Send Orders for Reprints to reprints@benthamscience.net

Infectious Disorders - Drug Targets, **XXXX**, *XX*,

RESEARCH ARTICLE

Human ACE-2, MCP1 and micro-RNA 146 as Novel Markers for COVID- 19 Affection and Severity

Amal Ahmed Mohamed[1], Sherief Abd-Elsalam[2,*], Ahmed Abdelghani[3], Mohamed Badr Hassan[3], Doaa Ghaith[4], Omnia Ezzat[5], Dalia Ali El-damasy[6], Norhan Nagdi Madbouli[7], Mohmoud Hamada[8], Mohamed Abdel Khalik Elkady[9], AL-Shaimaa M. AL-Tabbakh[10], Kareman Ahmed Ebrahim Eshra[11] and Nivin Baiomy[12]

[1]Biochemistry and Molecular Biology Department, National Hepatology & Tropical Medicine Research Institute, Cairo, Egypt; [2]Department of Tropical Medicine and Infectious Diseases, Faculty of Medicine, Tanta University, Tanta, Egypt; [3]Internal Medicine Department, Faculty of Medicine, Cairo University, Cairo, Egypt; [4]Clinical and Chemical Pathology Department, Faculty of Medicine Cairo University, Cairo, Egypt; [5]Biochemistry Department, Faculty of Pharmacy, Egyptian Russian University, Cairo, Egypt; [6]Microbiology Department, Faculty of Pharmacy, Egyptian Russian University, Cairo, Egypt; [7]Department of Internal Medicine and Nephrology, Ain Shams University, Cairo, Egypt; [8]Internal Medicine Department, Faculty of Medicine, Benha University, Cairo, Egypt; [9]Gastroenterology and Hepatology Department, Theodor Bilharz Research Institute, Warraq Al Hadar, Egypt; [10]Medical Microbiology and Immunology, Faculty of Medicine Benha University, Benha, Egypt; [11]Medical Microbiology and Immunology Department, Faculty of Medicine, Tanta University, Tanta, Egypt; [12]Clinical Pathology Department, Faculty of Medicine, Tanta University, Tanta, Egypt

Abstract: ***Background & Aims:*** Coronavirus disease - 2019 (COVID-19) is a major pandemic that causes high morbidity and mortality rates. Aim of this study: to detect the relations between many risk factors, ACE-2, MCP-1, Micro RNA 146 gene expression, and COVID-19 infection and disease severity.

Methods: This study was carried out on 165 cases of COVID-19 and 138 controls. ACE2 and MCP1 levels were measured in COVID-19 cases and control by ELISA and micro-RNA-146 expression by PCR.

Results: We found an increased blood level of ACE2 and MCP1 in COVID- 19 patients than in healthy persons and a significant down-regulation of micro-RNA 146 gene expression in cases than in controls. There was a significant correlation between increased blood level of ACE2, regulation of micro-RNA 146 gene expression and severity of lung affection, a significant correlation was found between increased blood level of MCP1 and thrombosis in COVID-19 patients. Neurological complications were significantly correlated with more viral load, more ACE2 blood level, and down regulation of micro RNA146 expression.

Conclusion: High viral load, increased blood level of ACE2, and down-regulation of micro-RNA 146 expression are associated with more severe lung injury and the presence of neurologic complications like convulsions and coma in COVID-19 Egyptian patients.

ARTICLE HISTORY

Received: December 10, 2021
Revised: February 10, 2022
Accepted: March 02, 2022

DOI:
10.2174/1871526522666220829153042

Keywords: COVID-19, viruses, thrombosis, ACE2, MCP1, micro RNA 146.

1. INTRODUCTION

Coronavirus disease - 2019 (COVID-19) is a major disease that firstly occurred in Wuhan, China, in December 2019, it started as an outbreak and then progressed to a pandemic [1]. It is considered a new zoonotic viral disease known as the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) caused by (2019-nCOV) [2]. All these coronaviruses are enveloped positive-strand RNA viruses that are isolated from bats that can be transferred from animals to humans, human to human, and animals to animals [3]. They share a similarity in the clinical symptoms in addition to specific differences that have been recently observed [4, 5]. The clinical presentation of the disease is variable but usually starts in the first seven days with mild symptoms such as fever, cough, shortness of breath, and fatigue [6]. Later on, critical symptoms may develop in some patients involving dyspnea and pneumonia that need special patient care in ICUs, especially for the need for mechanical ventilation to decrease death from respiratory fail-

*Address correspondence to this author at the Department of Tropical Medicine, Faculty of Medicine, Tanta University, Tanta, Egypt, El-Bahr Street, Tanta, Egypt; E-mails: sherif.abdelbaky@med.tanta.edu.eg; sherif_tropical@yahoo.com

