Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i19.3672

ACE II RECEPTOR GENE POLYMORPHISM, QUANTITATIVE IMMUNOLOGICAL DETECTION OF ACE II RECEPTORS AND MICRONUTRIENTS ASSOCIATION WITH HOSPITAL ACQUIRED COVID-19 PATIENTS

Amber Khan[1], Saira Yahya[2]*, Anum Siraj[3], Maliaka Arif[4], Muhammad Umair[5], Muhammad Saleem Iqbal Khan[6], Muhammad Abubakar[7], Rooma Ayyoub[8], Saleem Awan[9]

[1]Biosciences Department, Shaheed Zulfikar Ali Bhutto Institute of Science and Technology (SZABIST) Karachi, Email: amberkz303@gmail.com
[2]*Associate Professor, Biosciences Department, Shaheed Zulfikar Ali Bhutto Institute of Science and Technology (SZABIST) Karachi, Email: dr.saira.yahya@szabist.pk
[3]Biosciences Department, Shaheed Zulfikar Ali Bhutto Institute of Science and Technology (SZABIST) Karachi, Email: anumsiraj@hotmail.com
[4]MBBS. Dow International Medical College, Karachi, Email: maheen.arif48@gmail.com
[5]Center of Excellence in Molecular Biology, University of the Punjab, Email: umairkhan5328@gmail.com
[6]Department of Physiology and Department of Cardiology of the Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China, Email: msaleemkhan@zju.edu.cn
[7]Cancer Genetics and Epigenetic Lab, Biosciences Department, COMSATS University Islamabad, Email: abubakarbbt3@gmail.com
[8]Cancer Genetics and Epigenetic Lab, Biosciences Department, COMSATS University Islamabad, Email: roomaayyoub4646@gmail.com
[9]Biosciences Department, Shaheed Zulfikar Ali Bhutto Institute of Science and Technology (SZABIST) Karachi, Email: shezadaa60@gmail.com

***Corresponding Author: -** Saira Yahya
*Associate Professor, Biosciences Department, Shaheed Zulfikar Ali Bhutto Institute of Science and Technology (SZABIST) Karachi, Email: dr.saira.yahya@szabist.pk

Abstract

The outburst of the immensely communicable COVID-19 has proposed a consequential challenge to the world's health, particularly for those who are already countering any disease and are thus immunocompromised. COVID-19 uses human *ACE2* (angiotensin-converting enzyme 2), an epithelial cell of the lungs with receptors, to gain entry into human cells, which is the first step of viral infection. In this study, we have evaluated the levels of *ACE2* in serum and its gene expression to confirm that high *ACE2* levels and its gene polymorphism could be risk factors for COVID-19, or those patients, who were COVID-positive and immunocompromised, have more *ACE2* levels in comparison to non-COVID-patients with same population. Secondly, we assessed the levels of micronutrients, which showed the risk factors for COVID-19. Our data revealed that the gene expression of the ACE2 enzyme and its genotype G8790A polymorphism are associated with the progression of the disease. The levels of micronutrients were also found to be linked with COVID-19 progression in immunocompromised patients. The findings of the study have suggested that if levels

of ACE2 enzyme and micronutrients are controlled, then the progression of the disease can be decreased.

Keywords: COVID-19, ACE2, Vitamin D, Immunocompromised, Vitamin B12

1. INTRODUCTION

COVID-19 became a global pandemic and resulted in a huge lockdown globally [1]. The virus belongs to the family Coronoviridae, and is known to be the largest single-stranded RNA virus of its kind. The most common symptom of this disease is congestion of the respiratory system and shortness of breath [2], 3]. Its mortality rate is low, however, the age of an individual plays an important role in the severity of the disease. People with lower immunity face complications such as interstitial pneumonia and alveolar destruction [4–6]. The majority of the *ACE2* variant has demonstrated greater binding affinity with the COVID-19 virus S-protein [7]. The virus particle is known to enter the cells through the ACE-2 receptor, which is a type I transmembrane glycoprotein and consists of a single extracellular catalytic domain with 805 amino acids [8]. The lower level of ACE2 enzyme is observed in different organs of the body, such as the heart, kidneys, testes, and tissues, especially the lung and colon [9, 10]. The human Xp22 chromosome contains the gene that codes for the ACE2 protein. Its 40 kB of genomic DNA is made up of 20 introns and 18 exons [11]. The pathologies of the RAAS system, such as essential hypertension, are linked to polymorphisms in the *ACE2* gene [12]. The geographic polymorphism of a gene has been linked to the degree of ACE2 expression, and results show that ACE2 expression is higher in East Asian populations than in European populations. As a result, various populations can be connected to the virus' susceptibility. In the human genome, ACE2 is a polymorphic gene having about 140 single nucleotide polymorphisms (SNPs) [13]. Several studies have observed the ACE2 polymorphism associated with COVID-19 [14–16]2020), and the most commonly studied SNPs were rs2106809 and rs2285666 [17, 18]. The G8790A polymorphism (rs2285666) is found in intron 3 on chromosome Xp22. Since this intron is crucial in the splicing mechanism, changes to its polymorphism affect how genes are expressed [19]. Some ACE2 mutations affect the enzyme's affinity for the SARS-COV-2 virus [20,21]. The ACE2 binding affinity for SARS-CoV-2 is also known to be influenced by the SNP rs2285666 [22]. The second SNP that is frequently researched is A2350G (rs4343), which is found on exon 17 of the ACE1 gene [23], It can be inferred that this variant may be more vulnerable to COVID-9 based on the effects of this polymorphism on the serum levels of the ACE-1 enzyme [24,25]. The expression of this gene is found to be associated with the development of kidney diseases, hypertension, and viral diseases. Moreover, the polymorphism associated with this gene is also reported to increase the risk of COVID-19. A region of ACE2 on the cell membrane interacts with the SARS-CoV-1 spike protein [26]. That results in the increased expression of Ang II, which promotes cell growth and the proliferation of lung fibroblasts [27]. Ang II increases the expression of transforming growth factor-β (TGF-β), reactive oxygen species, and the release of pro-inflammatory cytokines [28]. This entire procedure demonstrates how ACE2 expression is decreased when a virus penetrates cells. The ratio ACE/ACE2, which is critical to cell physiology, serves as a marker for the expression of ACE and ACE2. A rise in this ratio during infection with COVID-19 may impair the cell's function [4]. Vitamin D3 is essential for the healthy functioning of the body, including muscles and bones. The loss of muscle mass and strength is associated with low levels of this vitamin [29]. Additionally, it is essential for preserving hormone levels and has been linked to bone turnover by raising certain hormones [30]. Lower back pain has also been linked to vitamin D3, and a Saudi Arabian study found that taking vitamin D pills reduced symptoms by 95% [31]. Vitamin D has a potential role in many metabolic processes, including the immune system. The immune cells possess targets for active vitamin D and also produce it. They also activate this hormone in a local fashion. Hence, it has an established role within the immune system [32]. Vitamin B12 is the most complex form among all the vitamins in its class [33, 34]. Vitamin B12 is mainly involved in the formation of red blood cells. Additionally, it has been linked to other metabolic pathways such as cell proliferation, division, and myelin production. Additionally, Vitamin

