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Alterations In Inflammatory Biomarkers Among Egyptian Covid-19 Patients

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Abstract

COVID-19 is a highly contagious disease with pathogenic characteristics; similar to pneumonia. It is caused by the SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), which led to the recent global pandemic. Persistent inflammation in COVID-19 patients is associated with Toll-Like Receptor-4 (TLR4) signaling. Host microRNAs can alter cytokine storm linked to SARS-CoV-2 infection and have been proposed as biomarkers for COVID-19 disease. This study aimed to investigate the expression levels of miR-182 and TLR4 along with their correlation with laboratory biomarkers and clinical features in COVID-19 patients. A total of 100 participants were included, comprising 70 COVID-19 patients and 30 healthy controls. The expression levels of miR-182 and TLR4 were determined using qRT-PCR. Significant differences were observed in laboratory biomarkers, including RBCs, Hemoglobin, Hematocrit (%), MCHC , PLTs, WBCs, INR, basophile , eosinophil, albumin, bilirubin ,LDH , ALT, AST, PT, creatinine, potassium (K) , random blood sugar , CRP, D-Dimer, RDW (%) and partial pressure of oxygen (pO2), and Bicarbonate (HCO_3) between COVID-19 patients and controls. Furthermore, miR-182 expression level was significantly upregulated, whereas TLR4 gene expression level was significantly downregulated in COVID-19 patients compared to controls. Besides, Receiver Operating Characteristic (ROC) curve analysis revealed diagnostic performance for miR-182 and TLR4, with cut off values of 3.2 (AUC =0.986 , 98.57 % sensitivity, 99.8% specificity) and 0.67 (AUC= 0.953 ,90 % sensitivity, 99.99 % specificity), respectively ($p<0.0001$). Based on these results, miR-182 and TLR4 may serve as potential diagnostic biomarkers for COVID-19.

Keywords: miR-182; Toll like Receptor-4 (TLR4); diagnostic biomarkers; COVID-19; SARS-CoV-2; inflammation.

1. Introduction

In December 2019, several acute atypical respiratory illnesses surfaced in Wuhan, China. This spread swiftly beyond Wuhan. It was found that the cause was a new type of coronavirus. The novel coronavirus was named the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2, 2019-nCoV) , because of its close resemblance (~80%) to SARS- CoV, which caused acute respiratory distress syndrome (ARDS) and high mortality during 2002–2003 [1]. Patients reported a variety of symptoms, such as dry cough, fever and tiredness , which are typically minor in 80% of cases. However, if the condition worsens, the patient may have respiratory distress or respiratory failure, necessitating the use of an intensive care unit (ICU) [2]. In severe cases, COVID-19-infected patients may exhibit a "cytokine storm," or hyperinflammation, defined by an excess of proinflammatory cytokines, which is often associated with poor outcomes [3]. The inflammatory response in severe COVID-19 cases begins with the release of cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), which may cause multiple organ failure and acute respiratory distress syndrome, which ultimately leads to death [4].

In order to trigger adaptive immune responses against pathogens, toll-like receptors (TLRs) are crucial innate immune system receptors. They are known pattern recognition receptors because they can identify conserved motifs on pathogens, viral double-stranded RNAs, gram-negative bacteria's lipopolysaccharide (LPS), and pathogen-associated molecular patterns (PAMPs) [5]. Many recent studies have investigated the role of cell surface TLRs, especially TLR4, that are necessary for virus-induced inflammatory responses by identifying molecular patterns [6]. TLR4 plays a crucial role in innate immunity as LPS causes an extensive inflammatory response by activating multiple intracellular signaling pathways, including NF-κB (Nuclear factor kappa B), and releasing numerous proinflammatory cytokines such as IL-6, IL-1β, IL-8, and TNFα [7].

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TLR4/NF-κB pathway activation is linked to many lung diseases, including Acute Lung Injury (ALI) [8]. Previous investigations demonstrated that lung tissue expresses high levels of Toll-Like Receptor-4 and NF-κB after an LPS challenge, and acute inflammatory lung injury was lessened when this pathway was blocked [9]. The spike protein S1 subunit binds to TLR4 in human and murine macrophages, causing pro-inflammatory responses and activating transcription factors such as AP-1 and NF-κB, which then encode proinflammatory cytokines and IFNs [10]. As a result, the body's immune system may respond to this strong binding by inducing exaggerated inflammation, particularly in patients exhibiting serious symptoms [11]. Therefore, the TLR4-spike protein connection would be disrupted by creating competitive TLR4 antagonists as immunopharmacological medications, which could serve as an immunotherapeutic modality for COVID-19 [12].

