Iraqi Journal of Biotechnology, 2022, Vol. 21, No. 2, 178-182

Determination of Angiotensin-Converting Enzyme 2 (ACE2) Receptor Level in Samples of Iraqi Patients Infected with COVID-19

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Received: 1/6/2022 Accepted: 31/8/2022 Published: December 20, 2022

Abstract: An outbreak with signs of pneumonia that caused by (acute respiratory syndrome coronavirus 2) (SARS–CoV-2) first appear in the city of Wuhan, China, at the end of 2019, then become a global, pandemic. The Angiotensin - converting enzyme-2 (ACE2) is not considered as only an enzyme but it is also a functional receptor on the surfaces of the cells through which the SARS-CoV-2 enters the cells of the host and it is highly expressed in (heart, kidneys, and lungs). The sputum samples were collected from 84 patients and 27 healthy individuals from Baghdad Medical City and Baqubah Teaching Hospital, in period of 28 April to 1 October 2021, after collection its stored at freeze until use, then measured by Sandwich Enzyme Linked Immune Sorbent Assay. The result revealed that the levels of ACE2 receptor was elevated in patient group as compared with healthy control with high significant difference($P<0.01$), the level reached to (37.20±1.796 ng/L) in COVID-19 patients, while in healthy control was (8.921±1.19 ng/L), as a conclusion Detection of ACE2 receptors in sputum of COVID – 19 patients revealed high significant difference when compared with healthy control and it is highly elevated range.

Keywords: COVID-19; ACE2; Sputum; ELISA.

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Introduction

In late of December 2019, SARS – CoV-2, was the etiology of the coronavirus disease in 2019 (COVID-19), it is originated in Wuhan, China and quickly spread to the majority of the countries in the world and lead to great of threats to the public health. The virus is shares about 79.5% of the genome identity with the SARSCoV-1 and it is also using the angiotensin-converting enzyme 2 (ACE2) as a cell entry receptor (1–5). Clinical symptoms of the COVID-19 patients include (fever, fatigue, dry cough, and pneumonia), whereas around of 20% of severe cases may lead to death from multi - organ failure. Such as part from respiratory system, multiple organs including immune system of the COVID-19 patients were also targeted by SARS CoV- 2 infection (6–9).

The outbreaks were caused by a highly pathogenic of coronaviruses, including the acute respiratory syndrome coronavirus (SARS-CoV) in (2002) and the infection with Middle East respiratory syndrome coronavirus

(MERS-CoV) in 2012. The current outbreak, denominated coronavirus disease 2019 (COVID-19), caused by the virus of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (6–9).

The SARS-CoV-2 is classified as an enveloped virus containing one positive-strand RNA genome that comprises 29.9 kb. SARS-CoV-2 shares about 80% similar to SARS-CoV. (10), and both of the viruses use the angiotensin - converting enzyme 2 (ACE2) as entry cellular receptor. The coronavirus has four structural proteins: envelope (E), membrane (M), nucleocapsid (N), and spike (S) proteins. The spike protein is forms the large protrusions from the virus surface, giving an appearance of the crown and the name of the coronavirus (2,11).

The (S) protein consists of the subunits (S1 and S2), it is responsible for attachment of virus and the membrane fusion, respectively. The spike is binds to the receptor of human ACE2 (hACE2) in the cell membrane through (S1) subunit of the receptor - binding domain (RBD). The SARS-CoV-2 RBD are binds to the soluble hACE2 is more strongly with SARS - CoV (3). The enhanced affinity for the hACE2 and may contribute to the SARS-CoV-2's with higher infectivity, as (COVID-19) is widespread globally and the number of the cases is increased. The transmembrane protease serine protease-2 (TMPRSS-2) and the ADAM metallo peptidase domain 17 (ADAM17) (12) of host cell required for the priming of the (S) protein to allow the fusion of viral to host membranes by the S2 subunit. Therefore, SARSCoV- 2 is internalized by the endocytosis, and viral RNA is to release for the replication and the translation by host cell machinery and further assembly and exocytosis of the new viral structures (13,14).

Methods

Patients

Sputum samples were collected from 84 confirmed cases infected with COVID 19 and diagnosis by using qRT-PCR Control 27 samples from normally individuals were collected to compare with the levels of receptor in patients.

Method

The sputum samples after collection were stored in deep freeze until used. The sample treated by equal size of buffer saline and centrifuged 4000 rpm \ 20 min, to separate the mucus from the solution then the solution obtained to measure the receptor with sandwich ELISA according to the kit manufacture (Sun long biotech).