ure [7]. Therefore, COVID - 19 is a major health problem that needs early and rapid diagnosis to start effective treatment as early as possible to prevent the occurrence of critical conditions which need costly special care and may lead to death especially with no specific symptoms to diagnose coronavirus infection. The accurate testing depends on the detection of the viral genome using the reverse transcription polymerase chain reaction (RT-PCR) analysis which is expensive and time consuming [1-8]. Both 2019-nCoV and SARS-CoV enter the host cell *via* the same receptor, angiotensin-converting enzyme 2 (ACE2) [9] and many studies showed that it is the host receptor for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [10]. Also, some studies showed that SARS-CoV-2 can enter ACE2 expressing cells only, but not cells without ACE2 [11]. The receptor-binding domain of the spike glycoprotein binds to the tip of subdomain I of ACE2, Membrane fusion of the virus and the host cell is activated after binding, and viral RNA is subsequently released into the cytoplasm, establishing infection [12]. ACE2 is mainly expressed on type II alveolar epithelial cells but weakly expressed on the surface of epithelial cells in the oral and nasal mucosa and nasopharynx, indicating that the lungs are the primary target of SARS-CoV-2. Moreover, ACE2 is highly expressed on myocardial cells, proximal tubule cells of the kidney, and bladder uroepithelial cells, and is abundantly expressed on the enterocytes of the small intestine, especially in the ileum. The cell-free and macrophage phagocytosis-associated virus may spread from the lungs to other organs with high ACE2 expression through blood circulation so ACE2 may be involved in multi-organ affection associated with COVID-19 infection [13]. Monocyte chemo attractant protein-1 (MCP-1) belongs to the C-C chemokine family and is a powerful monocyte chemotactic factor that is constitutively produced or induced by oxidative stress, cytokines, or growth factors. Monocytes and macrophages are the main sources of MCP-1, which regulates the migration and infiltration of monocytes, memory T cells, and natural-killer (NK) cells [14]. It is one of the cytokines related to thrombosis and some studies reported that the culture medium of pulmonary endothelial cells from patients with chronic thromboembolic pulmonary hypertension contains a high level of FGF-2 (fibroblast growth factor receptor), IL-1β, IL-6 and MCP-1 [15]. Several studies found that MCP-1 expression levels were higher in patients with COVID-19 and even higher among those admitted to the ICU [16]. It was also found that MCP-1 expression increases rapidly in the early acute phase of infection and then progressively decreases as the disease advances [17]. Xiong *et al*. detected elevated levels of MCP-1 in bronchoalveolar lavage fluid from COVID-19 patients and found it to be associated with the pathogenesis of the SARS-CoV-2 [18]. Also, some studies detected elevated levels of MCP-1 in the lung tissue of COVID -19 patients [19]. Therefore, monitoring MCP-1 levels early and acting upon any elevation might be a viable strategy to prevent COVID-19 from progressing from mild to severe. MicroRNAs (miRNAs) are a class of non-coding RNAs that regulate endogenous gene expression at the post-transcriptional level [20]. There are over 2,600 human miRNAs listed in the miRNA registry [21], which are estimated to collectively regulate 60% of all human protein-coding genes [22]. The foreign invasion alters the expression profile and functioning of various miRNAs, which are directly involved in the pathogenesis of infections and diseases [23]. Micro-RNA 146 worked by regulating the NFκB pathway, directing the expression of IRAK1 (Interleukin-1 receptor-associated kinase 1) and TRAF6 (Tumor necrosis factor receptor associated factor 6) in macrophages. IRAK1 and TRAF6 are adaptor molecules of the MyD88-dependent signaling pathway. Stimulation of TLR stimulated activator protein 1 (AP-1) and NF-κB transcription factors mediated various immune responses [24]. Micro-RNA146 targeted STAT1 (Signal transducer and activator of transcription 1) to cause Th1 effector cell differentiation, and suppress Th1 responses [25], targeted IRAK1, and TRAF6 to produce Negative regulator of the IFN pathway and immune response, reduce inflammatory cytokine production [26], targeted (AP)-1, IL-2 to produce Immune cell activation and cytokines production, a negative regulator of adaptive immunity [27]. In the current study, we aimed to detect the relationship between many risk factors with probability of infection with COVID-19 and to evaluate the correlations between blood level of ACE-2, MCP-1, expression of micro-RNA 146, lung disease severity and occurrence of neurologic complications in covid19 Egyptian patients.

2. MATERIALS AND METHODS

2.1. Subjects

This study was a cohort study, it was carried out in 165 cases and 138 controls, the cases were patients diagnosed with COVID -19 at the contagious disease control center (CDCC) and the controls were healthy participants. The duration of the study was 6 months from August 2020 to February 2021. This study was performed in the Clinical Pathology Department and Department of Tropical Medicine and Infectious diseases, Faculty of Medicine, Tanta University, Egypt. Patients' inclusion Criteria were OVID-19 infection confirmed by PCR Cobas 6800 Roch and exclusion criteria were pregnant or lactating women. An informed consent was obtained from all participants in this study. The ethical committee approval number was 34830/8/21 for this study.

2.2. Methods

All patients were clinically evaluated as the following: Complete history taking as regard age, sex, date of admission, date of onset of symptoms, history of hypertension, diabetes mellitus (DM), any cardiac problem, any renal problem, any chronic disease and a full clinical examination taken from every participant. According to the triage protocol of the ministry of health and population, a confirmed case is defined as a person with laboratory confirmation of COVID-19 infection, irrespective of clinical signs and symptoms. Molecular testing (PCR) with a deep nasal swab is the current test of choice for the diagnosis of acute COVID- 19 infection, which was done by Cobas 6800 equipment. Severity assessment: According to clinical, laboratory, and imaging data: Mild = mild symptoms, normal imaging, Moderate = positive imaging. Spo2 92%, Severe = Sp O_2 < 92%, Pa O_2 / Fi O_2 < 300, respiratory rate > 30 breath / minute, or lung infiltrates > 50% illness, Critical illness = Respiratory failure / septic shock, and / or multi-

organ dysfunction in this study. We measured the severity of COVID-19 infection by CT- severity score which divided the cases according to the severity of chest CT.

Body mass index (BMI), complete blood picture (CBC), serum ferritin, D-Dimer, C reactive protein (CRP), HbA1C, fasting blood glucose (FBS), post prandial blood glucose (PPBS), LDH, INR and RT-PCR for COVID-19. Quantitative determination of Human ACE2 concentrations was done by Sandwich ELISA according to the manufacturer's instructions. (Bio Techne Ltd. Catalog number NBP2-78734). Quantitative determination of MCP 1 concentrations by Sandwich ELISA according to manufacturer's instructions. (Bio-Techne Ltd.: Catalog number DCP00). Determination of serum level of miR-146 by RT-qPCR by a miRNeasy Mini Kit;cat no: 217004 (Qiagen, Hilden, Germany).

