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Angiotensin-Converting Enzyme Receptor Genotype and its Activity Level as Potential Predictors of the Severity COVID-19 among Iraqi Patients

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Abstract

Objectives: The purpose of this study is to determine whether the angiotensin-converting enzyme activity and various biomarkers are used to investigate the severity of Covid-19 and to study the genetic variation occurs in angiotensin-converting enzyme-2 (ACE-2) receptor in severe Covid-19-related genes in the Iraqi population of Kerbala Province.

Methods: This case-control study was conducted on 176 subjects who survived hospitalization and diagnosed by physician. Various biomarkers including ferritin, C-reactive protein, lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, angiotensin-converting enzyme-2 activity levels were determined. Accordingly, they were divided into three groups: 59 of them were infected with severe Covid-19, 54 of them were infected with moderate Covid-19 and 63 of them were checked and obtained as apparently healthy control. Severe and moderate patients were collected from Al-Hayat tertiary center at Al-Hussein Medical City, Kerbala Health Directorates, Kerbala – Iraq during Oct., 2020-July, 2021 with matched age ranged between (23–88) years. Blood samples of apparently healthy and Covid-19 samples were subjected to genomic DNA extraction within 24–48 hours of aspiration. The genomic DNA extracted was subjected to electrophoresis through 1.5% of agarose gels which was detected by staining with the fluorescent dye ethidium bromide and then visualized by illumination with UV light to confirm the presence and integrity of the extracted DNA.

Results: Genotyping of ACE-2 (I/D) polymorphism (rs4646994), which has a high prevalence, was performed by polymerase chain reaction assay. The amplification of an Alu repetitive element in an intron of the ACE-2 has shown three potential genotypes of I/I and D/D as homozygous, and I/D as heterozygous. Individuals with normal homozygous (DD) revealed band of (190 bp), while individuals with normal (II) revealed band of (490 bp), and the individuals with heterozygous (ID) revealed two bands (190, 490 bp) respectively. Every severe Covid-19 group carried (DD) allele genotype, moderate group carried (ID and II) alleles and finally the control group carried (DD, ID, II) alleles genotype.

Conclusion: In the ACE-2 polymorphism, the D/D genotype allele is implicated as a risk factor for severe Covid-19 patients, in Iraqi population.

Keywords: COVID-19, homozygote, ferritins, genotype

Introduction

More than 140 million cases of infection with Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been reported worldwide by April 19, 2021, with over 3 million deaths due to the virus[1] coronavirus disease situation dashboard. Coronavirus disease 2019 (Covid-19) which emerged in Wuhan, China, has spread to almost all countries and regions of the world, becoming one of the most lethal pandemic after the Spanish flu in 1918–1920. It is caused by an RNA virus (2019 novel coronavirus or 2019-nCoV or SARS-CoV-2). As of July 13, 2020, a total of 12,768,307 confirmed Covid-19 cases and 566,654 related deaths have been reported. Beyond its important morbidity and mortality and the huge burden of health care systems, Covid-19 has a massive societal and economic impact globally.[1,2] This pathogen was later renamed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the coronavirus study group (Gorbalenya, 2020),[3] and the disease was named coronavirus disease 2019 (Covid-19) by the WHO. Covid-19 can be either silent (asymptomatic) or associated with many symptoms, such as familiar cold symptoms (fever, stuffy nose, cough, weakness) bronchitis and pneumonia.[4] Covid-19 is moderately infectious with a relatively high mortality rate, but the information available in public reports and published literature is rapidly increasing.