B12's immune system benefits are well-established [35]. The powerful effect of Vitamin B12 in the removal of viral particles from host cells has been demonstrated in numerous investigations [36]. In this study, we measured the serum levels of ACE2 enzymes to better understand the function of ACE2 in COVID-positive and negative immunocompromised individuals. After contracting the COVID-19 infection, immunocompromised patients who are recognized for having weaker immunity owing to any cause, such as an organ transplant or prior chemotherapy sessions, create extreme symptoms. Therefore, we examined the ACE2 gene polymorphism as well as the serum concentrations of micronutrients including vitamin D3 and vitamin B12.

2. EXPERIMENTAL

Sample Collection and Processing

An ethical approval was obtained from the Ethics Review Board, SZABIST Karachi, Pakistan (ref. no. IERB(8)/SZABIST-KHI(LIFE)/1930104/210052) prior to the study. The blood samples were collected at Al-Mustafa Medical Center for immune-compromised patients, whowere dependent on malignant hemodialysis, and had chronic diabetes. Healthy controls (HCs) (n = 30) were>18 years and had no prior history of any type of infection within six months (i.e., viral bacteria). Whereas, immune-compromised patients with active COVID-19 infection (n = 30) (ICP-CP) and immune-compromised individuals with inactive COVID-19 infection (n = 30) (ICP-CN) were selected as cases. A total of 5 mL of whole blood was drawn into a purple top tuve from each patient by following all standard SOPs. The whole blood was centrifuged at 14000 g for 15 minutes at 4°C, and plasma was collected for the ELISA. Half of the whole blood was utilized for the DNA extraction. The samples were allotted 400 µL per tube and stored at -80°C for further experimentation.

DNA Extraction and Estimation

For the lysis of the cells, 100 µl of whole blood was mixed in 1 ml of DNAZOL reagent and gently mixed with pipetting up and down. The mixture was centrifuged at 10,000 g for 10 min. The obtained pellet was washed with 0.9% NaCl. DNA was precipitated with the addition of 0.5 ml of 100% ethanol per 1 ml of DNAzol reagent. Sample tubes were inverted and stored at room temperature (1-3 min). The solution was centrifuged at 4000 g for 1-2 min at 4 ºC for DNA precipitation. The DNA pellet was washed twice with 0.8-1.0 ml of 75% ethanol. During each wash, the suspended DNA was carefully inverted 3-6 times. After some time, the DNA was allowed to settle down and the etanol was collected by pipetting or decanting. The dried DNA pellet wasdissolved in a 100 µl TE buffer. The samples were run on 1% agarose gel electrophoresis to determine the integrity of the DNA and were quantified in a nano-drop.

Genotyping

ACE2 gene polymorphism Through RFLP: The *ACE2* gene in the DNA samples of participants was amplified using PCR. The allele-specific primers for the *ACE2* gene were selected as mentioned in [37]. The sequence of the forward primer was 5'-CATGTGGTCAAAAGGATATCT-3' and the reverse primer was 5'-AAAGTAAGGTTGGCAGACAT-3'. The PCR cycle program was set as initial denaturation at 95ºC for 1 minute, followed by 34 cycles of denaturation at 95 ºC for 40 seconds, annealing at 61ºC for 30 seconds, and extension at 72ºC for 30 seconds. For RFLP, the PCR products were digested at 37^0C for 8 hours straight by using 1.5 units of *AluI* restriction enzyme for the detection of SNP G879A in the *ACE2* gene. Only one DNA fragment of 466 bp in the agarose gel was considered the reference G allele genotype; while two DNA fragments of 281 and 185 bp were considered as the A allele genotype. **Enzyme Linked Immunosorbent Assay (ELISA)** The levels of *ACE2*, vitamin B-12, and vitamin D3 were evaluated by using a human ACE2 ELISA kit, 25 Vitamin B12 ELISA kit, and 25-OH Vitamin D ELISA kit, respectively, by following the manufacturer's instructions, **Statistical Analysis**: All the statistical analysis was done on SPSS v. 21. One-way ANOVA was applied to identify the significant differences between groups, and a value less than 0.05 was considered significant. For allele and genotype frequencies, Microsoft Excel was used

and values were identified manually by analyzing the gel electrophoresis images. The chi-square value and odd ratios (OR) were also identified on SPSS by applying multinomial logistic regression.