- Determination of serum level of miR-146 by RT-qPCR:

1. Total RNA extraction and purification was done using a miRNeasy Mini Kit;cat no: 217004 (Qiagen, Hilden, Germany) according to the manufacturer's protocol.
2. Reverse transcription: cDNA was synthesized by reverse transcription reaction using TaqMan MicroRNA Reverse Transcription Kit;cat no: 4366596 (Applied Biosystems, Foster city, USA) and the thermal cycler (Quanta Biotech).
3. Gene expression analysis: The quantification of miR-146 levels was amplified from cDNA using TaqMan universal Master Mix and TaqMan assay (has-miR-146; Catalog no: 4427975; Assay ID: 002623). The RNU49 was used as a housekeeper gene (RNU49; Cat no: PN4427975; ID: 001005). All samples were analyzed using the 5 plex Rotor- Gene PCR Analyzer (Qiagen, Germany). The 2ΔΔCt method was conducted for the analysis of gene expression levels using TaqMan microRNA Control Assays RNU49as an endogenous reference control for normalization purposes.

Statistical analysis of the data: Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Categorical data were represented as numbers and percentages. The chi-square test was applied to investigate the association between the categorical variables. Alternatively, Monte Carlo correction test was applied when the expected cell counts were less than 5. For continuous data, they were tested for normality by the Kolmogorov- Smirnov. Distributed data were expressed as a range (minimum and maximum), mean, standard deviation and median Student t-test was used to compare two groups for normally distributed quantitative variables while ANOVA was used for comparing the four studied groups and followed by Post Hoc test (Tukey) for pairwise comparison. On the other hand, Mann Whitney test was used to compare two groups for not normally distributed quantitative variables while Kruskal Wallis test was used to compare different groups for not normally distributed quantitative variables and followed by the Post Hoc test (Dunn's for multiple comparisons test) for pairwise comparison. The significance of the obtained results 0.05.

3. RESULTS

Table **1** shows that among 165 cases, 102 were male and 63 were female. When evaluating the blood parameters between patients and controls such as age, BMI, FBS, PPBS, HbA1c, it is suggested that impaired fasting glucose and glucose intolerance might be a risk factor for COVID-19 infection. WBC and neutrophil percentage (relative N count) was more in the patient than in the healthy control. Lymphocyte percentage (relative L count) was less in cases than in healthy control. The platelet count mean was higher in cases than in controls. Monocyte percentage (M relative count) was higher in the patient group than in the healthy group. For inflammatory markers, ferritin and LDH, serum ferritin was higher in the patient than in the control group. LDH was higher in cases than in control. CRP was much higher. ESR was higher in patients than in control. D-Dimer was higher in cases than in controls. INR was higher in cases than in control indicating that patients with COVID-19 are more liable for thrombosis formation than healthy personnel.

Table **2** describes the comparison between COVID19 patients and healthy persons as regards our markers of interest. ACE2 level was higher in patients than in healthy, 0.77 ±0.63 and 0.29 ±0.24, respectively. As regards MCP1 which is a cytokine and also considered a marker for increased thrombosis liability, it was higher in cases than in control, 312.79 ± 224.53 and 148.34 ± 18.95, respectively. Micro-146 RNA gene expression was less in cases than in control, 1.91 ±1.28 and 2.99 ± 2.0, respectively. There was a significant down regulation of micro-146-RNA in COVID- 19 patients compared with healthy persons.

Table **3** shows the distribution of the studied cases according to different parameters, chest CT severity score divided the cases into 4 scores from number 1 to number 4, cycle threshold of RT- PCR for COVID-19, neurological manifestations (taste impairment, coma, smell impairment, Headache, convulsion), different symptoms of COVID-19 infection (vomiting, Dyspnea, Headache, abdominal pain, expectoration, fatigue, nausea, myalgia, sore throat, fever Dizziness). Most patients are mild to moderate, 41.8% + 29.1% of cases belong to score 1+ 2 while only 29% of cases are in score 3+4. Many patients complained of mild neurological manifestations like taste impairment, smell impairment, and headache while less percentage of patients complained of more complications, coma and convulsions. The most incident symptom was fatigue, 44.2% of all cases followed by dyspnea 23.6 %.

Table **4** shows that cases are divided into 4 groups (score 1, score 2, score 3, and score 4) according to the severity of chest CT in cases of COVID-19 infection, the less cycle Review Version threshold (ct) of RT-PCR the more viral load and thus the more lung affection. The (ct) was 36.90 ± 2.04 in score 1 and 28.71 ± 0.75 in score 4. So there was a significant inverse correlation between the cycle threshold of RT-PCR of COVID-19 and chest CT severity score.

As regards neurological impairment, 58.3% of score 4 patients while 0.0% of score 1 patients had convulsions. About 33.3% of score 4 groups while 11.6% of score 1 group had coma. Many of score 1 group had taste impairment, smell impairment & headache (39.1%, 31.9% & 17.4% respectively). So, neurological complications are more prevalent with increasing lung affection and more viral load. ACE2 was 0.29 ± 0.13 in score 1 and 1.89 ± 0.39 in

Table 1. Comparison between the two studied groups according to demographic data and laboratory investigations.