Many risk factors have been described for this coronavirus such as elderly age, male gender, race, obesity, hypertension, diabetes and geographic region.[5,6]

Clinically, most of Covid-19 cases (80%) are either asymptomatic or have mild forms.[7] However, about 13.8 and 6.1% have severe and critical life-threatening disease that requires admission to hospital and sometimes in the intensive care unit. In the context of outstretched heath care systems and limited resources, risk stratification is pivotal to identify patients who the most need in-hospital and intensive management. Biomarkers along with some clinical factors might help to predict adverse outcomes among Covid-19 patients. Hence, we conducted this systematic review and meta-analysis to summarize available data on the association between some common hematological, inflammatory, biochemical parameters and the severity of Covid-19.[7,8] To date, there is no established curative treatment for Covid-19. Although some drugs such as hydroxychloroquine are integrated in treatment guidelines or under investigation in interventional studies, the management of Covid-19 is mostly supportive.[7,9] Identifying biological abnormalities induced by Covid-19 may contribute to a better understanding of the pathophysiology of the disease and ultimately guide the development of targeted adjuvant therapies besides antivirals drugs. Furthermore, such information on the biological profile of Covid-19 can guide clinicians in the assessment and treatment of these patients.[10] Genetic factors also play a major role in Covid19 infection. Inter-individual inherited differences in susceptibility to SARS-CoV-2 infection is linked to the presence of genetic polymorphisms (variants) in many

genes especially in those that code for the host receptors involved in viral entry process. These DNA changes are transmissible from one generation to another, detectable in at least 1% of individuals in a population and could explain the differences between individuals in the susceptibility to some multigenic, complex diseases like Covid19.[11,12] Two main approaches can be used in genetic epidemiology to establish a link between genetic variations and the risk of developing a disease: Genetic linkage analysis and association studies (candidate gene and genome-wide association studies).[13] Angiotensin-converting enzyme (chromosome Xp22.2) is the enzyme responsible for converting angiotensin-2 to angiotensin (1–7) form,[14] it is expressed in most organs such as, thyroid and lungs, heart, esophagus, kidney, adipose tissue, liver, retina, the vascular system, the small intestine, nasal and bronchial tissue, and alveolar type II epithelial cells.[15,16] ACE-2 is also known as a host cell receptor that contributes to the viral infection by corona viruses. The COVID-19 virus binds to the target cells through ACE-2 receptor which makes the COVID-19 attachment, invasion and penetration processes easier.[17] There are other receptors that can be used but, the virus has greater affinity for ACE-2 and weaker affinities for two other receptors, CD147 and Grp78 (Glucose-Regulate Protein 78).[16] The ACE-2 expression level has been reported to be significantly increased among men than women, which could explain the male predominance of COVID-19.[18,19] However, in another study ACE-2 expression was not significantly associated with gender/disease severity bias among Covid-19 Italian patients.[20] Similar results were obtained by another author, who didn't observe a disparity between age groups and gender groups (male *vs* female) in ACE-2 gene expression.[21] ACE-2 is very polymorphic gene with about 1700 polymorphisms and their frequencies vary between populations, some of these polymorphisms were correlated with increased expression of ACE-2 protein and were more frequent among the East-Asian populations.[17,22,23] Not only that, ACE deletion allele that is linked to alterations of ACE expression, also influences the spread of the virus and outcomes of infection with COVID-19 especially in the Asian populations.[24,25] Angiotensin converting enzyme-2 receptor (ACE-2) is a protein on the surface of many cell types. It is an enzyme that generates small proteins – by cutting up the larger protein angiotensinogen – that then go on to regulate functions in the cell. Using the spike-like protein on its surface, the SARS-CoV-2 virus binds to ACE-2 – like a key being inserted into a lock – prior to entry and infection of cells. Hence, ACE-2 acts as a cellular doorway – a receptor – for the virus that causes Covid-19. ACE-2 acts as the receptor for the SARS-CoV-2 virus and allows it to infect the cell. ACE-2 receptor is present in many cell types and tissues including the lungs, heart, blood vessels, kidneys, liver and gastro-intestinal tract. It is present in epithelial cells, which line certain tissues and create protective barriers. The purpose of the presented study is to investigate the activity levels of angiotensin-converting enzyme and various biomarker levels in adults infected with Covid-19 and study the molecular basis of angiotensin-converting enzyme-2 (ACE-2) receptor in Covid-19-related gene polymorphism in Iraqi populations of Kerbala Province.