MicroRNAs (miRNAs) are a family of single-stranded, non-coding small RNAs that range in length from 20 to 23 nucleotides [13]. MiRNAs can mediate mRNA transcriptional repression, degradation, or cleavage by detecting and binding to target mRNAs' 3′untranslated regions (UTRs). Thousands of miRNAs have been discovered to date in a wide range of species, and they are extensively involved in the regulation of numerous diseases as well as the metabolism and tissue development processes[14]. Many disorders have been associated with miRNA-182-5p. In a colon cancer model, for example, overexpression of miRNA182-5p directly reduced vascular endothelial growth factor C, inhibiting colon cancer development, angiogenesis, and lymphangiogenesis [15]. miRNA-182-5P reduced atherosclerosis by inhibiting apoptosis and oxidative stress in an atherosclerosis model generated by oxidized low-density lipoprotein and targeting toll-like receptor-4 [16]. Furthermore, it has been proposed that miR-182-5p acts as a TLR4 sponge to carry out its biological role. MiR-182-5p has been shown to alter damage caused by cerebral ischemia-reperfusion injury via TLR4 sponging [17].

The current study aimed to assess the expression levels of TLR4 and miR-182 in COVID-19 patients and to evaluate their correlations with various clinical and laboratory parameters in order to determine their association with COVID-19 severity.

1.1. Materials and methods

This study contained seventy COVID-19 positive patients were recruited from the ICU of Kasr-Al Ainy Internal Medicine Isolation Hospital, Cairo University. The study was conducted in accordance with the World Medical Association's Code of Ethics (Declaration of Helsinki) and approved by the Kasr-Al Ainy School of Medicine's Research Ethics Committee [code: N-297-2023]. Written agreement were obtained from participants. Both males and females between 32 and 88 years old were included. Those with malignancies, autoimmune disorders , chronic severe pulmonary diseases , chronic renal disorders, and pregnant women were also eliminated.Participants in the study were categorized into: Group 1 consisted of 30 controls of age and gender similar to group 2, which consisted of 70 COVID-19 patients.

All patients underwent a full history and clinical examination, which included age, gender, BMI, and comorbidities (DMII, HTN). The following tests were performed: complete blood count (CBC), kidney function tests (serum creatinine, sodium, and potassium), liver function tests (alanine aminotransferase [ALT] and aspartate aminotransferase [AST], Lactate dehydrogenase [LDH], Albumin, Bilirubin), Random blood sugar [RBS], D-dimer, and CRP levels. A complete blood count (CBC) was done with an automated cell counter (Sysmex KX-21N, TAO Medical Incorporation, Japan). Serum creatinine levels were determined using reagent kits acquired from Diamond Diagnostics (Cairo, Egypt) according to Henry's protocol [18], while sodium and potassium were assessed using a Medica EasyLyte Analyzer (United States).Additionally Liver function tests and RBS were also performed using a Fully Automated Biochemistry Analyzer (Diatron Pictus 500, Hungary).Moreover, D-dimer was assessed with a Sysmex 5100 analyzer (Siemens Healthcare Diagnostics, Marburg, Germany), and CRP was evaluated with a Cobas analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

I- RNA extraction and RT-qPCR

Total RNA, including miRNAs was extracted from serum by the miRNeasy extraction kit (Qiagen, USA).

Reverse transcription was carried out on 100 ng of total RNA in a final volume of 20 µl reverse transcription reaction using the miRNeasy Reverse Transcription kit (Qiagen, USA). Expression of miR-182 and mRNA of TLR-4 was evaluated by quantitative RT-PCR analysis using SYBR green PCR kit and specific primer assays (Qiagen, USA), miScript primer assays [hsamiR-182: (MS00008855), TLR4: TLR4, F:5-ATATTGACAGGAAACCCCATCCA-3, and R: AGAGAGATTGAGT AGGGGCATTT-3. For real-time PCR analysis of each miRNA, Two and a half µl of diluted RT products (cDNA template) were mixed with 7.5 µl RNase free water, 12.5µl QuantiTect SYBR Green PCR master mix and 2.5µl primer Assay (Qiagen, USA), Real-time PCR was performed using a Rotor gene Q Real Time PCR System (Qiagen, USA) The real-time cycler was programmed on 95 ◦C for 15 min, followed by a three-step cycling of 15 s on 94◦C, then 30 s on 55 ◦C and finally 30 s on 70 ◦C. After 40 cycles, analysis of melting curves was done to validate the specific production of the expected PCR product. No control miRNA is known in serum, so as an endogenous control, SNORD 68 was used for miR-182, while GAPDH was used for mRNA -TLR4.The expression levels were evaluated using the ΔCt method, ΔCT was calculated by subtracting the CT values of SNORD 68 or GAPDH from the CT values of the target miR-182 or TLR-4 respectively. miR-182 or TLR-4 expression levels were calculated using the formula 2-ΔΔCT.