4. RESULTS

Blood samples were collected from 90 individuals (including 30 HCs, 30 immune-compromised COVID-19 positives, and 30 immune-compromised COVID-19 negatives), with the mean ages of 27±2, 42±12, and 28±6 years, respectively. Demographic details of all three groups are shown in Table 1. The immune-compromised patients were cancer and/or hemodialysis patients *ACE2* G8790A Polymorphism through RFLPThe PCR amplification product of the *ACE2* gene was 466 base pairs (bp) in size. The genotypic pattern of the ACE2 gene was determined on the basis of the digestion pattern of PCR products with the restriction enzyme *AluI* at 37 °C for 8 h. The gel electrophoresis of all these groups; healthy population, COVID-19 positive, and COVID-19 negative, is shown in Figures 1A, B, and C. **Genotype and Allele Frequency of the *ACE2* G8790A Polymorphism Among the HC, ICP-CP, and ICP-CN patients** The genotype distribution of HC (HC) showed 76% GG, 8% GA, and 16% AA, while immunocompromised COVID positive (IC-CP) showed 48% GG, 12% GA, and 40% AA, and immunocompromised COVID negative (IC-CN) revealed 56% GG, 3% GA, and 40% AA. The results showed that the genotype distribution between HC and IC-CP was found to be significant (p-value <0.05), while the difference between HC and IC-CN was not significant (Table 3). Furthermore, the allelic frequency of the IC-CP group was significant (p-value <0.05) as compared to HC; IC-CN again showed a non-significant pattern (Table 2). We have found significant differences in the allelic frequency in all the groups with a *p*-value of <0.001 and an Odd Ratio (OR) of 4.413 in IC-CP and 5.63 in IC-CN patients (Table 3). The significant differences in both genotypic and allelic frequencies show the positive contribution of the *ACE2* G8790A polymorphism in the progression of the SAR-Cov-2 virus. The same polymorphism of the *ACE2* gene in diabetes mellitus is found, and the role of the allele is considered a risk factor in the progression of diabetes mellitus. **Enzyme-Linked Immunosorbent Assay (ELISA): *ACE2* Gene, Vitamin B12, and Vitamin D3 Levels in the Serum** ELISA was performed for the determination of ACE2 levels in the serum of a healthy population, Immune-compromised patients (ICP), COVID-19 positives, and COVID-19 negative patients. The results showed that immune-compromised patients with positive COVID-19 had significantly lower levels of ACE2 (*p-value*< 0.05) (Figure 7) compared to the healthy group. While the positive COVID-19 patients showed a significant decrease in ACE2 compared to the COVID-19 negatives (*p-value*< 0.001) (Figure. 2A). No significant difference was observed between HC groups and ICP-CN groups. In the comparative analysis of vitamin B12 in serum in the healthy population with ICP-CP and ICP-CN patients, the result showed an overall significant decrease of vitamin B12 in ICP-CP and ICP-CN (*p*-value <0.001) compared to the HC group (Figure 2B). Whereas, the comparative analysis of serum levels of vitamin D3 in the healthy population compared ICP-CP and ICP-CN patients, showed an overall significant decrease of vitamin D3 in both the IC-CP and IC-CN groups (p-value <0.001) compared to the HC group (Figure 2C). **Association of Three Genotypes With Serum ACE2, Vitamin B12, and Vitamin D3 Levels** To analyze the association of three genotypes (GG, GA, and AA) with the serum levels of ACE2, vitamin B12, and vitamin D3 in three groups, a one-way ANOVA test was applied between them. However, no significant difference in ACE2 expression was found among the genotypes. The serum ACE2 levels and genotypic polymorphism also revealed no association between them (Table 4). The One-way ANOVA test was performed to analyze the significant differences in vitamin B12 with all three genotypes. The data revealed that there was a significant difference in the AA genotype as compared to the reference genotype GG (P-value < 0.05). These results indicate that the A allele is a positive contributor and risk factor in the absorption of vitamin B12 in immunocompromised individuals (Table 4). The statistical data analysis of three genotypes with vitamin D3 levels revealed that the AA genotype is significant as compared to the GG genotype, which is set as a reference. These results indicate that the A allele is a positive contributor and risk factor in the absorption of vitamin D3 in immunocompromised individuals (Table 4). **Gender Association With*ACE2* Levels**To analyze the

expression pattern in males versus females, the statistical test was applied. The comparative analysis between the males and females showed that the Females had a higher serum concentration of *ACE2* as compared to men, however, the results were non-significant (p-value > 0.05) (Figure 3A). The serum levels of vitamin B12 were not significantly different (p-value >0.05) between males and females (Figure 3B). The independent student t-test was performed to find out the significance between males and females in their vitamin D3 levels, and the results showed that women have comparatively lower vitamin D3 levels as compared to men (*p*-value = 0.0064) (Figure 3C). **Correlation Analysis**: The Pearson correlation analysis was performed to find out the positive and negative correlation between *ACE2* serum levels, vitamin D3 levels, and vitamin B12 levels. Our results showed that *ACE2* levels were positively correlated with vitamin D3 levels (R2 = 0.0567, *p*-value = 0.033) (Figure 4A). The correlation analysis between both vitamins, B12 and D3, was also found to be significant and positively correlated with each other (R2 = 0.0235,*p-value* of < 0.01) (Figure 4B). However, *ACE2* serum levels were not found to be significantly correlated with Vitamin B12 levels (R2 = 0.4679, p-value > 0.05) (Figure 4C).