Materials and Methods

The current case-control study was conducted on 176 subjects who survived hospitalization and diagnosed by physician. Various biomarkers including ferritin, C-reactive protein, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), angiotensin-converting enzyme-2 (ACE-2) activity were determined. Accordingly, they were divided into three groups: 59 of them were infected with severe Covid-19, 54 of them were infected with moderate Covid-19 and 63 of them were checked and obtained as apparently healthy control. Severe and moderate patients were collected from Al-Hayat tertiary center at Al-Hussein Medical City, Kerbala Health Directorates, Kerbala–Iraq during Oct., 2020-July, 2021 with matched age ranged between (23–88) years. Blood samples of apparently healthy and Covid-19 samples were obtained from each case and used for some biomarkers determination (ferritin, ALT, AST and ALP activity levels) and molecular studies. One ml of whole blood was collected in EDTA containing tube and subjected for genomic DNA extraction within 24–48 hours of aspiration. Then DNA concentration and purity were measured by UV absorption at 260 and 280 nm (Bio Drop, U.K.).

The genomic DNA extracted was subjected to electrophoresis through 1.5% of agarose gels electrophoresis in 1X (TBE) buffer at 100 volts for 75 minutes or until dye markers have migrated an appropriate distance, depending on the size of the DNA to be visualized. The percentage of agarose used depends on the size of fragments to be resolved, where an agarose gels percentage are normally in the range of 0.5% to 2%; the ethidum bromide staining was done according to Robinson and Lafleche method.[26] DNA ladder 100 bp bands were used as standard for comparison with bands that resulted for allelic gene migration through gel electrophoresis and then detected at staining with the fluorescent dye ethidium bromide and then visualized by illumination with UV light to confirm the presence and integrity of the extracted DNA.

Genotyping for ACE-2 receptor gene polymorphism was performed by the polymerase chain reaction- Amplification Refractory Mutation System (PCR-ARMS) method using thermocycler (Biometra, Germany). The primers were taken in a lyophilized state; their units are known as a mass in picomoles then mixed by suitable vortex and their sequence of ACE-2 receptor gene was listed in Table 1.

The subsequent steps were done for the reconstitution and dilution of the primers: The tube was centrifuged at 10,000 rpm for 5–10 min before de-capping. The chosen volumes from nuclease free water were added according to the manufacturer to obtain a 100 *P*-moles/µl (master stock). Ten microliters of the master stock were transported to a 0.5 ml eppendorf tube that contained 90 µl of nuclease free water to obtain a 10 pmoles/µl a working primer stock solution. The master stock and working stock were kept at –20°C. The working stock was warmed up and kept on ice for use in PCR and then stored at –20°C after each use.

Table 1. **Primers used in molecular study of angiotensin-converting enzyme receptor-2 gene polymorphism**

Primer name	Sequence	Annealing temp. (°C)	Product size (bp)
ACE-rs4646994- F	5`-CTGGAGACCA CTCCCATCCTTTCT-3`	58	190
ACE-rs4646994-R	5`-GATGTGGCCATCA CATTCGTCAGAT-3`	58	490

The reaction setup and thermal cycling protocol of the PCR components and program for ACE-2 receptor gene show in Table 2.

Electrophoresis involves running a current through a gel loaded with the molecules of interest. The movement of the samples is directed based on the charge that the molecule carries. Based on the size and charge, the molecules will travel through the gel at different speeds, allowing them to be separated from one another as shown in Figure 1. Since, all the DNA molecules possess same amount of charge per mass, the gel electrophoresis separates them on the basis of size only.

Body mass index (BMI) was used to define obesity. The range of BMI (18.5–24.99 kg/m^2) that set it WHO but it does not accurately indicate the degree of fatness. The body mass index was measure by dividing weight in kilograms by length of individual in square meter.