1.2. Statistical Analysis

Data Analysis was performed using statistical package of social science (SPSS v.23). Data were subjected to the Kolmogorov-Smirnov test to determine the normality distribution and method of analysis. The data was expressed as mean ± standard deviation (SD). For quantitative parametric data, Independent student t-test was used to compare measure of 2-independent groups as well as One-way ANOVA test was used for comparing more than 2-independent groups with Benferroni Post-Hoc to test significance at p-value <0.05. For quantitative non-parametric data, the Kruskal-Wallis and Mann-Whitney tests were employed to compare more than two independent groups and assess significance at p-value < 0.05. For measuring the correlation between qualitative data, Bivariate Pearson correlation test to find out the association between different groups with a two-tailed to test the significance. Sensitivity and specificity test were developed to evaluate a new test with ROC Curve (Receiver Operating Character). The significance level was set at a P-value of <0.05 [19].

2. RESULTS

2.1. Demographic, Clinical, and Laboratory Features of COVID-19 Patients

In COVID-19 patients versus the control group showed that the age and gender were non significant. In contrast , there was statistically significant changes in systolic and diastolic blood pressures , RBCs, hemoglobin, Hematocrit , MCHC , PLTs, WBCs, INR, Basophile , Eosinophil , Albumin , Bilirubin , LDH , ALT, AST, PT, creatinine, potassium (K) , random blood sugar , CRP , D-Dimer , Glasgow Coma Scale (GCS) , RDW and partial pressure of oxygen (pO2), and Bicarbonate (HCO3) in COVID-19 patients compared to control as shown in (Table 1)

Table (1): Demographic, clinical and laboratory characteristics of COVID-19 patients

Parameters	COVID-19 (N=70)	Control (N=30)	P-value
Age (years)	62.57±11.17	59.90±2.73	0.20
Gender **Female** **Male**	32 (45.72%) 38 (54.28%)	10 (33.3%) 20 (66.7%)	1.00
Systolic blood pressure	142.14±29.60	104.33±10.72	**0.0001***
Diastolic blood pressure	88.06±21.51	70.0±6.94	**0.001***
RBCs (x 10*12/L)	4.35±0.78	4.90±0.001	**0.0001***
Hemoglobin (g/dl)	11.85±2.34	12.32±1.38	**0.003***
Hematocrit (%)	36.21±6.84	38.83±0.83	**0.040***
MCV (Fl/cell)	83.28±6.86	84.01±5.18	0.399
MCH (Pg/cell)	27.15±2.70	27.49±2.22	0.572
MCHC (g/dL)	32.59±1.46	34.15±0.59	**0.0001***
PLTs (x 10^9 cells /L)	227.30±89.35	286.57±58.12	**0.010***
WBCs (x 10^9 cells /L)	10.58±5.81	5.84±1.57	**0.0001***
INR	1.37±0.43	1.01±0.032	**0.0001***
Neutrophil (x 10^9 cells /L)	8.13±4.85	8.34±4.73	0.106
Lymphocyte (x 10^9 cells /L)	1.12±0.66	1.17±0.57	0.787
Monocyte (x 10^9 cells /L)	0.43±0.089	0.51±0.34	0.097

Basophil (x 10^9 cells/L)	0.01±0.011	0.11±0.081	**0.001***
Eosinophil (x 10^9 cells/L)	0.009±0.016	0.116±0.037	**0.001***
ALBUMIN (g/dl)	2.81±0.47	4.41±0.24	**0.0001***
Bilirubin (mg/dl)	0.72±0.51	1.16±0.067	**0.003***
LDH (U/L)	497.41±233.92	159.66±25.11	**0.0001***
ALT (U/L)	62.03±12.56	14.93±5.25	**0.004***
AST (U/L)	80.59±13.75	17.77±6.75	**0.001***
PT (seconds)	19.10±7.52	11.50±0.50	**0.0001***
Creatinine (mg/dl)	2.16±2.05	0.67±0.13	**0.002***
Sodium (Na) (mEq/L)	137.82±8.28	136.43±1.56	0.366
Potassium (K) (mmol/L)	4.32±1.16	3.80±0.20	**0.015***
Random Blood Sugar (mg/dL)	321.53±161.71	133.32±58.20	**0.0001***
CRP (mg/dL)	49.57±25.46	1.60±0.29	**0.0001***
D-Dimer (mg/L)	0.70±0.46	0.09±0.045	**<0.0001***
Glasgow Coma Scale/Score (GCS)	9.86±5.58	14.57±0.72	**0.001***
RDW (%)	14.36±1.21	13.72±0.53	**0.05***
PH	7.30±0.199	7.03±0.73	0.056
Partial pressure of carbon dioxide (PCO2) (mmHg)	46.13±24.0	38.37±3.75	**0.001***
Partial pressure of Oxygen (pO2) (mmHg)	54.61±21.56	87.63±6.05	**0.0001***
Bicarbonate (HCO3) (mEq/L)	20.96±6.29	22.91±3.64	**0.003***

Data are expressed as mean ± SD. P-value < 0.05 is considered statistically significant.