	Cases (n = 165)	Control (n = 138)	Test of Sig.	P
Gender				
Male	102 (61.8%)	78 (56.5%)	χ^2= 0.874	0.350
Female	63 (38.2%)	60 (43.5%)		
Age (years)				
Mean ± SD.	46.37 ± 14.82	38.54 ± 9.80	t= 5.502*	<0.001*
BMI (kg/m^2)				
Mean ± SD.	28.92 ± 4.65	27.48 ± 4.56	t= 2.702*	0.007*
WBCs				
Mean ± SD.	3.40 ± 1.23	7.36 ± 4.51	U= 2309.0*	<0.001*
PLT				
Mean ± SD.	280.59 ± 81.54	229.05 ± 59.44	t= 6.349*	<0.001*
Neutrophil				
Mean ± SD.	68.72 ± 3.76	56.31 ± 5.94	t= 21.235*	<0.001*
Lymphocyte				
Mean ± SD.	24.34 ± 4.35	37.35 ± 6.35	t= 20.401*	<0.001*
Monocyte				
Mean ± SD.	7.06 ± 2.63	6.36 ± 2.67	t= 2.306*	0.022*
Ferritin				
Mean ± SD.	209.05 ± 182.52	110.13 ± 36.50	U= 8919.50*	0.001*
D-Dimer				
Mean ± SD.	1.32 ± 0.51	0.30 ± 0.20	U= 460.0*	<0.001*
CRP				
Mean ± SD.	15.77 ± 13.86	4.97 ± 3.03	U= 3449.50*	<0.001*
HbA1c				
Mean ± SD.	5.63 ± 1.43	3.29 ± 0.82	t= 17.706*	<0.001*
FBS				
Mean ± SD.	101.82 ± 12.93	95.40 ± 11.0	t= 4.609*	<0.001*
PPBS				
Mean ± SD.	121.59 ± 14.13	114.91 ± 13.70	t= 4.160*	<0.001*
LDH				
Mean ± SD.	192.88 ± 26.10	182.80 ± 31.33	t= 3.054*	0.002*
1st hr. ESR				
Mean ± SD.	18.54 ± 13.79	12.04 ± 6.59	U= 8457.0*	<0.001*
2nd hr. ESR				
Mean ± SD.	43.08 ± 31.84	25.91 ± 11.70	U= 7893.50*	<0.001*
INR				
Mean ± SD.	1.23 ± 0.20	1.04 ± 0.10	t= 10.686*	<0.001*

SD: Standard deviation, t: Student t-test, U: Mann Whitney test, χ^2: Chi square test. p: p value for comparing between the studied groups, *: Statistically significant at $p \leq 0.05$.

Table 2. Comparison between the two studied groups according to markers.

	Cases (n = 165)	**Control (n = 138)**	**U**	**P**
ACE2				
Mean ± SD.	0.77 ± 0.63	0.29 ± 0.24	5327.50*	<0.001*
MCP1				
Mean ± SD.	312.79 ± 224.53	148.34 ± 18.95	1426.50*	<0.001*
Micro146				
Mean ± SD.	1.91 ± 1.28	2.99 ± 2.0	7827.50*	<0.001*

SD: Standard deviation U: Mann Whitney test.
p: p value for comparing between the studied groups.
*: Statistically significant at $p \leq 0.05$.

Table 3. Distribution of the studied cases according to different parameters (n = 165).

	No. (%)
Chest CT Severity score	
Score 1	69 (41.8%)
Score 2	48 (29.1%)
Score 3	24 (14.5%)
Score 4	24 (14.5%)
RT-PCR cycle threshold (ct)	
Mean ± SD.	34.61 ± 3.18
Median (Min. - Max.)	35.0 (28.0 - 39.0)
Neurological manifestations	
Taste impairment	50 (30.3%)
Coma	29 (17.6%)
Smell impairment	44 (26.7%)
Headache	25 (15.2%)
Convulsions	17 (10.3%)
Other symptoms	
Vomiting	11 (6.7%)
Dyspnea	39 (23.6%)
Headache	1 (0.6%)
Abdominal pain	4 (2.4%)
Expectoration	1 (0.6%)
Fatigue	73 (44.2%)
Nausea	2 (1.2%)
Myalgia	15 (9.1%)
Sore throat	16 (9.7%)
Fever	2 (1.2%)
Dizziness	1 (0.6%)

SD: Standard deviation.

Table 4. Relation between chest CT Severity score with neurologic manifestations and laboratory finding in cases group (n = 165).

	Score1 (n = 69)	**Score2 (n = 48)**	**Score3 (n = 24)**	**Score4 (n = 24)**		
BMI (kg/m^2)						
Mean ± SD.	28.43^a ± 4.76	29.10^a ± 5.02	28.83^a ± 3.90	30.0^a ± 4.33	F= 0.705	0.550
RT-PCR ct						
Mean ± SD.	36.90^a ± 2.04	34.58^b ± 1.16	34.0^b ± 2.0	28.71^c ± 0.75	F= 143.566*	<0.001*
Neurological						
Taste impairment	27 (39.1%)	16 (33.3%)	5 (20.8%)	2 (8.3%)	χ^2=9.258*	0.026*
Coma	8 (11.6%)	6 (12.5%)	7 (29.2%)	8 (33.3%)	χ^2=8.461*	^{MC}p=0.034*
Smell impairment	22 (31.9%)	17 (35.4%)	5 (20.8%)	0 (0.0%)	χ^2=11.985*	0.007*
Headache	12 (17.4%)	8 (16.7%)	5 (20.8%)	0 (0.0%)	χ^2=6.317	^{MC}p=0.096
Convulsions	0 (0.0%)	1 (2.1%)	2 (8.3%)	14 (58.3%)	χ^2=49.200*	^{MC}p<0.001*
ACE2						
Mean ± SD.	0.29 ± 0.13	0.76 ± 0.42	1.03 ± 0.41	1.89 ± 0.39	H= 116.256*	<0.001*
MCP1						
Mean ± SD.	316.36 ± 237.69	289.40 ± 183.55	291.96 ± 179.64	370.13 ± 293.68	H= 0.505	0.918
Micro146						
Mean ± SD.	2.54 ± 1.35	1.79 ± 0.83	1.86 ± 1.02	0.36 ± 0.27	H= 58.380*	<0.001*

SD: Standard deviation, χ^2: Chi square test, MC: Monte Carlo.
F: F for ANOVA test, Pairwise comparison bet. Each 2 groups was done using Post Hoc Test (Tukey).
H: H for Kruskal Wallis test, Pairwise comparison bet. Each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test).
p: p value for comparing between the studied scores.
*: Statistically significant at $p \leq 0.05$.
Means/ Medians with Common letters are not significant (*i.e.* Means/ Medians with Different letters are significant).

score 4, P value was 0.001*, so there was a significant increase in ACE2 in cases with severe lung affection and more viral load. MCP-1 was 316.36 ± 237.69 in score 1 and 370.13 ± 293.68 in score 4 of CT severity score, the P value was 0.918, so there was insignificant relation between increased MCP-1 in cases of COVID-19 infection and severity of lung affection according to CT severity score.