FIGURES, TABLES AND SCHEMES

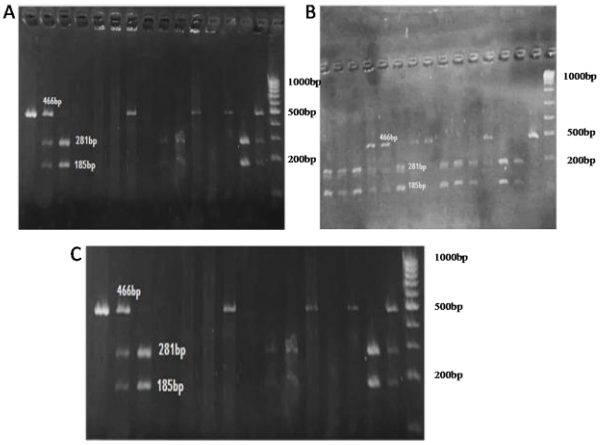

Fig. (1). Agarose gel (2%) electrophoresis results of HC (A), ICP-CP (B), and ICP-CN (C). The single band at 466 bp in a sample represents a dominant G allele genotype; two bands of 281 and 185 bp in a sample represent a recessive A allele genotype. All three bands (466, 281, and 185 bp) in a sample represent a heterozygous GA allele genotype

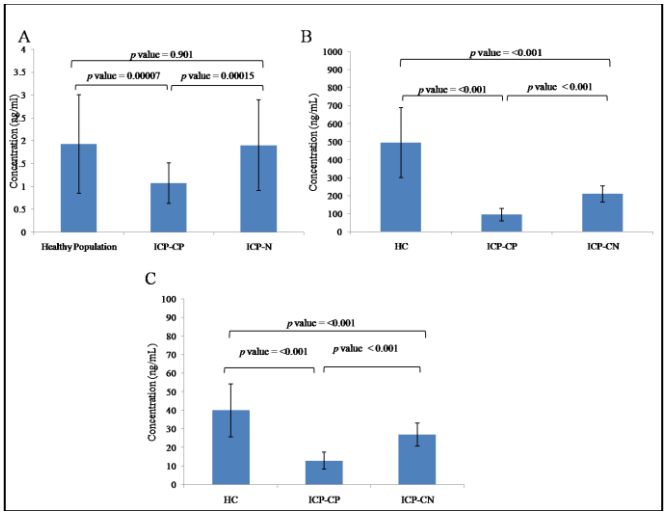

Fig. (2). The levels of (A) ACE2, (B) Vitamin B12, and (C) Vitamin D3 in HC, ICP-CP, and ICP-CN.

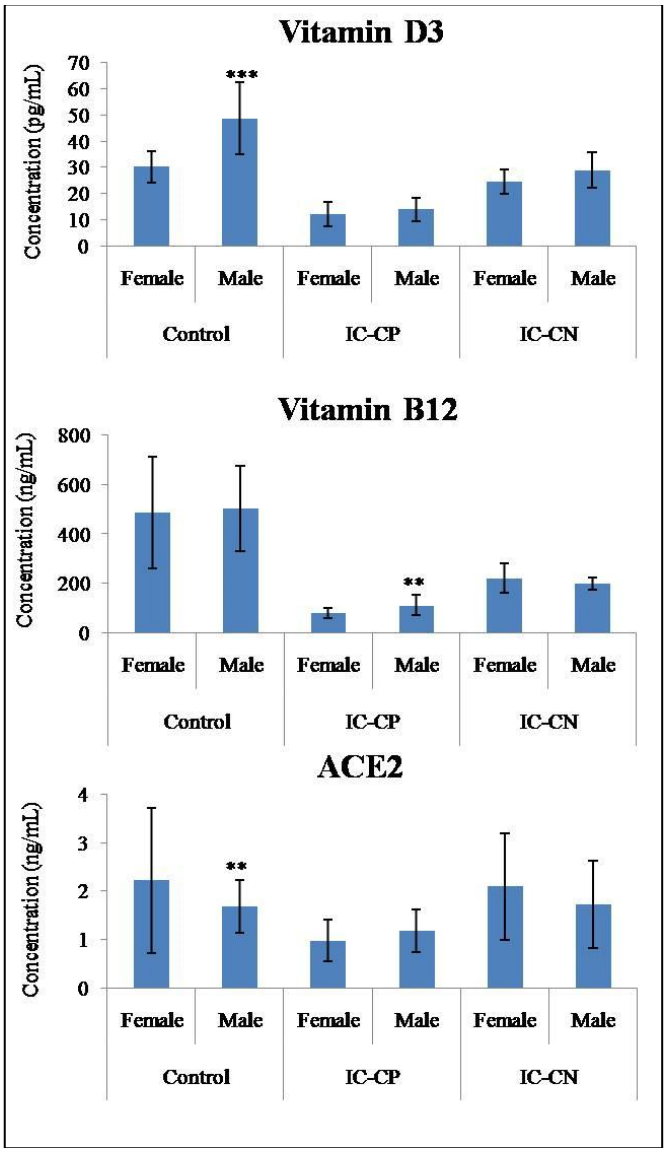

Fig. (3). Comparative analysis of serum levels of Vitamin D3, Vitamin B12, and ACE2 in males and females in male and female groups of controls, IC-CP, and IC-CN.

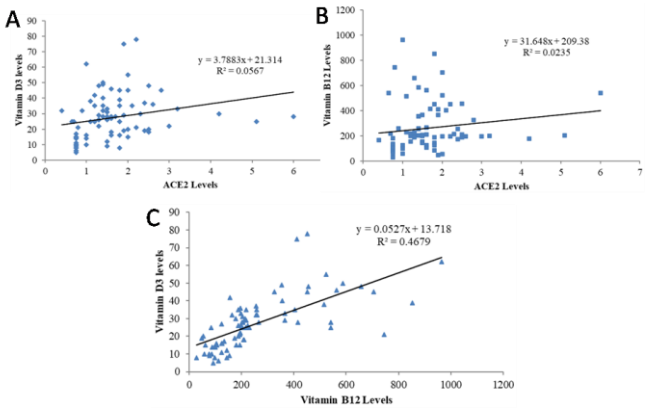

Fig. (4). The correlation analysis between the serum levels of (A) ACE2 and vitamin D3, (B) vitamin B12 and vitamin D3, and (C) ACE2 and vitamin 12.

