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Short communication

Angiotensin-converting enzyme-2 and interleukin-12 serum level as indicator to severity between COVID-19 patients

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Abstract
The Coronavirus, one of the most rapidly spreading respiratory viruses, caused a worldwide epidemic that killed about six million people. This led to the fast development of several vaccines and drugs to reduce disease severity and speed patient recovery. This study aimed to identify the serum levels of each of the angiotensin-converting enzyme-2 and interleukin-12 .The severity of infection in coronavirus COVID-19 patients was compared to immune levels of these cytokines and receptors in the different cases of COVID-19 patients. This case-control study included 90 blood samples from COVID-19 patients with ages between 15-80 years. Results revealed that the serum levels of both angiotensin-converting enzyme-2 (ACE-2) and interleukin-12 (IL-12) were measured in COVID-19 patients and the results were compared using an independent T-test, it was found that their levels for interleukin-12 revealed a significant difference (P ≤0.05) in the serum levels of severe cases when compared with non-severe cases. There was an increase in the serum level of IL-12 in severe cases was 33.340 ng/L, in the serum level and in non-severe cases was 20.913 ng/L. (P ≤0.000), and for angiotensin-converting enzyme-2 this study revealed a significant difference in ACE-2 serum levels in severe cases (P ≤0.05) when compared with the non-severe cases of patients with COVID 19. The serum level of ACE-2 in severe cases was 11.023 ng/ml, and in non-severe cases, it was 5.443ng/ml (P ≤0.000). It was concluded that the emerging coronavirus works to create an immune storm represented by raising the serum levels of both ACE-2 and IL-12 that contribute to the damage to the alveoli in severely COV-19 patients.

Keywords: Covid-19, Interleukin-12 (IL-12), Angiotensin-Converting Enzyme-2 (ACE-2), ELSA test

INTRODUCTION

The COVID19 has been a source of concern for all countries throughout the world, including our own, for the past three years, as an economic and commercial movement has been halted, education in schools has been halted, and e-learning has evolved. It became evident to us that the virus enters the human body through infected people's droplets and that it then requires the angiotensin-converting enzyme to bind to the cells of the alveoli

The spike protein's attachment to the human body cell's ACE2 receptor (ACE2) is the initial and crucial step in virus entry. The S protein's receptor-binding domain (RBD) comprises the receptor-binding motif (RBM), which is responsible for docking to the receptor (Zhang *et al*., 2020). Human host cells have ACE-2 receptors on their membranes. Though to varying degrees, it can be present within every one of the bodily organs. Since the SARS-COV virus's primary target is the alveolar cells, it is clear that ACE-2 is strongly expressed in alveolar cells but not in membranes of the nose, mouth, or eye cells. Both a pH-dependent and a pH-independent pathway have been postulated to be possible entry points for the virus into cells. Genetic material is transmitted to the host cell without the virion or host cell membrane fusing in the pH-dependent route. When the virus binds to the cleavage site of the ACE2 receptor, the endocytosis route is triggered, and this is the final step (Zou *et al*., 2020). Both the innate and adaptive immune systems activated by COVID-19, which results in the release of cytokines and chemokines and the production of pro-inflammatory cytokines by T and B lymphocytes. (Crisci *et al*.,2020). When comparing critical and non-critical patients, it was revealed that Intensive care unit patients' plasma had

greater IL-12 concentrations than mild patients' plasma (Yang *et al*.,2020). Interleukin-12 (IL-12) is the primary regulator of adaptive type 1 cell-mediated defense (IL-12). One of the most important mechanisms in neoplasia and virus defenses. Human clinical research (Galon *et al*.,2006). Examining several studies attributing improved clinical outcomes substantiates this claim and IL-12-based therapeutic strategies (van Herpen *et al*.,2004). This research aimed at study reveal and identify the serum levels of each of the angiotensin-converting enzyme-2 and interleukin-12 in severe and non-severe C0VID-19 patients

MATERIALS AND METHODS

Sample collection

Nighty blood samples from COVID-19 patients with ages between 15-80 years were distributed according to the severity as the following (45 severe and 45 non-severe) who were hospitalized at the COVID 19 Wards in Merjan Medical City and Imam Sadiq Hospital in Babylon Province for 2 months (November and December 2022). All subjects had five ml of venous blood drawn after sterilization with 70% ethyl alcohol on the skin over the vein, which was then placed into a Gel tube for serum separation. After 30 min at room temperature, the blood was centrifuged for five min at 3000 rpm. The serum was then collected in two repeaters in a sterile Eppendorf tube and kept refrigerated at -20 C.

Immunological Study

ELISA kit was applied to the in vitro quantitative determination of IL-12 and ACE-2 concentrations in serum according to the manufacturer protocol (Korain Biotech CO.). The lower limit of detection for each was 2.5ng/l, and 0.75ng/ml, respectively.

Ethical consideration

The approvals were obtained from all the participants and after obtaining the fundamental approval from the official authorities. The following information was recorded (patient name, age, sex, date of infection, and chronic disease).