Micro 146RNA gene expression was 2.54± 1.35 in score 1 and 0.36 ± 0.27 in score 4, so there was down-regulation of micro 146 RNA gene expression in severe cases of COVID-19 infection according to CT- severity score.

Table **5** shows that 17 patients had convulsions + 29 patients with coma, these patients had more 19 viral load (29.71±2.64 and 33.69±3.44 respectively), more ACE2 blood levels (1.62±0.60 and 1.02±0.74 respectively), and more down regulation of Micro RNA146 (0.66±0.82 and 1.47±1.18 respectively) in comparison with patients with mild neurologic symptoms like taste impairment, smell impairment and headache who had a less viral load, less ACE2 blood level and less down regulation of microRNA146 than patients with convulsion and coma. MCP1 blood level was interestingly more in patients with headache and taste impairment (408.6±268.9 and 330.6±239.6 respectively) than its level in patients with more neurological complications like convulsion and coma (333.6±259.4 and 280.1±191.4 respectively) suggesting that different mechanism is involved in its mechanism of action.

Table **6** showed that there was a significant relationship between the level of MCP1 and D - dimer level as p value was 0.007*.

Table **7** and Fig. (**1**) showed that the overall accuracy of ACE2 blood level at a cutoff > 0.36 was 76.6% with sensitivity and specificity of 63.64%, and 84.06% respectively, and the overall accuracy of micro RNA gene expression at cutoff < 2.2 was 65.6 % with sensitivity and specificity 62.42% and 55.07% respectively, The overall accuracy of MCP1 at cutoff >166 was 93.7% with sensitivity and specificity 89.70% and 89.13 % respectively. At the above mentioned cutoffs, ACE2 positive and negative predictive values were 82.7% and65.9% respectively. Micro RNA146 gene expression positive and negative predictive values were 62.4% and 55.1% respectively. MCP1 positive and negative predictive values were 90.8% and 87.9% respectively.

4. DISCUSSION

In the current study, we aimed to detect the relations between many risk factors and with the probability of infection with COVID-19, also to evaluate the correlations

Table 5. Relation between Neurological manifestations with markers in cases group (n = 165).

	Neurological					Test of Sig.	p
	Taste impairment (n = 50)	**Coma (n = 29)**	**Smell impairment (n = 44)**	**Headache (n = 25)**	**Convulsions (n = 17)**		
RT-PCR ct							
Mean ± SD.	35.66[a] ± 2.39	33.69[b] ± 3.44	35.73[a] ± 2.07	34.96[ab] ± 2.91	29.71[c] ± 2.64	F= 19.683*	<0.001*
ACE2							
Mean ± SD.	0.54 ± 0.45	1.02 ± 0.74	0.60 ± 0.50	0.63 ± 0.41	1.62 ± 0.60	H= 35.932*	<0.001*
MCP1							
Mean ± SD.	330.6 ± 239.6	280.1 ± 191.4	251.6 ± 164.8	408.6 ± 268.9	333.6 ± 259.4	H= 6.510	0.164
MicroRNA146 gene expression							
Mean ± SD.	2.37 ± 1.35	1.47 ± 1.18	2.42 ± 1.07	1.43 ± 0.90	0.66 ± 0.82	H= 41.994*	<0.001*

SD: Standard deviation.
F: F for ANOVA test, Pairwise comparison bet. Each 2 groups was done using Post Hoc Test (Tukey).
H: H for Kruskal Wallis test, Pairwise comparison bet. Each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test).
p: p value for comparing between the studied scores.
*: Statistically significant at $p \leq 0.05$.
Means/ Medians with Common letters are not significant (*i.e*. Means/ Medians with Different letters are significant).

Table 6. Correlation between MCP1 with D-Dimer and INR in cases group (n = 165).

	r_s	p
MCP1 vs. D-Dimer	-0.210	0.007*
MCP1 vs. INR	0.046	0.559

r_s: Spearman coefficient.
*: Statistically significant at $p \leq 0.05$.

Table 7. Validity (AUC, sensitivity, specificity) for ACE2, Micro146 and MCP1 to discriminate patients (n = 165) from control (n = 138).

	AUC	p	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
ACE2	0.766	<0.001*	0.713 0.819	>0.36	63.64	84.06	82.7	65.9
Micro146	0.656	<0.001*	0.595 0.717	≤2.2	62.42	55.07	62.4	55.1
MCP1	0.937	<0.001*	0.909 0.965	>166	89.70	89.13	90.8	87.9

AUC: Area Under a Curve
p value: Probability value
CI: Confidence Intervals
NPV: Negative predictive value
PPV: Positive predictive value
*: Statistically significant at $p \leq 0.05$

between blood levels of ACE-2, MCP-1, expression of micro RNA 146 and lung disease severity and occurrence of neurologic complications in covid19 Egyptian patients.

Our study was carried out on 165 cases of COVID 19 infected patients, 102 (61.8%) were male and 63 (38.2%) were female, their age mean was 46.37 ± 14.82, we detected in our study that there was a significant increase in serum ferritin in cases of COVID-19 infection. There was a significant increase in both fasting and postprandial blood glucose levels in COVID-19 patients indicating that impaired glucose tolerance might be a risk factor for COVID-19 acquiring infection [28].