Table 2. **Program of polymerase chain reaction of ACE-2 receptor gene in Covid-19 and healthy control**

Steps	Temp., ℃	Time	Cycle
Initial denaturation	95	5 min.	1
Denaturation	95	30 sec.	30
Annealing	58	30 sec.	
Extension	72	30 sec.	
Final extension	72	5 min	1

Results

Main features of 176 individuals included in this study were divided into 63 cases as apparently health control and the other 113 cases of them were infected with Covid-19 as summarized in Table 3. The Covid-19 patients were sub-divided into two groups depending on the severity of the disease and some biomarker levels changes. Mild Covid-19 includes 54 patients and the remaining 59 patients were infected with severe Covid-19 and its complications. The mean ± SD of age and BMI of each groups were indicated in Table 3.

In this table a significant differences between each of severe and moderate Covid-19 group as compared with control group was observed with respect to the age ($P < 0.001$), while BMI data show a non-significant results ($P = 0.817$ and $P = 0.495$) respectively. The mean ± SD values of angiotensin-converting enzyme-2 (ACE-2) activity levels was higher in the severe and moderate Covid-19 as compared with that found in healthy control (4.74 ± 0.85, 3.83 ± 0.82, and 3.03 ± 0.82 ng/ml) respectively. A non-significant differences between moderate and control groups (***P* = 0.721**) was obtained and

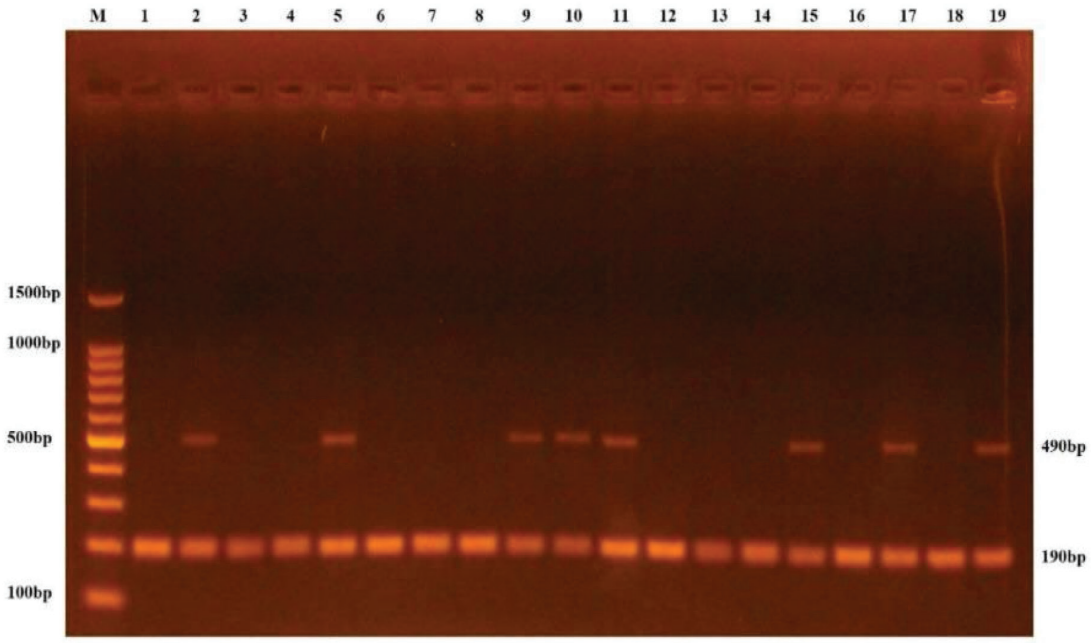

Fig. 1 **The amplification of rs4646994 region of human samples were fractionated on 1.5% agarose gel electrophoresis stained with ethidium bromoide. M: DNA Marker (Ladder 100 bp). Lanes 1-19 resemble 190, 490 bp of PCR products.**

