Usage of ACE2 as a biomarker in patients with COVID-19 severe infection in relation with LDH

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Abstract
Background: Coronavirus disease (COVID-19) is an infectious disease caused by the severe acute respiratory syndrome coronavirus-2. Most of the people infected with the virus will have moderate respiratory illness and they recover without requiring special treatment. Aim: The aim of this study was to evaluate the role of ACE2 as a biomarker in patients with COVID-19 severe infection. Materials and methods: A case-control retrospective study included 90. subjects who were divided as the following : 60 COVID-19 patients who aged between 13-90 years with a positive real time –polymerase chain reaction (RT-PCR) for COVID-19,under monitoring the specialist doctor in terms of giving treatment and observing the clinical condition of the patient. patients with COVID-19 were divided in to 20 patients with severe symptoms who were admitted into the intensive care unit in the hospital, 20 patients with moderate symptoms who were admitted to the hospital ,and 20 patients with mild symptoms who were treated as an out patients. 30 individuals who were apparently healthy as a control group. Blood blood sample were collected from each patients fir determination of Serum Angiotensin Converting Enzyme 2 (ACE2) and LDH by ELISA. Results: The highest proportion of study group were aged ≥ 60years (55% and 40% respectively) . According to gender the highest proportion of the study group were female s(51% and 56.7% respectively), regarding the smoking status 28.3% of patient group and 50 % of control were current smokers , while the highest proportion of the patient group complained from severe COVID-19 infection . A significant difference $p<0.05$ in ACE2 levels between patient and control group so significant increase was observed in the level of the biomarkers (ACE2, LDH,) with means (261.47pg/ml,495.28 iu/l , when measured in COVID-19 patients , in comparison with control group means (50.07 pg/ml, 288.16 iu/l,) respectively, which meaning that a positive correlations between ACE2 level and LDH ,Ferritin Conclusions: Present study revealed that ACE2 has a vital role in COVID-19 patients and in severity of systemic inflammation , , LDH , play an essential role in severity of COVID- 19.
Keywords: Covid-19; ACE; LDH; Severe infection

Introduction

Coronaviruses are single-stranded RNA viruses with a diameter of 80–220 nm, transmission of SARS-CoV-2 occurs either through exposure to micro-droplets from infected individuals or by contact transmission through contaminated fomites, virus reaches the smaller airways and alveoli, and targets the bronchial and alveolar epithelial cells. [(1)] The spike surface glycoprotein S on the virus binds to angiotensin-converting enzyme 2 (ACE-2), a membrane carboxyl peptidase present in distal airways and alveoli, which have the highest expression of ACE-2, along with alveolar macrophages and dendritic cells.[(2)] Angiotensin-converting enzyme 2 is expressed on the vascular endothelium, nasal, oral, nasopharyngeal, pharyngeal epithelia, gut epithelia, cardiac prices, renal proximal tubular cells ,in the skin, reticule endothelial and the central nervous system, angiotensin-converting enzyme 2 expression depends on age, gender, genetic factors, and presence of comorbid conditions such as obesity, chronic cardiopulmonary disease, cancer, and use of immunosuppressive drugs.[(3)] Renin cleaves angiotensinogen to produce angiotensin I which is further cleaved by ACE to produce angiotensin II having a dual role, action through Angiotensin II Type 1 Receptor (AT1R), facilitates vasoconstriction, fibrotic remodeling, and inflammation, while that through Angiotensin II Type 2 Receptor (AT2R) leads to vasodilation and growth inhibition, angiotensin II is cleaved by ACE2 to Ang 1–7 which counteracts the harmful effects of the ACE/Ang II/AT1 axis.[(4)] Angiotensin converting enzyme 2 primarily plays a key role to physiologically counterbalance ACE and regulate angiotensin II, Internalization of the ACE-2 after viral interaction leads to its down regulation, and consequent up regulation of angiotensin II, the latter acting through AT1R, activates the downstream inflammatory pathways, leading to the "cytokine storm" that adversely affects multiple organs. [(5)] Angiotensin-converting enzyme 2 is a vital element in a biochemical pathway that is critical to regulating processes such as blood pressure, wound healing and inflammation, called the renin-angiotensin-aldosterone system (RAAS) pathway. [(6)] Angiotensin-converting enzyme 2 helps modulate the many activities of a protein called angiotensin II (ANG II) that increases blood pressure and