RESULTS AND DISCUSSION

Estimation of IL-12 serum level between COVID-19 patients

Getting rid of COVID-19 patients includes a strong immune response capable of controlling the infection and appropriate treatment. The present results revealed a significant difference (P ≤0.05) in the serum levels of IL-12 in severe cases when compared with non-severe cases. There was an increase in the serum level of IL-12 in severe cases was 33.340 ng/L, in the serum level of non-severe cases was(20.913 ng/L, P ≤ 0.000), as shown in Table 1.

Long *et al*. (2020) agreed with the present study and confirmed that IL-12 serum level was increased in the symptomatic group of COVID-19 patients when compared with the asymptomatic group was, observing a reduction of IL-12 serum level and other inflammatory cytokines and chemokines

The results of the Tjan *et al*. (2021) study, which contradict the current findings, showed that serum levels of IL-12 were increased in asymptomatic patients and moderate patients, while low serum levels of IL-12 were found in severely symptomatic COVID-19 patients. The discrepancy between the two studies may be attributable to the time of sample collection after infection and the number of samples collected for severe cases. From these findings, the increase in serum IL-12 levels in respiratory care unit (RCU) patients as they approach the normal limit in non-severe patients resulted in the formation of an immune storm that contributed to the damage to the alveoli in severely COVID -19 patients.

Estimation of ACE-2 serum level between COVID-19 patients

On the other hand, in the present study, the findings revealed a significant difference in ACE-2 serum levels in severe cases (P ≤0.05) when compared with the non -severe cases of patients with COVID 19. The serum level of ACE-2 in severe cases was 11.023 ng/ml and in non-severe cases, it was (5.443ng/ ml, P ≤ 0.000), as shown in Table 1. Sriram and Insel (2020) support this finding and stated that ACE2 is found in all people, but the amount varies between individuals, tissues, and cells. ACE2 may be elevated in hypertension, diabetes, and coronary heart disease patients. According to a study conducted on a cohort of 12 COVID-19 patients, circulating Ang II levels were significantly higher than in healthy controls and were linearly related to viral load, indicating a direct link between tissue ACE2 downregulation and systemic RAS imbalance and facilitating the development of multiorgan damage from SARS-CoV-2 infections (Liu *et al*.,2020). Another study found that COVID-19 participants had an imbalance in the renin-angiotensin-aldosterone system, with an increased expression of the ACE2, renin, and kallikrein enzymes in their lavage fluids (Garvin *et al*., 2020). Xu *et al*. (2020) observed that the high prevalence of pneumonia and bronchitis in patients with severe COVID-19 infection can be attributed to the increased expression of ACE2 in the lung, intestine, kidney, and blood vessel epithelial cells.

The present study compared ACE-2 and IL-12 between males and females in both severe and non-severe patients and found there were no significant differences

Table 1. Estimation of IL-12 and ACE-2 serum levels between severe and non-severe COVID-19 patients and the correlation between these parameters

Type of patients	No. of patients	IL-12 ng/L Mean± S.D	P-value	ACE-2 ng/ml Mean± S.D	P-value	Correlation	r- value	P-value
Severe	45	33.340±11.987	0.000	11.023 ±5.731	0.000	IL-12&ACE-2	0.586**	0.000
Non-severe	45	20.913±4.361		5.443±1.211				

*Significant (P≤0.05). ** Significant correlation at the level of significance (0.01)

Table 2. Estimation of IL-12, ACE-2 between male and female severe and non-severe COVID-19 patients

Parameters		Males Mean± S. D	Females Mean± S. D	P-value
IL-12 ng/L	Severe	35.044±12.948	31.636±10.962	0.341
	Non-severe	21.164± 4.838	20.722±3.873	0.372
ACE-2 ng/ml	Severe	12.128±7.330	9.917±3.303	0.194
	Non-severe	5.411±0.996	5.403±1.409	0.166

*Significant (P≤0.05)

between males and females in these two parameters in both severe and non-severe patients, as shown in Table 2.

Inferring from this, it can be concluded that these two variables are not associated with an increase in symptoms of infection in males when compared to females and that the cause of male infection may be stronger than that of female infection because females have a strong immune response; are less susceptible to viral infections; because of that, they have a high level of the protective hormone estrogen and progesterone (Hussain *et al*., 2020; Valencia, 2020).

Correlation among the immunological parameter

The correlation of ACE-2 and IL-12 serum levels in COV-19 patients showed that there is a direct correlation between the cytokine and this immune receptor, and the results showed the Correlation was significant at the 0.01 level (2-tailed) as shown in Table 1.

Yao *et al*. (2020) support the present study that clarifies that antigen presentation stimulates the body's humoral and cellular immunity, mediated by virus-specific immune cells. One of the main causes of death from coronavirus was cytokine storm, which was an uncontrolled systemic inflammatory response. COVID-19 induced a strong immune response by releasing pro-inflammatory cytokines and chemokines, similar to SARS-CoV and MERS-CoV infections.

Conclusion

The present study concluded that both ACE-2 and IL-12 are involved in the pathogenesis of COV-19 infection. They are one of the factors that contribute to the development of acute and fatal infections in COV-19 patients.

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Conflict of interest

The authors declare that they have no conflict of interest.

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