RBCs: Red blood cells, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin Concentration, PLTs: Platelets, WBCs: White blood cells, INR: International normalized ratio, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, LDH: Lactate dehydrogenase, PT: Prothrombin time, CRP: C-reactive protein, RDW (%): Red cell distribution width, PH: Potential of hydrogen, TLR4: Toll-like receptor 4.

Table (2): Serum biomarker levels between the COVID-19 study group and the control group

Biomarkers	COVID-19	Control	p-value
mir-182	8.54±4.28	1.06±0.12	**<0.0001***
TLR4	0.45±0.27	0.98±0.008	**0.0001***

SD is used. * significant (P<0.05)
miR-182 MicroRNA-182 TLR4 Toll Like Receptor 4.

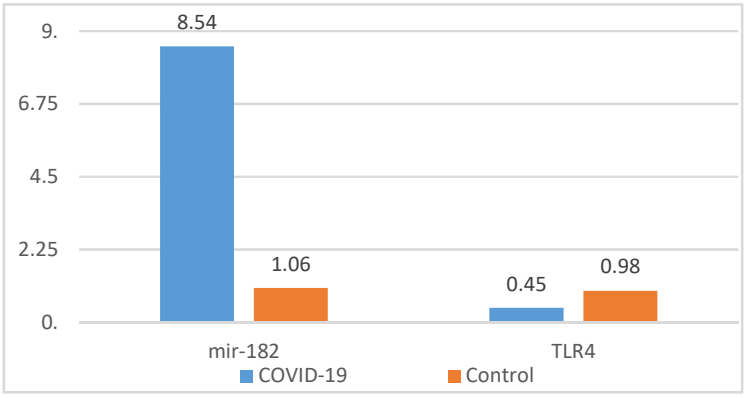

Figure 1: Expression level of MIR-182 and TLR4 among COVID-19 studied group and control group

Table (2) and figure (1) showed that the relative expression level of miR-182 was significantly upregulated (p <0.0001). On the other side, the relative gene expression of TLR4 was significantly downregulated in COVID-19 patients versus controls (p=0.0001)

2.2. Correlation of miR-182 and TLR4 expression levels with various COVID-19 patients characteristics

miR-182 expression levels showed statistically significant correlation to Radiological Society of North America (RSNA) criteria, Consolidation, Incidental-CT-findings, Chest CT distribution, and number of lobes affected. A similar statistically significant correlation was found between TLR4 expression levels and clinical investigations conducted on the patients, including RSNA criteria, Ground glass opacities (GGO), Consolidation, Incidental-CT-findings, Chest CT distribution and number of lobes affected as indicated in Table (3).

Table (3): Relationship of miR-182 and TLR4 expression levels with clinical data of COVID-19 patients

Parameters		MIR-182	p-value	TLR4	p-value
Duration of admission	≤10 days (N=49) >10 days (N=21)	8.69±4.59 8.19±3.51	0.256	0.448±0.27 0.476±0.27	0.670
Heart Rate	<60 bpm (N=3)	7.91±2.31	0.247[a] 0.512[b] 0.180[c]	0.570±0.29	0.446[a] 0.953[b] 0.080[c]
	Between 60 and 100 bpm (N=50)	8.74±4.63		0.458±0.289	
	>100 (N=27)	8.07±3.50		0.431±0.24	
RSNA	Typical (N=62)	8.25±4.34	**0.05**	0.428±0.275	**0.002**
	Indetremined (N=8)	10.78±3.14		0.672±0.186	
Drug treatment	Negative (N=46)	8.56±3.59	0.158	0.495±0.272	0.788
	Positive (N=24)	8.50±5.44		0.382±0.275	
Comorbidity					