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inflammation, increasing damage to blood vessel linings and various types of tissue injury, angiotensin-converting enzyme 2 converts ANG II to other molecules that counteract the effects of ANG II. [7]. The aim of this study was to evaluate the role of ACE2 as a biomarker in patients with COVID-19 severe infection.

Materials and Methods

A Case- control retrospective study was carried out in Tikrit General Hospital - isolation unit /Tikrit City and Kirkuk General Hospital - isolation unit starting from the period 1st of January to the end of March 2022 on patients infected with the Coronavirus . The study included 90 subjects who were divided as the following : 60 patients who aged between 13-90 years with a positive real time –polymerase chain reaction (RT-PCR) for COVID-19, under the monitoring of specialist doctor in terms of giving treatment and observing the clinical condition of the patient. patients with COVID-19 were divided in to 20 patients with severe symptoms who were admitted in to the intensive care unit in the hospital, 20 patients with moderate symptoms who were admitted to the hospital ,and 20 patients with mild symptoms who were treated as an outpatients. 30 individuals who were apparently healthy as a control group. Laboratory tests were conducted for both groups of patients and control. Each patient was educated about the research's specifics, filled out questionnaire , and singed out form the participate in the study according to Tikrit University /College of Medicine Committee. Tikrit directorate of health has also granted permission to visit Tikrit General Hospital and collect samples from patients. Five milliliters of blood sample were collected from each patients. Blood was distributed into sterile test tubes. Each sample was collected centrifuged at 3800 rpm for 10 minutes to get the serum. The separated serum stored at –20 °C for the subsequent assay of Serum Angiotensin Converting Enzyme 2 (ACE2**)** by ELISA (Enzyme-Linked Immuno Sorbent Assay) kit, Immediate measurement of lactate dehydrogenase, these analyses were measured by fully automated. Hemolysis samples were rejected.

Results

The present study revealed that the highest rate of patients and control group were within the age group ≥ 60 years as shown in table 4.1.

Table 1: Distribution of the Study Group According to Age

Age (Year)	Patients No. (%)	Control group No. (%)
<40	8 (13.3)	7 (23.3)
40-59	19 (31.7)	11 (36.7)
≥ 60	33 (55)	12 (40)
Total	60 (100)	30 (100)

The present study revealed that the highest rate of the patient and the control group were females, as shown in table 4.2.

Table 2: Distribution of Study Group According to Sex.

Sex	Patients No. (%)	Control group No .(%)
Male	29 (48.3)	13 (43.3)
Female	31 (51.7)	17 (56.7)
Total	60 (100)	30 (100)

The present study revealed that the 28.3% of the patient and 50% of the control group were current smokers .while the 71.7% of patients and 50% of control group were nonsmokers as shown in Table 3

Table 3: Distribution of Study Group According to the Smoking Status.

