. This finding is reflected in our study as well as we have pointed out that most of the patients in the SIRS group have significantly higher rates of comorbidities like diabetes mellitus, hypertension, CAD and chronic respiratory diseases. Li et al. found out that the expression of miRNA 627-5p was significantly decreased in COVID-19 patients with a 2.29 fold downregulation as compared with healthy controls.[28]In our study, we did not have any healthy controls. However we observed that the mean plasma level of miRNA 627-5p was significantly reduced among COVID-19 complicated with SIRS as compared with COVID-19 patients without SIRS. Furthermore, a significant reduction in the expression of miRNA 627-5p was observed in the presence of co-morbidities like diabetes mellitus and hypertension. However other comorbidities, did not show an independent effect on the circulating levels of miRNA 627-5p. In our study, we also did an ROC curve analysis to check whether miRNA-27b and miRNA-627-5p could accurately and precisely predict SIRS in covid-19. The results showed that a cut-off of >26.91 for miRNA-27b gave 67.8% sensitivity and 75% specificity in predicting SIRS with an AUC of 0.76. Similarly for miRNA-627-5p, a cut-off of ≤31.1 gave a sensitivity of 61.4% and a specificity of 69.8% in predicting SIRS with an AUC of 0.68. To the best of our knowledge, we searched the literature for similar studies but could not find any such one to validate our findings.Analysing our study on the background of various previous studies, we concluded that the levels of miRNA-27b were significantly increased ($p<0.001$) among COVID-19 patients complicated with SIRS as compared to COVID-19 patients without SIRS. Also the levels of miRNA-627-5p were significantly decreased ($p=0.02$) among COVID-19 patients complicated with SIRS as compared to COVID-19 patients without SIRS.

CONCLUSION

Our study established an association between circulating levels of miRNA-27b and miRNA-627-5p in patients affected by COVID-19 infection complicated with SIRS. While the levels of up-regulating miRNA-27b were significantly higher in COVID-19 infection with SIRS when compared with COVID-19 infection without SIRS, the levels of down-regulating miRNA 627-5p were significantly lower in COVID-19 infection with SIRS than COVID-19 infection without SIRS. A cut of value of >26.91 for miRNA-27b gave 67.8% sensitivity and 75% specificity in predicting SIRS with an AUC of 0.76 and a cut-off of ≤31.1 for miRNA-627-5p gave a sensitivity of 61.4% and a specificity of 69.8% in predicting SIRS with an AUC of 0.68. In our study we could not establish baseline levels of micro RNAs under investigation in the general population due to widespread COVID-19 infection and the presence of asymptomatic carriers. Also enrolment of more patients and follow-up with repeat samples and reassessment will throw more light on the association of miRNA and COVID-19 infection.

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