Table 1. Demographic Characteristics of Immuno-compromised Patients and HCs

Characteristics	HC	IC-CP	IC-CN	Total
N (relative percentage)	30	30	30	90 (100%)
Age (mean)	27±2	42±12	28±6	
Male (n)	16	14	16	
Female (n)	14	16	14	

Table 2. The genotypic distribution of three alleles (GG, GA, and AA) in all three groups; Control, Immunocompromised COVID Positive, and Immunocompromised COVID Negative).

Gene	Groups (n)	Genotype Frequencies (n %)				
		GG	GA	AA	X2	p-value
	Control	20 (74.1)	2 (7.4)	5 (18.5)		
	IC-CP	10 (38.5)	3 (11.5)	13 (50)	0.029	0.036
	IC-CN	14 (46.7)	0	16(53.3)	0.013	0.186

Table 3. The allelic distribution of the three alleles; (GG, GA, and AA) in all the three groups.

Gene	Groups (n)	Allelic frequencies (%)				
		G	A	X2	p-value	OR (95% CI)
ACE2 G8790A	Control	29 (82.9)	6 (17.1)			
	IC-CP	22 (56.4)	17 (43.6)	0.0003921	0.023	3.735 (1.264 to 11.03)
	IC-CN	25 (58.1)	18 (41.9)	0.0006569	0.026	5.63 (2.46 to 12.86)

Table 4: Association of GG, AA, and GA genotypes with serum ACE2, Vitamin B12 and Vitamin D3 levels

Gene	Genotypes	HC	IC-CP	IC-CN	P-value
ACE2	GG	1.9±1.18	1.31±0.55	1.72±1.01	
	AA	1.64±0.42	1.03±0.38	2.06±0.98	1
	GA	1.5±0.14	0.75±0	0	0.374
Vitamin B12	GG	491±194	92±29	214±26	
	AA	421±213	213±114	207±57	0.03586
	GA	459±148	148±67	0	0.94481
Vitamin D3	GG	42±16	17±4	28±4	
	AA	37±7	11±3	26±7	0.005
	GA	48±2	11±3	0	0.972

5. DISCUSSION

COVID-19 became a global pandemic, and a challenge to health care [38]. Angiotensin-converting enzyme 2 (*ACE2*) in humans (and rodents) is an endothelium-binding carboxymonopeptidase with a single active catalytic region. Its expression is predominantly restricted to animal endothelial cells of the arteries, arterioles, and venules in various organs, including the heart, lungs, and kidneys [39].

The *ACE2* gene encoded endothelial receptor plays a vital role in the entrance of Sars-2 virus [38,40,41]. Several studies have been conducted to check the *ACE2* expression pattern in COVID-19 patients [39,41–49], These studies suggest the *ACE2* expression pattern is an important factor not only for the entrance of the virus, but also that it may result in other complications, i.e., cardiac diseases (arrhythmias, sudden cardiac death, and systolic and diastolic dysfunction) [39], hypertension, and acute respiratory distress syndrome (ARDS) [43]. This study was conducted to evaluate the serum levels of *ACE2* in 90 participants who were normal/healthy individuals (HC), immunocompromised patients with COVID-19 positives (ICP-CP), and COVID-19 negatives (ICP-CN). Moreover, along with the serum levels of the *ACE2* enzyme, other micronutrients such as vitamin B12 and D3 were also evaluated. The significantly lower serum levels of ACE2in the ICP-CP compared to the HC and ICP-CN were observed in our results. These findings are consistent with the findings of Chaudhry et al (2020). The lower levels of the ACE2 receptor, which is the main entry point for SARS-CoV-2, and SARS-CoV, might lead to other complications like cardiac diseases, hypertension, and ARDS. Moreover, SARS-CoV-2 infection was reported to lower the ACE2 enzyme, which leads to ACE1/ACE2 imbalance [50]. The SNP tested in our study, G8790A (rs2285666), has an endogenous structure and this mutation can alter mRNA binding as well as affect gene expression and ACE2 protein levels [11,51]. In a study conducted to find the association between the polymorphism of ACE2 and T2D patients, the AA genotype was found to play a significant role in T2D progression compared to the other genotypes [52]. As per our study, the genetic polymorphism of ACE2 G8790A indicates that the significant association of the A allele with the levels of vitamin B12 and D3 can be a risk factor in the progression of severe symptoms in COVID-19. A significant role of G allele was reported to be associated with the prevalence and risk of SARS-CoV-2 infection by Alimoradi et al., conducted in Iran [53], and also in Indian and Caucasian populations [18,54]. However, the variables, which confirm previous studies, did not influence disease severity or COVID-19 mortality [55]. Our study also revealed the non-significant association of genotypic and allelic frequency with the expression of ACE2 protein levels. The gender-wise statistical analysis was also performed to see the impact of the COVID-19 on serum level of *ACE2.* Several studies report that the expression of ACE2 is dependent on gender (Sex differences in renal angiotensin-converting). Men who have conditions such as diabetes, hypertension, and other conditions are more likely to have severe COVID-19 symptoms as compared to women [56]. However, some studies have shown that gender is not linked to the severity of the disease [55]. The differences in the lifestyles of both women and men are also responsible for catching infectious diseases such as COVID-19 [57]. The males are more involved in smoking tobacco, which could trigger the development of pneumonia. On the contrary, females are known to have stronger innate immune systems compared to males, which could justify the lower expression of *ACE2* in males than in females [55,58]. These micronutrients, which are classified as vitamins or minerals, play an important and necessary role in metabolism, immune function, wound healing, antioxidant activity, cellular differentiation and proliferation, and blood coagulation. Therefore, additional therapies must be introduced to compensate for the loss created by virus molecules in a cell [59]. Micronutrients in our body modulate and manipulate the immune response of an individual. The lower level of these micronutrients demands additional supplements for the immunocompromised individuals in order to strengthen their immune system and fight back the virus with greater efficiency [60–62]. Which may increase the survival rate, and decrease the mortality rate in elderly individuals as well as immunocompromised patients [63,64]. Micronutrients such as Vitamin B12 and D3 were also found to be associated with the expression of ACE2 enzymes. The fluctuations in the vitamins of immunocompromised individuals also increase their susceptibility to the virus. For instance, Vitamin B12, which is usually obtained from meat, plays an important role in immunity. A deficiency of vitamin B12 affects the outcomes and progression of infectious diseases [65]. There are several studies that explain the potent role of Vitamin D3 in the body. A meta-analysis and systematic review suggested micronutrient deficiency, i.e., vitamin D3, is associated with the progression of COVID-19 disease. The elderly people with Vitamin D3 deficiency are especially likely to get severe symptoms of COVID-19 [66]. Similarly, the lower levels of vitamin D3, found in