Results and discussion

The result revealed that the level of ACE2 receptor was elevated in patient group as compared with healthy control with high significant difference ($P<0.01$), the level of ACE2 receptor was reached to (37.20±1.796 ng/L) in COVID-19 patients, while in healthy control was (8.921±1.19 ng/L) as illustrated in Figure (1).

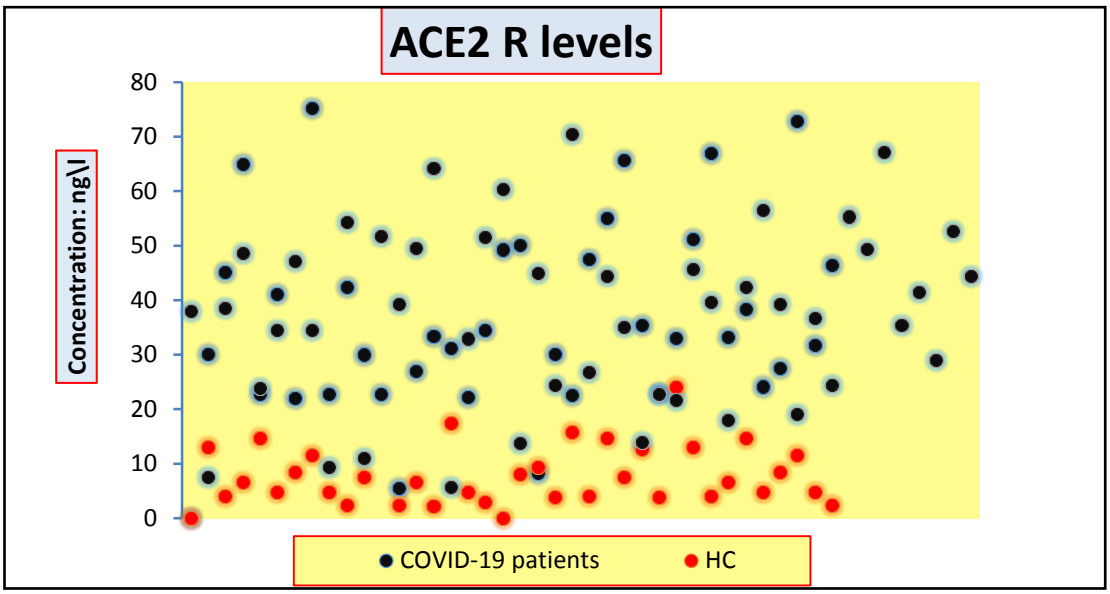

Figure (1): Comparison between ACE2 receptor levels in COVID-19 patients and healthy control.

The Angiotensin-Converting Enzyme 2 (ACE2) has been proved to the main host cell receptor for binding of SARS-CoV-2. The SARS-CoV-2 spike protein (S) is binds to the ACE2 to start the process of the replication (15). Most of people present a respiratory difficulty in response to SARS-CoV-2 infection, these findings were detected by the fact of that the release of the inflammatory cytokines, such as the interferons (IFNs) that caused by the SARS-CoV-2, can rise the expression of the ACE2 and the potentiate the infection (16,17).

The current results were agreed with recent study conducted by (18) from the United Arab Emirates, who showed that the (ACE2) receptor were at significantly raised in the non-diabetic and the diabetic COVID-19 patients in compared to the healthy individuals. Also, (19) noted that the ACE2 R levels were elevated in COVID-19 patients with the severe disease than the mild and the moderate. The period of the hospital stay is correlated with the ACE2 levels. Higher ACE2R levels are related to higher levels pf CRP and the D-dimer levels.

In the fact, the patients with more severe (COVID-19) have the higher viral loads in the respiratory tract (throat, Broncho alveolar lavage fluid, or the sputum) and longer viral persistence than those who are experience the milder disease (20,21). Not surprisingly, expression levels of the viral of the host receptor ACE2 are thought to be the important and the relevant factor influencing the viral loads and the infection (22, 23).

The age-dependent increases in SARS2-CoV-2 receptors in respiratory epithelium part may be responsible for increased severity of the COVID-19 lung disease in the elderly of people. Regarding the children, research reports that showed of the ACE2 expression in the nasal epithelium was in significantly higher in the older children (10 to 17 years old), and the adults (more than 18 years old) when it compared with the younger children (less than 10 years old) (24).