Smoking Status	Patients No. (%)	Control group No. (%)
Current Status	17 (28.3)	15 (50.0)
Non Smoker	43 (71.7)	15 (50.0)
Total	60 (100)	30 (100)

The present study revealed that the highest proportion of patient complained from severe COVID-19 infection was (36.7%) as shown in Figure 1

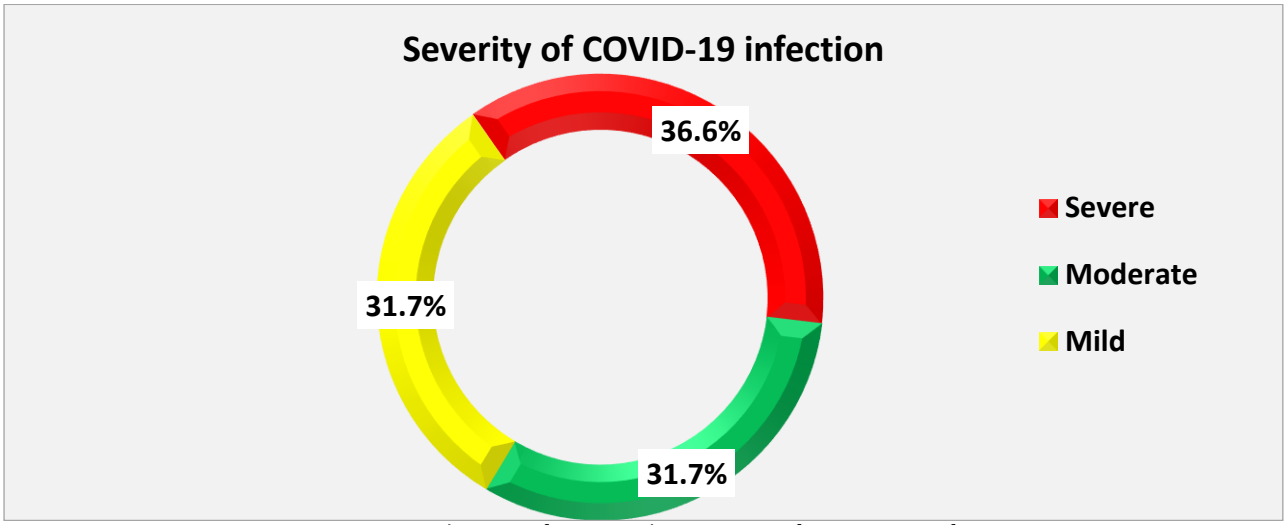

Figure 1: Distribution of Patients by Severity of COVID-19 Infection.

The present study revealed that the mean of ACE2 Level was significantly higher in patients than that in control group (261.47 versus 50.07 pg/ml, P = 0.001). as shown in Figure 2

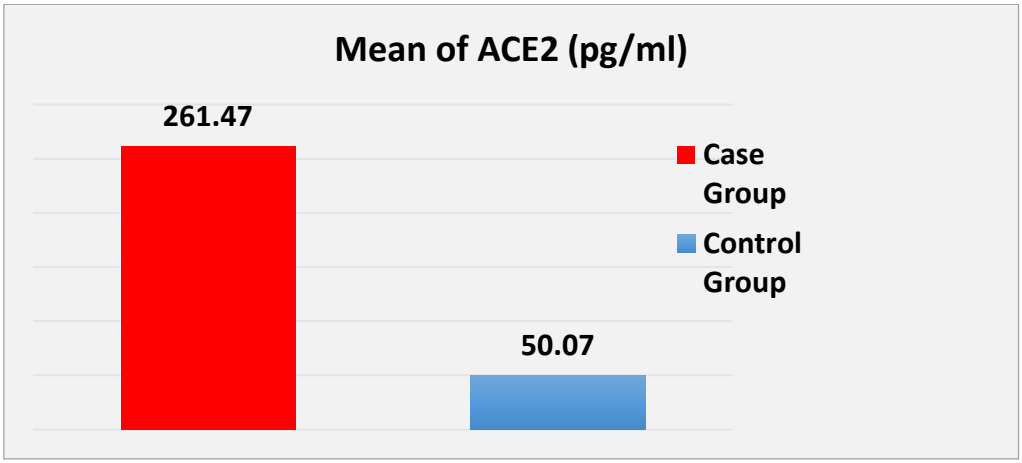

Figure 2: Mean of ACE2 in Study Group.