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Heart Rate	<60 bpm (N=3)	7.91±2.31	0.247[a] 0.512[b] 0.180[c]	0.570±0.29	0.446[a] 0.953[b] 0.080[c]
	Between 60 and 100 bpm (N=50)	8.74±4.63		0.458±0.289	
	>100 (N=27)	8.07±3.50		0.431±0.24	
Hypertension	Negative (N=26)	8.31±4.11	0.898	0.43±0.27	0.688
	Positive (N=44)	8.67±4.42		0.46±0.28	
Diabetes Mellites	Negative (N=14)	7.86±3.66	0.386	0.432±0.244	0.236
	Positive (N=56)	8.71±4.43		0.46±0.286	
CT Findings					
GGO	Negative (N=51)	8.48±4.17	0.262	0.43±0.28	**0.05**
	Positive (N=19)	8.69±4.67		0.51±0.24	
Consolidation	Negative (N=67)	8.33±4.25	**0.001**	0.448±0.279	**0.016**
	Positive (N=3)	13.11±1.04		0.640±0.151	
mixed GGR and consolidation	Negative (N= 35)	9.06±4.27	0.561	0.499±0.25	0.095
	Positive (N=35)	8.01±4.28		0.413±0.29	
pl effusion	Negative (N=49)	8.34±4.30	0.476	0.451±0.27	0.475
	Positive (N=21)	8.99±4.30		0.468±0.297	
nodal enlargement	Negative (N=54)	8.51±4.19	0.229	0.460±0.266	0.073
	Positive (N=16)	8.65±4.70		0.444±0.321	
Pattern bronchial dilatation	Negative (N=68)	8.57±4.27	0.632	0.457±0.274	0.241
	Positive (N=2)	7.41±5.59		0.430±0.34	
Incidental_CT_findings_	Negative (N=38)	7.3±3.58	**0.001**	0.346±0.25	**0.0001**
	Positive (N=32)	10.33±4.39		0.587±0.24	
Chest CT distribution	Central (N=5)	10.77±1.43	**0.05[x]** **0.008[y]** 0.726[z]	0.640±0.26	**0.05[x]** 0.23[y] 0.871[z]
	Peripheral (N=25)	8.94±4.92		0.438±0.27	
	Mixed (N=40)	8.01±4.03		0.444±0.27	
No. of lobe affected	2 (N=1)	11.90	0.98[i] 0.63[ii] **0.05[iii]** 0.165[iv] 0.082[iiv] 0.548[v]	0.81	0.43[i] 0.26[ii] **0.05[iii]** **0.008[iv]** **0.007[iiv]** 0.444[v]
	3 (N=2)	11.94±1.27		0.71±±0.063	
	4 (N=4)	10.09±3.07		0.420±0.25	
	5 (N=63)	8.28±4.37		0.044±0.27	

Significant p in bold. significant between- a (<60 bpm, 60-100 bpm), b between (<60 bpm, >100), c between (60-100 bpm, >100). significant between chest CT distribution- x between (central, Peripheral) y between (Central, mixed) z between (Peripheral and mixed) - significant between the number of affected lobes-i between (2,3) ii between (2,4) iii between(2,5), iv between (3,4), iiv between (3,5), v between (4,5). RSNA Radiological Society of North America, GGO Ground-glass opacity, Pl Pleural, CT Computed tomography , mixed GGR stands for a mix of ground-glass opacity (G) and reticular pattern (R) and "consolidation" refers to areas of increased lung density that obscure the underlyingvasculature .

2.3. Significant correlations of miR-182 and TLR4 expression levels with serum biomarkers and clinical data of COVID-19 patients

Following the measurement of the bivariate correlation between MIR-182 and serum biomarkers, and clinical information among COVID-19 patients utilizing Pearson correlation (r). The results demonstrated the existence of significant positive correlations with ALT, AST, Creatinine and partial pressure of oxygen (pO2) ($p<0.05$) . On the other side, there are significant negative correlations with Hematocrit , WBCs , Neutrophil , D-Dimer and CXR score ($p<0.05$). Furthermore, there are significant negative correlations between TLR4 and RBCs , Hematocrit , D-Dimer , CO-RADS and CXR score ($p<0.05$) . Additionally, there is a positive correlation between TLR4 and creatinine, and Random blood sugar ($p<0.05$) displayed in Table(4).

Table (4): Correlations of miR-182 and TLR4 expression levels with serum biomarkers and clinical data of COVID-19 patients

Parameters	miR-182 r (p-value)	TLR4 r (p-value)
RBCs	-0.190 (0.115)	-0.298 (**0.012**)
Hematocrit (%)	-0.235 (**0.050**)	-0.253 (**0.035**)
WBCs	-0.258 (**0.031**)	-0.177 (0.143)
Neutrophil	-0.230 (**0.05**)	-0.151 (0.212)
ALT	0.305 (**0.010**)	0.116 (0.340)
AST	0.280 (**0.019**)	0.189 (0.117)
Creatinine	0.243 (**0.043**)	0.334 (**0.005**)
Random Blood Sugar	0.098 (0.419)	0.266 (**0.026**)
D-Dimer	-0.246 (**0.040**)	-0.270 (**0.024**)
Partial pressure of Oxygen (pO^2)	0.291 (**0.014**)	0.166 (0.168)
CO-RADS	-0.214 (0.075)	-0.270 (**0.024**)
CXR score	-0.349(**0.003**)	-0.366(**0.002**)

r Pearson correlation; P values in bold are statistically significant ($p<0.05$).
RBCs: Red blood cells, WBCs: White blood cells, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, CO-RADS coronavirus disease 2019 Reporting and Data System, CXR score: chest X-ray score.