There was a significant increase in BMI in cases of COVID-19 infection, thus indicating that obesity might be a risk factor for COVID-19 infection, this is in agreement with another study [29], which also detected a significant

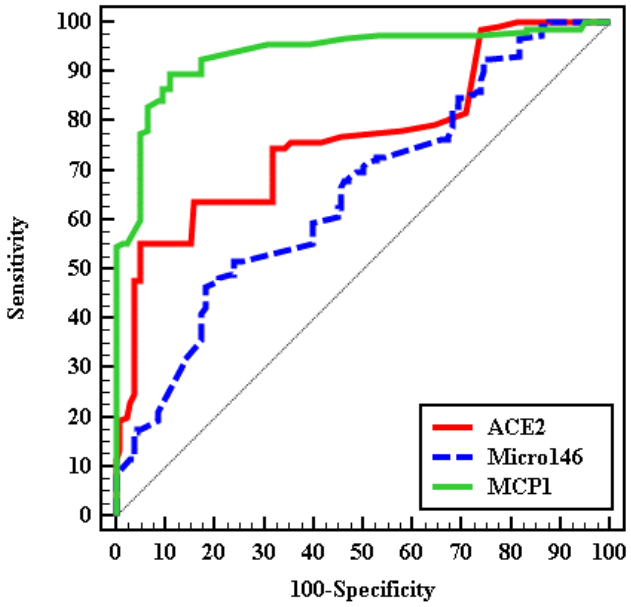

Fig. (1). ROC curve for ACE2, MCP1 and Micro146 to discriminate patients (n = 165) from control (n = 138). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

increase in platelet count, total WBC count with a significant decrease in lymphocyte percentage in cases compared to control, this is in agreement with another study [30]. There was a significant increase in D-Dimer, INR which was in agreement with another study [31], the increased level of D-dimer and INR in COVID-19 patients indicated that these patients were more liable for thrombosis.

As regards the ACE2 level, there was a significant increase in ACE2 level in cases than in the control group, and this can be explained by SARS-CoV-2 entering ACE2expressing cells only, confirming that ACE2 is the cell receptor for SARS-CoV-2 [12, 32]. Further studies showed that the binding affinity of the SARS-CoV-2 spike glycoprotein to ACE2 is 10- to 20 fold higher than that of SARS-CoV to ACE2 [13, 33, 34], Also, there was a significant relationship between increased ACE 2 in cases of COVID-19 infection and severity of lung affection and this can be explained by another study [35], which detected the same results similar to our results and this lung injury might be due to increased ACE2 led to high Ang-II levels in the lungs which could increase vascular permeability and cause pulmonary edema, ACE insertion/deletion polymorphism may be correlated with the severity of ARDS [36].

As regards to MCP1 level, there was a significant increase in MCP1 level in cases than in controls, this was in agreement with another study [37], also there was significant relationship between this increased MCP1 and increased D-dimer level in cases, and this indicated that MCP1 might have a role in thrombosis which occur in COVID-19 patients as MCP1 is a cytokine related to thrombosis [15]. As regards to micro-146 RNA gene expression results, there was a significant decrease in micro-146-RNA gene expression in cases than in controls, this was in agreement with another study [38], there was a significant relationship between this decreased gene expression and severity of lung affection in COVID-19 patients as micro RNA-146 gene negatively regulate the acquired immune system [27] and thus its down regulation, which occurred in COVID-19 patients might be responsible for cytokine storm. Also, there was a significant relationship between a load of SARS-CoV-2 infection and the occurrence of neurological manifestations in cases, this can be explained by increased ACE2, which is widely present in the brain, predominantly in neurons, and participates in the neural regulation of broad physiological functions [39], in a mouse model, SARS-CoV invaded the brain through the olfactory bulb and then spread transneuronal to other areas [40], olfactory and gustatory dysfunctions have been reported in many patients with COVID-19, suggesting the involvement of the olfactory bulb in SARS-CoV-2 infection, Autopsies showed edema and focal degeneration of neurons in the brains of patients with SARS [41].

Many patients had neurologic manifestations in COVID-19, and SARS-CoV-2 was detected in the cerebrospinal fluid of a patient with encephalitis [42-48]. Considering that SARS-CoV-2 has a much higher affinity for its receptor (ACE2) than SARS-CoV, the former could be capable of infecting and damaging the central nervous system.

CONCLUSION

Obesity and diabetes mellitus are risk factors for COVID-19 infection, increased level of ACE2 and down regulation of micro RNA 146 gene expression is associated with more severe lung injury, and increased level of MCP1 is associated with more thrombosis**.** High viral load, increased blood level of ACE2, and down-regulation of micro-RNA 146 expression are associated with more severe lung injury and with the presence of neurologic complications like convulsions and coma in COVID-19 Egyptian patients.

LIST OF ABBREVIATIONS

COVID-19	=	Coronavirus disease - 2019
ICUs	=	Intensive Care Units
RT-PCR	=	Reverse Transcriptase - Polymerase Chain Reaction
ACE-2	=	Angiotensin-Converting Enzyme 2
RAS	=	Renin-Angiotensin System
SARS-CoV-2	=	Severe Acute Respiratory Syndrome Coronavirus 2
MCP-1	=	Monocyte Chemo Attractant Protein-1
NK	=	Natural-Killer
MiRNAs	=	MicroRNAs
IRAK1	=	Interleukin-1 Receptor-Associated Kinase 1
TRAF6	=	Tumor Necrosis Factor Receptor Associated Factor 6

STAT1	=	Signal Transducer and Activator of Transcription 1
AP-1	=	Activator Protein 1
CDCC	=	Contagious Disease Control Center
BMI	=	Body Mass Index
FBS	=	Fasting Blood Glucose
PPBS	=	Post Prandial Blood Glucose
INR	=	International Normalized Ratio

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Research Ethics Committee of Tanta University faculty of medicine. The ethical committee approval number was 34830/8/21 for this study.

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All human research procedures were followed in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

STANDARDS OF REPORTING

STROBE guideline has been followed.

CONSENT FOR PUBLICATION

Informed consent was signed by every patient before enrolment in the study.