The present study revealed that there was no significant difference (p =0.474) according to the level of ACE2 between severe groups (243.95 ± 129.0) and non-severe group (271.61 ± 165.0) respectively, as shown in Table 4.6.

Table 4: Level of ACE2 according to severity of COVID-19 .		
Severity of COVID-19	Level of ACE2 pg/mL Mean ± SD	*P - Value*
Severe	243.95 ± 129.0	p = 0.474
Non-severe	271.61 ± 165.0	

The present study revealed that there was a significant difference (p≤ 0.05) in LDH levels between the patient (495.28 ± 124.9) and the control group (288.16 ± 53.8) respectively, as shown in Table 5.

Table 5: Comparison of LDH Levels between Study Group.		
Study group	Level of LDH IU/L Mean ± SD	*P - Value*
Patients	495.28 ± 124.9	p≤ 0.05
Control	288.16 ± 53.8	

The present study revealed that statistically significant positive correlations were detected between ACE2 level and LDH Figure 3.

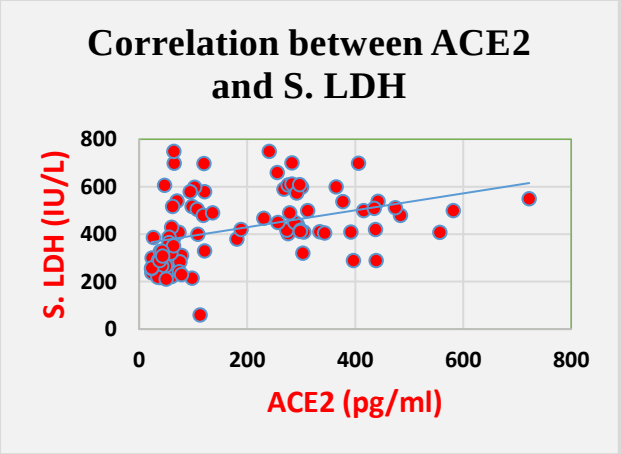

Figure 3: Correlation between ACE2 and LDH.

Discussion

According to the findings in this study the highest rate of patients and control group were within the age group ≥ 60 years was 55% and 40% respectively. In agreement with Inde et al.,2020 [8] which found that ACE2 is lower in children than adults. Dis agreement with Ortiz et al .,2020 [9] who found the opposite conclusion. They proposed decrease of ACE2 across age and the observed decrease of the ACE2/ACE ratio in chronic inflammatory diseases often associated with severe COVID-19, support a negative relationship between COVID-19 severity and ACE2 expression.[10] In dis agreement with Guo et al., 2020[11] who found COVID-19, caused by the SARS-CoV-2 virus, is affecting a disproportionally higher number of men than women. Epidemiological data show that a much larger number of men are severely affected by the disease, and there is an even more substantial gender difference in the mortality of patients with COVID-19.[12] Although differences in the immune system might account for the differences in the morbidity and mortality between men and women,this large gender difference has been shown by Jin et al.,2020.[13] Sex hormones regulate the immune response to infections and also regulate expression of the ACE2 receptor, which are responsible for viral entry and priming, respectively.[14] In agreement with Rossato et al.,2020[131] who was demonstrated that none of the hospitalized patient was a current smoker, and confirmed that there was no difference in the disease severity between patients who never smoked and currentsmokers. Hanfany et al.,2018[15] who showed that ACE-2 gene expression was reduced in non-smokers compared to smokers, it is also known that ACE-2 down regulation may be dependent on inflammatory mediators increased production. Cardinale et al.,2018 [16] Cigarette smoker promote inflammation inducing the production of pro-inflammatory cytokines, such a TNF-α, IL-1, IL-6, IL-8 and granulocyte-macrophage colony-stimulating factor (GM-CSF), the reduction of ACE-2 on lung