Table (5): Correlation between miR-182 and TLR4 expression levels among COVID-19 patients

Parameters	miR-182 r(p-value)
TLR4	0.792 (**<0.0001**)

miR-182 MicroRNA-182 TLR4 Toll Like Receptor 4

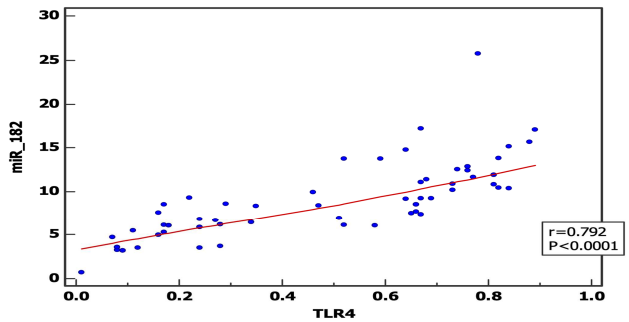

Figure 2: Correlation between miR-182 and TLR4 among COVID-19 patients

Table (5) and figure (2) indicate a significant positive correlation (r=0.792, P<0.0001) between miR-182 and TLR4 expression levels.

2.4. Diagnostic performances of miR-182 and TLR4 among COVID-19 patients

The best diagnostic performance to distinguish between COVID-19 patients and normal controls was found by ROC curve analysis for miR-182, at a cut-off value of 3.2 fold with(AUC = 0.986, sensitivity 98.57%, and specificity of 99.8), Additionally, TLR4 showed significant diagnostic performance at cut-off value of 0.67 fold (AUC = 0. 953, sensitivity= 90.0% and specificity= 99.99%) as shown in (Table 6) and Figures (3,4).

Table (6) : Diagnostic and prognostic performances of miR-182 and TLR4 among COVID-19 patient groups

Biomarkers	AUC	Cut-off value	Sensitivity (%)	Specificity (%)	95% C.I	Accuracy	p-value
miR-182	0.986	3.2	98.57%	99.8%	0.939 to 0.999	99.20%	<0.0001*
TLR4	0.953	0.67	90.0%	99.99%	0.892 to 0.985	95.0%	<0.0001*

* Significant (P<0.05). miR-182 MicroRNA-182, TLR4 Toll Like Receptor 4 , AUC Area under the curve.

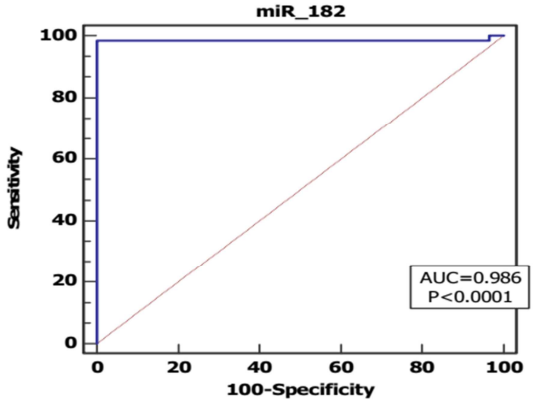

Figure (3): ROC Curve of MIR-182 for COVID-19 patient group

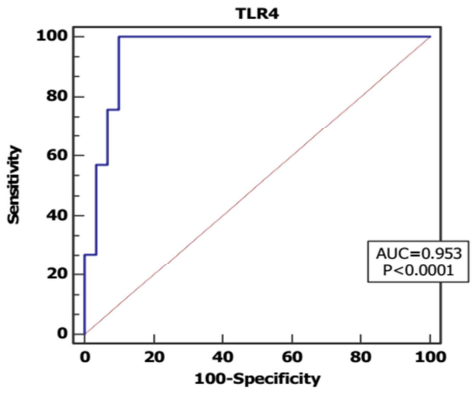

Figure (4): ROC Curve of TLR4 for COVID-19 patient group

3-DISCUSSION

The coronavirus disease 2019 (COVID-19) outbreak has caused significant disruptions due to its rapid spread and high mortality rate, deeming it a worldwide emergency. SARS-CoV-2, the virus that causes COVID-19, was rapidly spreading worldwide. COVID-19 individuals could develop pneumonia, severe acute respiratory distress syndrome (ARDS), and multiple organ failure [20]. The common signs of SARS-COV-2 infection were fever, dry cough, and dysgeusia. These symptoms developed into GIT (gastrointestinal tract) distress, such as vomiting and diarrhea in rare cases. Adaptive immunity and innate immunity are both components of the SARS-CoV-2 protective mechanism. Symptomatic patients showed elevated levels of cytokines in their serum such as tumor necrosis factor (TNF-α), interferon (IFN-γ), interleukin(IL-6), IL-2, IL-4, IL-6, IL-8, and IL-1β in their serum, and so on referred to as a "cytokine storm" that may have been caused by an immune system imbalance [21]. Toll-like receptors (TLRs) are pattern recognition receptors found in several innate immune cells in the alveolar environment, such as monocytes, macrophages, and dendritic cells. In patients with COVID-19, these receptors cause systemic inflammation [22].

Toll-Like Receptor-4 is an innate immune receptor that has the strongest interaction with the SARS-CoV-2 virus's spike glycoprotein, inducing immune system overreaction and systemic inflammation. COVID-19 individuals have been diagnosed with myocarditis, ARDS, and organ damage [23]. TLR-4 stimulates cytokine release, particularly IL-6 and TNF-a, resulting in cytokine storm and acute respiratory distress syndrome (ARDS) [24].