AVAILABILITY OF DATA AND MATERIALS

Data are available upon reasonable request from the corresponding author.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Mohamed AA, Mohamed N, Abd-Elsalam S, *et al.* COVID-19 in pediatrics: A diagnostic challenge. Curr Pediatr Rev 2021; 17(3): 225-8. http://dx.doi.org/10.2174/1573396317666210329153515 PMID: 33781192

[2] Mohamed AA, Mohamed N, Mohamoud S, *et al.* SARS-CoV-2: The path of prevention and control. Infect Disord Drug Targets 2020; (20): 1-5. http://dx.doi.org/10.2174/1871526520666200520112848 PMID: 32433010

[3] Rodriguez-Morales AJ, Cardona-Ospina JA, Gutiérrez-Ocampo E, *et al.* Clinical, laboratory and imaging features of COVID-19: A systematic review and meta-analysis. Travel Med Infect Dis 2020; 34: 101623. http://dx.doi.org/10.1016/j.tmaid.2020.101623 PMID: 32179124

[4] Rodriguez-Morales AJ, Bonilla-Aldana DK, Balbin-Ramon GJ, *et al.* History is repeating itself: Probable zoonotic spillover as the cause of the 2019 novel Coronavirus epidemic. Infez Med 2020; 28(1): 3-5. PMID: 32009128

[5] Chen N, Zhou M, Dong X, *et al.* Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. Lancet 2020; 395(10223): 507-13. http://dx.doi.org/10.1016/S0140-6736(20)30211-7 PMID: 32007143

[6] Yin Y, Wunderink RG. MERS, SARS and other coronaviruses as causes of pneumonia. Respirology 2018; 23(2): 130-7. http://dx.doi.org/10.1111/resp.13196 PMID: 29052924

[7] Lechien JR, Chiesa-Estomba CM, Place S, *et al.* Clinical and epidemiological characteristics of 1420 European patients with mild-to-moderate coronavirus disease 2019. J Intern Med 2020; 288(3): 335-44. http://dx.doi.org/10.1111/joim.13089 PMID: 32352202

[8] Chen T, Wu D, Chen H, *et al.* Clinical characteristics of 113 deceased patients with coronavirus disease 2019: Retrospective study. BMJ 2020; 368: m1091. http://dx.doi.org/10.1136/bmj.m1091 PMID: 32217556

[9] Donoghue M, Hsieh F, Baronas E, *et al.* A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. Circ Res 2000; 87(5): E1-9. http://dx.doi.org/10.1161/01.RES.87.5.e1 PMID: 10969042

[10] Patel S, Rauf A, Khan H, Abu-Izneid T. Renin-Angiotensin-Aldosterone System (RAAS): The ubiquitous system for homeostasis and pathologies. Biomed Pharmacother 2017; 94: 317-25. http://dx.doi.org/10.1016/j.biopha.2017.07.091 PMID: 28772209

[11] Zhou P, Yang XL, Wang XG, *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020; 579(7798): 270-3. http://dx.doi.org/10.1038/s41586-020-2012-7 PMID: 32015507

[12] Wrapp D, Wang N, Corbett KS, *et al.* Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 2020; 367(6483): 1260-3. http://dx.doi.org/10.1126/science.abb2507 PMID: 32075877

[13] Kuba K, Imai Y, Ohto-Nakanishi T, Penninger JM. Trilogy of ACE2: A peptidase in the renin-angiotensin system, a SARS receptor, and a partner for amino acid transporters. Pharmacol Ther 2010; 128(1): 119-28. http://dx.doi.org/10.1016/j.pharmthera.2010.06.003 PMID: 20599443

[14] Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol 2004; 203(2): 631-7. http://dx.doi.org/10.1002/path.1570 PMID: 15141377

[15] Kong SL, Chui P, Lim B, Salto-Tellez M. Elucidating the molecular physiopathology of acute respiratory distress syndrome in severe acute respiratory syndrome patients. Virus Res 2009; 145(2): 260-9. http://dx.doi.org/10.1016/j.virusres.2009.07.014 PMID: 19635508

[16] Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte Chemoattractant Protein-1 (MCP-1): An overview. J Interferon Cytokine Res 2009; 29(6): 313-26. http://dx.doi.org/10.1089/jir.2008.0027 PMID: 19441883

[17] Huang C, Wang Y, Li X, *et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020; 395(10223): 497-506. http://dx.doi.org/10.1016/S0140-6736(20)30183-5 PMID: 31986264

[18] Lin L, Lu L, Cao W, Li T. Hypothesis for potential pathogenesis of SARS-CoV-2 infection-a review of immune changes in patients with viral pneumonia. Emerg Microbes Infect 2020; 9(1): 727-32. http://dx.doi.org/10.1080/22221751.2020.1746199 PMID: 32196410

[19] Xiong Y, Liu Y, Cao L, *et al.* Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. Emerg Microbes Infect 2020; 9(1): 761-70. http://dx.doi.org/10.1080/22221751.2020.1747363 PMID: 32228226

[20] Chu H, Chan JF, Wang Y, *et al.* Comparative replication and immune activation profiles of SARS-CoV-2 and SARS-CoV in human lungs: An *ex vivo* study with implications for the pathogenesis of COVID-19. Clin Infect Dis 2020; 71(6): 1400-9. http://dx.doi.org/10.1093/cid/ciaa410 PMID: 32270184