Table 3. **Comparison of age and body mass index and angiotensin-converting enzyme between moderate and severe Covid-19 groups as compared with control group**

Parameters	Moderate Covid-19 *N* = 54 Mean ± SD	Control group *N* = 63 Mean ± SD	*P*-value	Control group *N* = 63 Mean ± SD	Severe Covid-19 *N* = 59 Mean ± SD	*P*-value
Age, year	52.06 ± 14.35	40.03 ± 11.86	**<0.001**	40.03 ± 11.86	59.1 ± 12.64	**<0.001**
BMI, kg/m^2	32.59 ± 5.52	33.31 ± 5.83	**0.495**	33.31 ± 5.83	33.07 ± 5.72	**0.817**
ACE-2 activity, ng/ml	3.83 ± 0.82	3.03 ± 0.82	**0.721**	3.03 ± 0.82	4.74 ± 0.85	**0.001**

BMI, Body mass index; N, Number; Significant $P < 0.05$; SD, Standard Deviation.

significant differences between severe Covid-19 and apparently health control group (***P* < 0.001**).

The angiotensin-converting enzyme gene (ACE-2) was located on chromosome 17q23.3, spansv21 kb, and comprises 26 exons and 25 introns. Exon 26 encodes for the functionally important membrane-anchoring domain of the ACE-2 protein.

Insertion (I allele) polymorphism had band in (490 bp) of an Alu repetitive element in an intron of the ACE-2 receptor gene that called homozygotes. Deletion genotype (D allele) had band in (190 bp) that lack the repetitive element also called homozygotes, while (ID) genotype had two band I and D in the same gene that mean in the same gene had insertion and deletion of an Alu repetitive element in intron of the ACE-2 receptor gene and it is called heterozygotes, so the two band in the same location differ in speed of migration as shown in Figure 1. The (DD) genotype showed band at 190 bp, (II) genotype showed band at 490 bp while (ID) genotype showed both bands at 190 and 490 bp. The size of each of (DD, II and ID) genotypes was determined as indicated in Table 4.

Therefore, individuals with normal homozygous deletion/deletion (DD) revealed band size of (190 bp), while individuals with normal insertion/insertion (II) revealed band size of (490 bp), and the individuals with heterozygous insertion/deletion (ID) revealed two bands size at each of (190, 490 bp) respectively, Figure 1.

Table 4 shows the comparison of age and body mass index in moderate Covid-19 group with the ACE-2 receptor gene allele types by unpaired t test. In this table show age and BMI are non-significant correlated with moderate Covid-19 group according to the allele (ID) and (II).

Table 5 shows a significant result for mean ± SD of age between severe Covid-19 as compared with apparently control groups according to the ACE-2 receptor gene polymorphism allele deletion/deletion (DD), but the relation with BMI was non-significant.

Table 6 show the results obtained for the relation between hypertension and T2DM associated with Covid-19 are significant as compared with control groups according to the ACE-2 receptor gene polymorphism allele (DD) but for moderate Covid-19 group the relation was non-significant with ACE-2 receptor gene polymorphism alleles (ID) and (II).

As shown in Table 6 the effect of smoking nicotine on the renin-angiotensin system was observed which indicated that 8.7% of angiotensin-converting enzyme receptor gene polymorphism allele (ID) in moderate Covid-19 and 8.5% of (DD) allele in severe Covid-19 infection were smokers respectively as compared with control group. Nicotine can impact the angiotensin-converting enzyme (ACE-2), which is relevant because coronaviruses bind to ACE-2. Current and past tobacco smoking is associated with changes in ACE-2 receptor expression. Table 6 indicated that most of patients whether it is severe or moderate infection with Covid-19 nonsmokers, the reason is due to nicotine may bind with the ACE-2 receptor and decrease levels of ACE-2 in multiple organs.[27] Smoking is associated with increased susceptibility and mortality in MERS-CoV infection, potentially due to up-regulation of dipeptidyl peptidase-IV, the host receptor for MERS-CoV, in smokers. In the context of respiratory viruses, smoking has been reported to cause increased hospital and ICU admissions with influenza infection, greater severity with respiratory syncytial virus bronchiolitis and increased mortality with viral pneumonia.[28] In Table 7 all parameters (ferritin, LDH, CRP, ACE2, ALT and AST) are significantly elevated in moderate group according to the allele ID compared with control group expect ALP, while they are significantly elevated in severe Covid-19 group as compared with control according to the DD allele expect ACE-2 which was non-significantly changes.