The current study indicated that the expression level of TLR4 mRNA was significantly lower in COVID-19 patients compared to controls. This result is consistent with a recent study, which was the first to demonstrate that the TLR4 signaling pathway is downregulated in peripheral blood mononuclear cells (PBMCs) of COVID-19 patients [25]. However, their result was in contradiction with other researches that reported higher TLR4 activation and downstream signaling [26,27] . A study determined that the improvement of immoderate TLR4-mediated innate signaling may be helpful for the therapy of COVID-19, suggesting that downregulating TLR4 pathway activation might work as an effective adaptability with superabundant inflammatory reaction [26]. In the corresponding research that is compatible with our result demonstrated that TLR4 pression was shown to be lower in PBMCs of COVID-19 as compared to Non-COVID-19 patients, which contradicts the high levels

of circulating IL-6 that were discovered in their COVID-19 patients [25]. One explanation was endotoxemia, which is elevated in COVID-19 patients [28], which has a negative feedback loop on TLR4 receptor activation and expression [29]. The intestinal permeability may be increased by binding of SARS-CoV-2 to ACE2 (Angiotensin converting enzyme 2) in the gut [30]. This enables the release of recognized TLR4 ligands, such as LPS. Excessive LPS levels may reduce TLR4 expression and activation, increasing tolerance to LPS [29].

Another investigation illustrated that hypoxia decreased the expression of TLR4 in many cell types and organs, such as cultured pulmonary artery endothelial cells. They observed that in cultivated pulmonary artery endothelial cells, extended(48–72 h) hypoxic exposure resulted in lower expression of TLR4 [31]. Their findings were in good accordance with those of other results on SV40-HCEs cultivated under 2% O2 for 48 hours before being exposed to lipopolysaccharides; in addition, the downregulated expressions of TLR4 in hypoxic HCECs (Hepatic cystic echinococcosis) are in line with certain in vitro and in vivo findings [32]. A study revealed that cells exposed to 5% O2 had lower NF-κB activation and cytokine release than cells exposed to 18% O2, suggesting that THP-1 (human leukaemia monocytic cell line) macrophages are better ready to respond to an inflammatory signal under normoxic circumstances [33]. Their results imply that moderate hypoxia may be able to modulate TLR4 expression, which in turn may control the inflammatory response [34]..Furthermore, research found that severe chronic obstructive pulmonary disease (COPD) was significantly more closely associated with decreased TLR4 expression than less severe COPD [35]. However, A study contradicted our result, demonstrating that TLR4 expression level in the blood of severe and moderate COVID-19 patients was shown to be significantly higher than in controls [36].

Inflammation had been linked to aberrant miR-182 expression [37]. Additionally, it has been shown that miR-182 contributes to the development of tumors and their metastases by inhibiting the transcription of proinflammatory genes that are triggered by TNF-α [38]. Moreover, it affects the clonal growth of activated helper T cells and adversely controls immunological responses in atherosclerosis by targeting TLR4 [16]. Importantly, miR-182 may influence the immune response, apoptosis, inflammation, and cell proliferation in relation to BM-MSCs' (Bone marrow mesenchymal stem cells) therapeutic effects in ALI [39]. Research on alcoholic hepatitis (AH), which is more prevalent in drinkers who also have underlying alcoholic liver disease (ALD), indicates that miR-182 can cause upregulation of the expression of inflammatory mediators in patients with AH, increasing liver cell damage [40]. Furthermore, additional research revealed that miR-182-5p protects against cerebral and hepatic ischemia-reperfusion damage. By targeting TLR4, an intrinsic immune signaling receptor, miR-182-5p inhibits the release of proinflammatory cytokines like TNF-α and IL-6, thereby mitigating the side effects of cerebral and liver ischemia-reperfusion injuries, including the suppression of the inflammatory response [17].

Our data indicated that the expression level of miRNA-182 increased eight times more in COVID-19 patients than in healthy individuals (Table 2), similarly Arghiani et al. [41] showed that the miR 182 expression was elevated in COVID-19 patients . Also it was reported that HMPV (Human metapneumovirus) causes significantly upregulation of miR-182-5p expression in moDCs (monocyte-derived dendritic cells) [42], where they highlighted the overexpression of miR-182-5p [43] , which may contribute to the imbalance of Th17 (Type 17 helper) cells [44] , also releasing of cytokines related to a Th2-like profile is stimulated by miR-182 , causing increased secretion of cytokines such as IL-13 and IL-5 and recruitment of neutrophils in the lung [45], and protects against several intracellular infections by boosting the expression of interferons and other proinflammatory cytokines [46]. Another study found that miR-182-5p expression was increased in COPD (chronic obstructive pulmonary disease) [47].Additionally, a study found that miR-182-5p was overexpressed in mice induced with bleomycin (BLM)-induced fibrotic mouse model. They also discovered that blocking miR-182-5p could help to reduce fibrosis. Inhibiting miR-182-5P could be a viable therapy option for idiopathic pulmonary fibrosis IPF or normal pulmonary fibrosis [48]. Besides, the overexpression of miR-182-5p in RAW264.7 cells suppressed pro-inflammatory cytokines, hence inhibiting the inflammatory response generated produce its anti-inflammatory effects. These findings imply that miR-182-5p is a viable therapeutic target for ALI , which could be beneficial for ALI patients' prognosis [49]. Additionally according to earlier research, TLR4 is a miR-182-5p target gene [16,17] , that was congruent with our result showed a correlation between miR-182 and TLR4 that was significant (Table 5) . A study found that upregulating miR-182-5p inhibited LPS-induced activation of the TLR4/NF-κB pathway in RAW264.7 cells. Based on the available information, miR-182-5p may inhibit the TLR4/NF-κB pathway, thus reducing the inflammatory response triggered by LPS [49] .