this study, could cause severe illness in people with lower immunity. Moreover, its significance can be highlighted as lower levels of this vitamin can cause mortality [67]. The genotypic profiles, along with the levels of micronutrients in the body, were found to be positively linked with the progression of the disease. In order to understand the complete pathophysiology of the disease, the levels of other micronutrients must be studied. Manipulating these micronutrients could stop the progression of the disease, which can ultimately help in lowering the mortality rate.

CONCLUSION

Low levels of ACE2 and gene polymorphism were found to be associated with COVID-19 progression and severity as lower levels of serum ACE2 levels, vitamin D3, and B12 were observed in COVID-positive immunocompromised patients as compared to healthy controls. In COVID-negative individuals the levels were also found to be comparatively lower as compared to normal, however, non-significant suggestions after treatment ACE2, Vitamin B12, and Vitamin D3 levels were restored to the normal condition. It is also noteworthy that the lower levels of ACE2, Vitamin B12, and Vitamin D3 were associated with the severity of the disease. As per our findings, the severity of the disease could be controlled by giving vitamin D3 and vitamin B12 supplements, which may strengthen the immune system of COVID-19 patients.

REFERENCES

1. 20200604-covid-19-sitrep-136.pdf. Available: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200604-covid-19-sitrep-136.pdf
2. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA. 2020;323: 1239–1242.
3. Banerjee A, Kulcsar K, Misra V, Frieman M, Mossman K. Bats and coronaviruses. Viruses. 2019; 11 (1): 41. 2019.
4. Imai Y, Kuba K, Penninger JM. The discovery of angiotensin-converting enzyme 2 and its role in acute lung injury in mice. Exp Physiol. 2008;93: 543–548.
5. Chi Y, Ge Y, Wu B, Zhang W, Wu T, Wen T, et al. Serum Cytokine and Chemokine Profile in Relation to the Severity of Coronavirus Disease 2019 in China. J Infect Dis. 2020;222: 746–754.
6. Lake MA. What we know so far: COVID-19 current clinical knowledge and research. ClinMed . 2020;20: 124–127.
7. Cao Y, Li L, Feng Z, Wan S, Huang P, Sun X, et al. Comparative genetic analysis of the novel coronavirus (2019-nCoV/SARS-CoV-2) receptor ACE2 in different populations. Cell Discov. 2020;6: 11.
8. 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. PharmacolTher. 2010;128: 119–128.
9. Xu X, Chen P, Wang J, Feng J, Zhou H, Li X, et al. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Sci China Life Sci. 2020;63: 457–460.
10. Harmer D, Gilbert M, Borman R, Clark KL. Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. FEBS Lett. 2002;532: 107–110.
11. Velkoska E, Patel SK, Burrell LM. Angiotensin converting enzyme 2 and diminazene: role in cardiovascular and blood pressure regulation. CurrOpinNephrolHypertens. 2016;25: 384–395.
12. Luo Y, Liu C, Guan T, Li Y, Lai Y, Li F, et al. Association of ACE2 genetic polymorphisms with hypertension-related target organ damages in south Xinjiang. Hypertens Res. 2019;42: 681–689.
13. Chaoxin J, Daili S, Yanxin H, Ruwei G, Chenlong W, Yaobin T. The influence of angiotensin-converting enzyme 2 gene polymorphisms on type 2 diabetes mellitus and coronary heart disease. Eur Rev Med Pharmacol Sci. 2013;17: 2654–2659.