Moreover, the previous study by henonin *et al.*, (25) showed that the preterm and term of newborns have a lower expression of ACE2 in the nasal epithelium than the adults (25). This may

lead to explain why COVID-19 is less prevalent and less severe in the children (26).

Peters *et al.* (27) record that the higher level of the ACE2 in the male patients is notable cause higher mortality of (COVID-19) in the males. Perhaps relatedly, the ACE2 gene is on the X chromosome, and differences in the sex chromosome dosage could affect the ACE2 expression through the X-inactivation or the differences in the parental imprinting. However, other studies in rodents have found that the ACE2 levels are high relatively in the males, because ACE2 levels are suppressed in the females by female sex hormones (28).

It is necessary to acquire more knowledge of the role that the ACE2 plays in different organs and the physiological pathways because of its broad spread in tissue expression (29). This may shed the light on the factors that modulate the cell surface ACE2 receptor affecting the viral cell entry and consequently susceptibility to the SARS-CoV-2 infection. This, in turn, could have significant implications for identifying better therapies and screening tools to assess disease progression and the severity (30).

References

1. Hoffmann, M.; Kleine-Weber, H.; Schroeder, S.; Krüger, N.; Herrler, T.; Erichsen, S., *et al*. (2020). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell, 181(2): 271–280.e8.
2. Li, W.; Moore, M. J.; Vasilieva, N.; Sui, J.; Wong, S. K.; Berne, M. A., *et al*. (2003). Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature, 426(6965): 450–454.
3. Monteil, V.; Kwon, H.; Prado, P.; Hagelkrüys, A.; Wimmer, R. A.; Stahl, M., *et al*. (2020). Inhibition of SARS-CoV-2 Infections in Engineered Human Tissues Using Clinical-Grade Soluble Human ACE2. Cell, 181(4): 905–913.e7.
4. Wang, Q.; Zhang, Y.; Wu, L.; Niu, S.; Song, C.; Zhang, Z., *et al*. (2020). Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2. Cell, 181(4): 894–904.e9.
5. Zhou, P.; Yang, X. L.; Wang, X. G.; Hu, B.; Zhang, L.; Zhang, W., *et al*. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature, 579(7798): 270–273.
6. Guan, W. J.; Ni, Z. Y.; Hu, Y.; Liang, W. H.; Ou, C. Q.; He, J. X., *et al*. (2020). Clinical Characteristics of Coronavirus Disease 2019 in China. The New England Journal of Medicine, 382(18): 1708–1720.
7. Huang, C.; Wang, Y.; Li, X.; Ren, L.; Zhao, J.; Hu, Y., *et al*. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet (London, England), 395(10223): 497–506.
8. Wang, D.; Hu, B.; Hu, C.; Zhu, F.; Liu, X.; Zhang, J., *et al*. (2020). Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA, 323(11): 1061–1069.
9. Xu, Z.; Shi, L.; Wang, Y.; Zhang, J.; Huang, L.; Zhang, C., *et al*. (2020). Pathological findings of COVID-19 associated with acute respiratory distress syndrome. The Lancet. Respiratory Medicine, *8*(4): 420–422.
10. Zhou, P.; Yang, X. L.; Wang, X. G.; Hu, B.; Zhang, L.; Zhang, W., *et al*. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature, *579*(7798): 270–273.
11. Tai, W.; He, L.; Zhang, X.; Pu, J.; Voronin, D.; Jiang, S., *et al*. (2020). Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. *Cellular & molecular immunology*, *17*(6), 613–620.
12. Wrapp, D.; Wang, N.; Corbett, K. S.; Goldsmith, J. A.; Hsieh, C. L.; Abiona, O., *et al*. (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science (New York, N.Y.), 367(6483): 1260–1263.
13. Xu, J.; Xu, X.; Jiang, L.; Dua, K.; Hansbro, P. M. and Liu, G. (2020). SARS-CoV-2 induces transcriptional signatures in human lung epithelial cells that promote lung fibrosis. *Respiratory Research, 21*(1): 182.
14. Hoffmann, M.; Kleine-Weber, H.; Schroeder, S.; Krüger, N.; Herrler, T.; Erichsen, S., *et al*. (2020). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell, 181(2): 271–280.e8.

15. Li F. (2016). Structure, Function, and Evolution of Coronavirus Spike Proteins. Annual Review of Virology, *3*(1): 237–261.

16. Rodrigues, R. and Costa de Oliveira, S. (2021). The Impact of *Angiotensin-Converting Enzyme 2* (*ACE2*) Expression Levels in Patients with Comorbidities on COVID-19 Severity: A Comprehensive Review. Microorganisms, 9(8): 1692.