[21] Bartel DP. Metazoan microRNAs. Cell 2018; 173(1): 20-51.2018; http://dx.doi.org/10.1016/j.cell.2018.03.006
[22] Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ. miRBase: MicroRNA sequences, targets and gene nomenclature. Nucleic Acids Res 2006; 34(Database issue): D140-4.
[23] Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 2009; 19(1): 92-105. http://dx.doi.org/10.1101/gr.082701.108
[24] Lai FW, Stephenson KB, Mahony J, Lichty BD. Human coronavirus OC43 nucleocapsid protein binds microRNA 9 and potentiates NF-kappaB activation. J Virol 2014; 88(1): 54-65.
[25] Ma Y, Wang C, Xue M, Fu F, Zhang X, Li L, *et al.* The coronavirus transmissible gastroenteritis virus evades the type I interferon response through IRE1alpha-mediated manipulation of the microRNA miR-30a-5p/SOCS1/3 axis. J Virol 2018; 92(22)
[26] Tribolet L, Kerr E, Cowled C, *et al.* MicroRNA biomarkers for infectious diseases: From basic research to biosensing. Front Microbiol 2020; 11
[27] Ambyah PA, Sepramaniam S, Mohamed Ali J, *et al.* MicroRNAs in circulation are altered in response to influenza A virus infection in humans. PLoS One 2013; 8(10): e76811.
[28] Peng X, Gralinski L, Ferris MT, *et al.* Integrative deep sequencing of the mouse lung transcriptome reveals differential expression of diverse classes of small RNAs in response to respiratory virus infection. mBio 2011; 2(6).
[29] Holman N, Knighton P, Kar P, *et al.* Risk factors for COVID-19-related mortality in people with type 1 and type 2 diabetes in England: A population-based cohort study. Lancet Diabetes Endocrinol 2020; 8(10): 823-33. http://dx.doi.org/10.1016/S2213-8587(20)30271-0 PMID: 32798471
[30] Querol-Ribelles JM, Tenias JM, Grau E, *et al.* Plasma d-dimer levels correlate with outcomes in patients with community-acquired pneumonia. Chest 2004; 126(4): 1087-92. http://dx.doi.org/10.1378/chest.126.4.1087 PMID: 15486368
[31] Li W, Moore MJ, Vasilieva N, *et al.* Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 2003; 426(6965): 450-4. http://dx.doi.org/10.1038/nature02145 PMID: 14647384
[32] Papageorghiou AT, Deruelle P, Gunier RB, *et al.* Preeclampsia and COVID-19: Results from the INTERCOVID prospective longitudinal study. Am J Obstet Gynecol 2021; 225(3): 289.e1-289.e17. http://dx.doi.org/10.1016/j.ajog.2021.05.014 PMID: 34187688
[33] El-Raey F, Alboraie M, Youssef N, *et al.* Predictors for severity of SARS-COV-2 infection among healthcare workers. J Multidiscip Healthc 2021; 14: 2973-81. http://dx.doi.org/10.2147/JMDH.S335226 PMID: 34729011
[34] Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, *et al.* Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan. China JAMA 2020; 323(11): 1061-9. http://dx.doi.org/10.1001/jama.2020.1585
[35] Yang X, Yu Y, Xu J, *et al.* Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: A single-centered, retrospective, observational study. Lancet Respir Med 2020; 8(5): 475-81. http://dx.doi.org/10.1016/S2213-2600(20)30079-5 PMID: 32105632
[36] Marshall RP, Gohlke P, Chambers RC, *et al.* Angiotensin II and the fibroproliferative response to acute lung injury. Am J Physiol Lung Cell Mol Physiol 2004; 286(1): L156-64. http://dx.doi.org/10.1152/ajplung.00313.2002 PMID: 12754187
[37] Tan WSD, Liao W, Zhou S, Mei D, Wong WF. Targeting the renin-angiotensin system as novel therapeutic strategy for pulmonary diseases. Curr Opin Pharmacol 2018; 40: 9-17. http://dx.doi.org/10.1016/j.coph.2017.12.002 PMID: 29288933
[38] Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost 2020; 18(4): 844-7. http://dx.doi.org/10.1111/jth.14768 PMID: 32073213
[39] Curtale G, Rubino M, Locati M. MicroRNAs as molecular switches in macrophage activation. Front Immunol 2019; 10: 799. http://dx.doi.org/10.3389/fimmu.2019.00799 PMID: 31057539
[40] Santos RAS, Sampaio WO, Alzamora AC, *et al.* The ACE2/angiotensin-(1-7)/MAS axis of the renin-angiotensin system: Focus on angiotensin-(1-7). Physiol Rev 2018; 98(1): 505-53. http://dx.doi.org/10.1152/physrev.00023.2016 PMID: 29351514
[41] Netland J, Meyerholz DK, Moore S, Cassell M, Perlman S. Severe acute respiratory syndrome coronavirus infection causes neuronal death in the absence of encephalitis in mice transgenic for human ACE2. J Virol 2008; 82(15): 7264-75. http://dx.doi.org/10.1128/JVI.00737-08 PMID: 18495771
[42] Gu J, Korteweg C. Pathology and pathogenesis of severe acute respiratory syndrome. Am J Pathol 2007; 170(4): 1136-47. http://dx.doi.org/10.2353/ajpath.2007.061088 PMID: 17392154
[43] Mao L, Jin H, Wang M, *et al.* Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. JAMA Neurol 2020; 77(6): 683-90. http://dx.doi.org/10.1001/jamaneurol.2020.1127 PMID: 32275288
[44] Abd-Elsalam S, Salama M, Soliman S, *et al.* Remdesivir efficacy in COVID-19 treatment: A randomized controlled trial. Am J Trop Med Hyg 2021; 106(3): 886-90. http://dx.doi.org/10.4269/ajtmh.21-0606 PMID: 34649223
[45] El-Bendary M, Abd-Elsalam S, Elbaz T, El-Akel W, Cordie A, Elhadidy T, *et al.* Efficacy of combined sofosbuvir and Daclatasvir in the treatment of COVID-19 patients with pneumonia: A multicenter Egyptian study. Expert Rev Anti Infect Ther 2021; 1-5 http://dx.doi.org/10.1080/14787210.2021.1950532 PMID: 34225541
[46] Abd-Elsalam S, Noor RA, Badawi R, *et al.* Clinical study evaluating the efficacy of ivermectin in COVID-19 treatment: A randomized controlled study. J Med Virol 2021; 93(10): 5833-8. http://dx.doi.org/10.1002/jmv.27122 PMID: 34076901
[47] Abd-Elsalam S, Soliman S, Esmail ES, *et al.* Do zinc supplements enhance the clinical efficacy of hydroxychloroquine?: A randomized, multicenter trial. Biol Trace Elem Res 2021; 199(10): 3642-6. http://dx.doi.org/10.1007/s12011-020-02512-1 PMID: 33247380
[48] Malkova A, Kudlay D, Kudryavtsev I, Starshinova A, Yablonskiy P, Shoenfeld Y. Immunogenetic predictors of severe COVID-19. Vaccines (Basel) 2021; 9(3): 211. http://dx.doi.org/10.3390/vaccines9030211 PMID: 33802310