14. Barash A, Machluf Y, Ariel I, Dekel Y. The Pursuit of COVID-19 Biomarkers: Putting the Spotlight on ACE2 and TMPRSS2 Regulatory Sequences. Front Med. 2020;7: 582793.
15. Alimoradi N, Firouzabadi N. impact of genetics on predisposition and prognosis of COVID-19. Trends in Pharmaceutical Sciences. 2021;7. Available: https://journals.sums.ac.ir/article_47482_ea202aa2ea8b29f2944eed3e2d3d45c6.pdf
16. Gemmati D, Tisato V. Genetic Hypothesis and Pharmacogenetics Side of Renin-Angiotensin-System in COVID-19. Genes . 2020;11. doi:10.3390/genes11091044
17. Çelik SK, Genç GÇ, Piskin N, Acikgoz B, Altınsoy B, İşsiz BK, et al. ACE I/D and ACE2 receptor gene (RS2106809, RS2285666) polymorphisms is not related to the clinical course of COVID-19; a case study. Authorea Preprints. 2021. Available: https://www.authorea.com/doi/full/10.22541/au.161572992.20220722
18. Srivastava A, Bandopadhyay A, Das D, Pandey RK, Singh V, Khanam N, et al. Genetic Association of ACE2 rs2285666 Polymorphism With COVID-19 Spatial Distribution in India. Front Genet. 2020;11: 564741.
19. Patel SK, Velkoska E, Freeman M, Wai B, Lancefield TF, Burrell LM. From gene to protein—experimental and clinical studies of ACE2 in blood pressure control and arterial hypertension. Front Physiol. 2014;5. doi:10.3389/fphys.2014.00227
20. Calcagnile M, Forgez P, Iannelli A, Bucci C, Alifano M, Alifano P. Molecular docking simulation reveals ACE2 polymorphisms that may increase the affinity of ACE2 with the SARS-CoV-2 Spike protein. Biochimie. 2021;180: 143–148.
21. Ashoor D, Ben Khalaf N, Marzouq M. A computational approach to evaluate the combined effect of SARS-CoV-2 RBD mutations and ACE2 receptor genetic variants on infectivity: The COVID-19 …. Frontiers in cellular. 2021. Available: https://www.frontiersin.org/articles/10.3389/fcimb.2021.707194/pdf
22. Pouladi N, Abdolahi S. Investigating the ACE2 polymorphisms in COVID-19 susceptibility: An in silico analysis. Molecular Genetics & Genomic Medicine. 2021;9: e1672.
23. Firouzabadi N, Shafiei M, Bahramali E, Ebrahimi SA, Bakhshandeh H, Tajik N. Association of angiotensin-converting enzyme (ACE) gene polymorphism with elevated serum ACE activity and major depression in an Iranian population. Psychiatry Res. 2012;200: 336–342.
24. Firouzabadi N, Tajik N, Shafiei M, Ebrahimi SA, Bakhshandeh H. Interaction of A-240T and A2350G related genotypes of angiotensin-converting enzyme (ACE) is associated with decreased serum ACE activity and blood pressure in a healthy Iranian population. Eur J Pharmacol. 2011;668: 241–247.
25. Zhu X, Bouzekri N, Southam L, Cooper RS, Adeyemo A, McKenzie CA, et al. Linkage and Association Analysis of Angiotensin I–Converting Enzyme (ACE)–Gene Polymorphisms with ACE Concentration and Blood Pressure. Am J Hum Genet. 2001;68: 1139–1148.
26. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 2020;181: 271–280.e8.
27. Voors AA, Pinto YM, Buikema H, Urata H, Oosterga M, Rooks G, et al. Dual pathway for angiotensin II formation in human internal mammary arteries. Br J Pharmacol. 1998;125: 1028–1032.
28. Uhal BD, Kyong Kim J, Li X, Molina-Molina M. Angiotensin-TGF-1 Crosstalk in Human Idiopathic Pulmonary Fibrosis:Autocrine Mechanisms in Myofibroblasts and Macrophages. Curr Pharm Des. 2007;13: 1247–1256.
29. Visser M, Deeg DJH, Lips P, Longitudinal Aging Study Amsterdam. Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. J ClinEndocrinolMetab. 2003;88: 5766–5772.
30. Diamond TH, Levy S, Smith A, Day P. High bone turnover in Muslim women with vitamin D deficiency. Med J Aust. 2002;177: 139–141.