cells may be a direct effect of nicotine. Wrapp et al., 2020[17] showed in smokers the chronic exposure to cigarette smoke triggers a compensatory mechanism increasing the ACE-2 expression in order to balance the inflammatory effects induced by tobacco smoke in the long term. Angiotensin Converting Enzyme (ACE2) is elevated in patients with active COVID-19 disease and in the period after infection. [18] Coronavirus disease 2019 (COVID-19) patients classified as severe cases displayed higher viral loads in nasopharyngeal swab samples during the early stages of disease onset compared to mild. Patients ACE2 is expressed in the type II alveolar pneumocytes.[19] The binding of the virus to ACE2 on the type II alveolar pneumocytes and subsequent infection leads to depletion of these cells, resulting in a decrease in the production and secretion of surfactant, as well as a lack of ability to regenerate and repair injured lung tissue, leading to the exacerbation of lung injury in severe COVID-19. [20]
According to the findings in this study mean of ACE2 Level was significantly higher in patients than that in control group (261.47 versus 50.07 pg/ml, P = 0.001). Angiotensin Converting Enzyme 2 serves as the main receptor for severe acute respiratory syndrome coronavirus 2 which causes COVID-19.[21] In agreement with current study finding Garvin et al., 2020 [22] a critical imbalance in the renin-angiotensin-aldosterone system with the upregulated expression of ACE2, renin enzymes in COVID-19 subjects. Angiotensin Converting Enzyme2 is also the key enzyme of the alternative renin–angiotensin system (RAS) and counterbalances angiotensin II activity by enzymatically converting angiotensin II to angiotensin 1–7, anchored in the membrane of type II alveolar and lung epithelial cells, ACE2 can be cleaved by the metalloproteinase ADAM17.[23] High serum ACE2 may contribute to the resolution of the disease, binding of SARS-CoV-2 to membrane ACE2 has at least 2 effects: First it downregulates membrane ACE2 causing a dysregulated local renin angiotensin system (RAS) that favours inflammation and ongoing tissue damage secondary to excess angiotensin II Second the dysregulated local RAS is associated with prolonged shedding of the catalytically active site of ACE2 into the circulation.[24]
A strong correlation was found between the level of LDH and lung damage as well as disease severity. [25] Elevation of LDH, reflected a poor prognosis in severe COVID-19 patients, its elevation generally indicates tissue damage, although the virus binds to human angiotensin converting enzyme 2 (ACE2) receptor in the lung ,which explains the lungs are the first organs affected. [26]
Elevated LDH could be used as a severity indicator in patients with COVID-19, In agreement with a study conducted by Brandon Michael Henry et .al [27] demonstrated an association between elevated LDH values and worse outcomes in patients with COVID-19. Elevated LDH levels during infection may be immunologic changes after SARS-COV2 infection of respiratory tract which result in an early acute respiratory inflammatory response with consequent release of pro-inflammatory cytokines, which is a mediator of lung inflammation and fibrosis, LDH increases production of lactate, leads to enhancement of immune-suppressive cells, including macrophages and dendritic cells (DCs) and inhibition of cytolytic cells, such as natural killer (NK) cells and cytotoxic T-lymphocytes. [28] Lactate Dehydrogenase is often induced upon T cell activation and proliferation ,it was also hypothesized that change in lactate modulated the inflammatory response in macro-phages.[29] Suppression of LDH has anti-inflammatory effects due to the down regulation of several inflammatory mediators including cytokines.[30] Lactate dehydrogenase increased significantly in serious and critical patients with COVID-19.[31] Wu et al.[32] reported LDH was one of the risk factors correlated with the development of acute respiratory distress syndrome (ARDS) and progression from ARDS to death in patients with COVID-19.

Conclusions

Present study revealed that ACE2 has a vital role in COVID-19 patients and in severity of systemic inflammation, LDH, play an essential role in severity of COVID- 19.
Recommendations: Advice to use serum level of ACE and biomarker of management and severity of the COVID-19 infection

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