In our study ROC curve analysis showed that AUC, sensitivity and specificity for miR-182 were 0.986 , 98.57%, and 99.8% , respectively with $p<0.0001$ and for TLR4 were 0.953 ,90% ,and 99.99% , respectively with $p<0.0001$ So their expression can be changed in individuals infected with covid-19 even in the early stages of the disease, indicating that they are specific for covid-19 and may have a diagnostic ability for covid-19 (Table 6) , Furthermore, Another studies were in compatible with our results indicated that miR-182-5p shown an exceptional combinatory prediction capacity (AUC 0.91, $p < 0.0001$) in distinguishing between mild COPD patients and healthy [47]. While TLR4 demonstrated moderate diagnostic performance as determined by the AUC for sTLR4 (AUC = 0.655) with sensitivity 27.16% and specificity 68.97%) for COVID-19 patients [50] .

TLRs have been linked to acute kidney injury, which correlates with renal disease severity and inflammatory indicators [51]. Furthermore, Rivero et al. and Choudhury and Mukherjee showed that TLR4 can enhance chemokine production in renal tubule epithelial cells [52]. A study found a positive correlation between TLR4 and creatinine ($p < 0.01$) [36].

Our study found that COVID-19 patients had significantly higher systolic and diastolic blood pressure compared to the control group (p value < 0.0001, 0.001), which was consistent with another research, which implies that COVID-19 raises both the diastolic and systolic blood pressure and may result in newly developed hypertension [53]. Additionally, our analysis revealed that COVID-19 patients had significantly decreased levels of RBCs, Hemoglobin, Hematocrit, MCHC and PLTs. Moreover, WBCs , INR , LDH , CRP , D-dimer , RDW, and PT were significantly increased than control group , These results were in accordance with research conducted by [54] . Albumin and bilirubin levels were decreased , while ALT , AST and Creatinine were increased as compared to control, Our findings were in harmony with an investigation made by [55]. Nakanishi H et al. observed that potassium level was elevated in COVID-19 patients as compared to the control group [56], which was consistent with our analysis.

SARS-CoV-2 might affect potassium levels because of the action of furin, a type of proprotein protease, which is responsible

for SARS-CoV-2's greater infectivity when compared to other coronaviruses [57]. Furin cleaves the virus's Spike protein, allowing it to attach to ACE2 [58]. Furin activates ENaC (ENaC Epithelial sodium channels) by cleaving its α-subunit at two locations [59]. Recent research [60] suggests that the furin-cleavable peptide sequence on the ENaC α-subunit corresponds to the furin-cleavage region of the SARS-CoV-2 spike protein. Furin is required for ENaC function and expression; however, in this case, it is hijacked by SARS-CoV-2, resulting in competition for furin utilization following virus infection [61]. Without furin-mediated cleavage, ENaC's efficacy is likely to decline. This may have an adverse effect on epithelial cells and disrupt water or electrolyte equilibrium, resulting in higher serum potassium levels. ENaC dysfunction can be better understood by investigating its genetic abnormalities. Pseudo-hypoaldosteronism occurs when ENaC subunits lose a function mutation, resulting in hyperkalemia, metabolic acidosis, and hypertension [62]. Furthermore, ENaC is necessary for lung fluid clearance, therefore, lower ENaC activity may explain why COVID-19 patients sometimes die from severe pulmonary edema [63].

4- Conclusion. In COVID-19 patients, miR-182 expression was observed to be significantly elevated, although TLR4 expression was significantly decreased as compared to normal individuals. There is a significant correlation between miR-182 and TLR4, Additionally, miR-182 and TLR4 serum expression levels could be used as diagnostic markers for COVID-19, according to the results of the ROC curve. Future research is required to verify these findings on a large group of patients and to prove the predictive significance of these parameters as a prospective target for immunotherapy in patients with COVID-19 infection.

5-Conflicts of interest

There is no conflicts to declare

6-Formatting of funding sources

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