31. Al Faraj S, Al Mutairi K. Vitamin D deficiency and chronic low back pain in Saudi Arabia. Spine . 2003;28: 177–179.
32. Vitamin D. deficiency. Holick MF. N Engl J Med. 2007;357: 266–281.
33. Zhang M, Han W, Hu S, Xu H. Methylcobalamin: a potential vitamin of pain killer. Neural Plast. 2013;2013: 424651.
34. Andres E, Dali-Youcef N. Chapter 19 - Cobalamin (vitamin B12) malabsorption. In: Patel VB, editor. Molecular Nutrition. Academic Press; 2020. pp. 367–386.
35. Herrmann W, Obeid R. Causes and early diagnosis of vitamin B12 deficiency. DeutschesÄrzteblatt International. 2008;105: 680.
36. Maggini S, Pierre A, Calder PC. Immune Function and Micronutrient Requirements Change over the Life Course. Nutrients. 2018;10. doi:10.3390/nu10101531
37. Yi L, Gu YH, Wang XL, An LZ, Xie XD, Shao W, et al. Association of ACE, ACE2 and UTS2 polymorphisms with essential hypertension in Han and Dongxiang populations from north-western China. J Int Med Res. 2006;34: 272–283.
38. Ni W, Yang X, Yang D, Bao J, Li R, Xiao Y, et al. Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19. Crit Care. 2020;24: 422.
39. Oudit GY, Kassiri Z, Jiang C, Liu PP, Poutanen SM, Penninger JM, et al. SARS-coronavirus modulation of myocardial ACE2 expression and inflammation in patients with SARS. Eur J Clin Invest. 2009;39: 618–625.
40. Getachew B, Tizabi Y. Vitamin D and COVID-19: Role of ACE2, age, gender, and ethnicity. J Med Virol. 2021;93: 5285–5294.
41. Chaudhry F, Lavandero S, Xie X, Sabharwal B, Zheng Y-Y, Correa A, et al. Manipulation of ACE2 expression in COVID-19. Open Heart. 2020;7. doi:10.1136/openhrt-2020-001424
42. Li G, He X, Zhang L, Ran Q, Wang J, Xiong A, et al. Assessing ACE2 expression patterns in lung tissues in the pathogenesis of COVID-19. J Autoimmun. 2020;112: 102463.
43. Gagliardi MC, Tieri P, Ortona E, Ruggieri A. ACE2 expression and sex disparity in COVID-19. Cell Death Discov. 2020;6: 37.
44. Fan C, Lu W, Li K, Ding Y, Wang J. ACE2 Expression in Kidney and Testis May Cause Kidney and Testis Infection in COVID-19 Patients. Front Med. 2020;7: 563893.
45. Pinto BGG, Oliveira AER, Singh Y, Jimenez L, Gonçalves ANA, Ogava RLT, et al. ACE2 Expression is Increased in the Lungs of Patients with Comorbidities Associated with Severe COVID-19. medRxiv. 2020. doi:10.1101/2020.03.21.20040261
46. Higham A, Singh D. Increased ACE2 Expression in Bronchial Epithelium of COPD Patients who are Overweight. Obesity . 2020;28: 1586–1589.
47. Tavares C de AM, Avelino-Silva TJ, Benard G, Cardozo FAM, Fernandes JR, Girardi ACC, et al. ACE2 Expression and Risk Factors for COVID-19 Severity in Patients with Advanced Age. Arq Bras Cardiol. 2020;115: 701–707.
48. Patel AB, Verma A. Nasal ACE2 Levels and COVID-19 in Children. JAMA: the journal of the American Medical Association. jamanetwork.com; 2020. pp. 2386–2387.
49. Chen L, Li X, Chen M, Feng Y, Xiong C. The ACE2 expression in human heart indicates new potential mechanism of heart injury among patients infected with SARS-CoV-2. Cardiovasc Res. 2020;116: 1097–1100.
50. Verdecchia P, Cavallini C, Spanevello A, Angeli F. The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. Eur J Intern Med. 2020;76: 14–20.
51. Wysocki J, Ye M, Soler MJ, Gurley SB, Xiao HD, Bernstein KE, et al. ACE and ACE2 activity in diabetic mice. Diabetes. 2006;55: 2132–2139.
52. Wu Y-H, Li J-Y, Wang C, Zhang L-M, Qiao H. The ACE2 G8790A Polymorphism: Involvement in Type 2 Diabetes Mellitus Combined with Cerebral Stroke. J Clin Lab Anal. 2017;31. doi:10.1002/jcla.22033

53. Wang Z, Yuan Z, Matsumoto M, Hengge UR, Chang Y-F. Immune responses with DNA vaccines encoded different gene fragments of severe acute respiratory syndrome coronavirus in BALB/c mice. BiochemBiophys Res Commun. 2005;327: 130–135.
54. Möhlendick B, Schönfelder K, Breuckmann K, Elsner C, Babel N, Balfanz P, et al. ACE2 polymorphism and susceptibility for SARS-CoV-2 infection and severity of COVID-19. Pharmacogenet Genomics. 2021. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/pmc8415730/
55. Alimoradi N, Sharqi M, Firouzabadi D, Sadeghi MM, Moezzi MI, Firouzabadi N. SNPs of ACE1 (rs4343) and ACE2 (rs2285666) genes are linked to SARS-CoV-2 infection but not with the severity of disease. Virol J. 2022;19: 48.
56. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020;395: 507–513.
57. Vardavas CI, Nikitara K. COVID-19 and smoking: A systematic review of the evidence. TobInduc Dis. 2020;18: 20.
58. Ghosh S, Klein RS. Sex Drives Dimorphic Immune Responses to Viral Infections. J Immunol. 2017;198: 1782–1790.
59. Prelack K, Sheridan RL. Micronutrient supplementation in the critically ill patient: strategies for clinical practice. J Trauma. 2001;51: 601–620.
60. Fletcher RH, Fairfield KM. Vitamins for chronic disease prevention in adults: clinical applications. JAMA. 2002;287: 3127–3129.
61. Fairfield KM, Fletcher RH. Vitamins for chronic disease prevention in adults: scientific review. JAMA. 2002;287: 3116–3126.
62. National Research Council, Division on Earth and Life Studies, Commission on Life Sciences, Committee on Diet and Health. Diet and Health: Implications for Reducing Chronic Disease Risk. National Academies Press; 1989.
63. Annweiler G, Corvaisier M, Gautier J, Dubée V, Legrand E, Sacco G, et al. Vitamin D Supplementation Associated to Better Survival in Hospitalized Frail Elderly COVID-19 Patients: The GERIA-COVID Quasi-Experimental Study. Nutrients. 2020;12. doi:10.3390/nu12113377
64. Long KZ, Montoya Y, Hertzmark E, Santos JI, Rosado JL. A double-blind, randomized, clinical trial of the effect of vitamin A and zinc supplementation on diarrheal disease and respiratory tract infections in children in Mexico City, Mexico. Am J ClinNutr. 2006;83: 693–700.
65. Calder PC, Carr AC, Gombart AF, Eggersdorfer M. Optimal Nutritional Status for a Well-Functioning Immune System Is an Important Factor to Protect against Viral Infections. Nutrients. 2020;12. doi:10.3390/nu12041181
66. Alipio M. Vitamin D supplementation could possibly improve clinical outcomes of patients infected with coronavirus-2019 (COVID-19). Available at SSRN. 2020;3571484. Available: https://media-01.imu.nl/storage/energiekevrouwenacademie.nl/2851/vitamine-d-suppletie-2020.pdf
67. Pereira M, DantasDamascena A, GalvãoAzevedo LM, de Almeida Oliveira T, da Mota Santana J. Vitamin D deficiency aggravates COVID-19: systematic review and meta-analysis. Crit Rev Food SciNutr. 2020; 1–9.