17. Zhuang, M. W.; Cheng, Y.; Zhang, J.; Jiang, X. M.; Wang, L.; Deng, J., *et al*. (2020). Increasing host cellular receptor-angiotensin-converting enzyme 2 expression by coronavirus may facilitate 2019-nCoV (or SARS-CoV-2) infection. Journal of Medical Virology, 92(11): 2693–2701.

18. Ziegler, C. G. K.; Allon, S. J.; Nyquist, S. K.; Mbano, I. M.; Miao, V. N.; Tzouanas, C. N., *et al*. (2020). SARS-CoV-2 Receptor ACE2 Is an Interferon-Stimulated Gene in Human Airway Epithelial Cells and Is Detected in Specific Cell Subsets across Tissues. Cell, 181(5), 1016–1035.

19. Elemam, N. M.; Hasswan, H.; Aljaibeji, H.; Sharif-Askari, N. S.; Halwani, R.; Taneera, J., *et al*. (2022). Profiling Levels of Serum microRNAs and Soluble ACE2 in COVID-19 Patients. Life (Basel, Switzerland), *12*(4): 575.

20. A Elrayess, M.; T Zedan, H.; A Alattar, R.; Abusriwil, H.; Al-Ruweidi, M. K. A. A.; Almuraikhy, S., *et al*. (2022). Soluble ACE2 and angiotensin II levels are modulated in hypertensive COVID-19 patients treated with different antihypertension drugs. Blood pressure, *31*(1): 80–90.

21. Liu, Y.; Yan, L. M.; Wan, L.; Xiang, T. X.; Le, A.; Liu, J. M., *et al*. (2020). Viral dynamics in mild and severe cases of COVID-19. The Lancet. Infectious diseases, *20*(6): 656–657.

22. Liu, Y.; Yang, Y.; Zhang, C.; Huang, F.; Wang, F.; Yuan, J., *et al*. (2020). Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. Science China. Life sciences, 63(3): 364–374.

23. Pinto, B. G. G.; Oliveira, A. E. R.; Singh, Y.; Jimenez, L.; Gonçalves, A. N. A.; Ogava, R. L. T., *et al*. (2020). ACE2 Expression Is Increased in the Lungs of Patients With Comorbidities Associated With Severe COVID-19. The Journal of Infectious Diseases, 222(4): 556–563.

24. Gheblawi, M.; Wang, K.; Viveiros, A.; Nguyen, Q.; Zhong, J. C.; Turner, A. J., *et al*. (2020). Angiotensin-Converting Enzyme 2: SARS-CoV-2 Receptor and Regulator of the Renin-Angiotensin System: Celebrating the 20th Anniversary of the Discovery of ACE2. Circulation Research, *126*(10): 1456–1474.

25. Bunyavanich, S.; Do, A. and Vicencio, A. (2020). Nasal Gene Expression of Angiotensin-Converting Enzyme 2 in Children and Adults. JAMA, 323(23): 2427–2429.

26. Heinonen, S.; Helve, O.; Andersson, S.; Janér, C.; Süvari, L. and Kaskinen, A. (2022). Nasal expression of SARS-CoV-2 entry receptors in newborns. Archives of disease in childhood. Fetal and neonatal edition, *107*(1): 95–97.

27. Castagnoli, R.; Votto, M.; Licari, A.; Brambilla, I.; Bruno, R.; Perlini, S., *et al*. (2020). Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection in Children and Adolescents: A Systematic Review. JAMA pediatrics, *174*(9): 882–889.

28. Peters, M. C.; Sajuthi, S.; Deford, P.; Christenson, S.; Rios, C. L.; Montgomery, M. T., *et al*. (2020). COVID-19-related Genes in Sputum Cells in Asthma. Relationship to Demographic Features and Corticosteroids. American Journal of Respiratory and Critical care Medicine, *202*(1): 83-90.

29. Liu, J.; Ji, H.; Zheng, W.; Wu, X.; Zhu, J. J.; Arnold, A. P., *et al*. (2010). Sex differences in renal angiotensin converting enzyme 2 (ACE2) activity are 17β-oestradiol-dependent and sex chromosome-independent. Biology of sex differences, *1*(1): 6.

30. Scialo, F.; Daniele, A.; Amato, F.; Pastore, L.; Matera, M. G.; Cazzola, M., *et al*. (2020). ACE2: The Major Cell Entry Receptor for SARS-CoV-2. Lung, 198(6): 867–877.