Table 4. **Comparison of age and body mass index in severe Covid-19 cases according to ACE-2 gene polymorphism allele, ID and II obtained by polymerase chain reaction amplification as compared with control DD allele**

Parameters	Moderate Covid-19, *N* = 54		*P*-value
	ID allele *N* = 46 Mean ± SD	II allele *N* = 8 Mean ± SD	
Age, Year	52.91 ± 14.62	47.13 ± 12.32	0.297
BMI, (kg/m²)	33.07 ± 5.6	29.83 ± 4.38	0.126

N, Number; BMI, Body Mass Index; SD, Standard Deviation; *P*-value, Prober value; ID, Insertion/Deletion; II, Insertion/Insertion.

Table 5. **Shows a comparison between severe Covid-19 and control groups according to the type of ACE-2 receptor gene allele deletion/deletion for age and BMI**

Parameters	Severe Covid-19 DD allele *N* = 59 Mean ± SD	Control DD allele *N* = 27 Mean ± SD	*P*-value
Age, Year	59.1 ± 12.64	41.96 ± 12.62	**<0.001**
BMI, (kg/m²)	33.07 ± 5.72	33.93 ± 6.41	0.537

N, Number; BMI, Body Mass Index; SD, Standard Deviation; *P*-value, Prober value; DD, Deletion/Deletion allele found in sever Covid-19 and control group.

Table 6. **Comparison of gene polymorphism of ACE-2 alleles (ID and II) with hypertension, type 2 diabetes mellitus and smoking in moderate Covid-19 cases and compared with severe and control alleles (DD)**

Parameters		Moderate Covid-19		*P*-value	Severe Covid-19	Control	*P*-value
		ID allele *N* = 46 (%)	II allele *N* = 8 (%)		DD allele *N* = 59 (%)	DD allele *N* = 27 (%)	
Hypertension	with	25 (54.3%)	5 (62.5%)	0.720	26 (44.1%)	0 (0.0)	**<0.001**
	without	21 (45.7%)	3 (37.5%)		33 (55.9%)	27 (100%)	
T2DM	with	16 (34.8%)	4 (50.0%)	0.450	28 (47.5%)	0 (0.0)	**<0.001**
	without	30 (65.2%)	4 (50.0%)		31 (52.5%)	27 (100%)	
Smoking	Yes	4 (8.7%)	0 (0.0)	1.000	5 (8.5%)	0 (0.0)	0.320
	No	42 (91.3%)	8 (100%)		54 (91.5%)	27 (100%)	

N, Number; T2DM, Type 2 Diabetes Mellitus; ID, Insertion/Deletion; II, Insertion/Insertion; *P* value, Prober value.

Table 7. **Comparison of biochemical in ACE-2 Alleles, ID allele in moderate Covid-19 group as compared to ID allele in control and also the DD allele in severe Covid-19 as compared with control DD allele**

Parameters	ID allele in moderate Covid-19 group as compared with control			DD allele in severe Covid-19 group as compared with control		
	Group	Mean ± SD	*P*-value	Group	Mean ± SD	*P*-value
Ferritin, (ng/ml)	Moderate	602.24 ± 509.41	**<0.001**	Severe	765.04 ± 359.37	**<0.001**
	Control	81.36 ± 47.31		Control	70.08 ± 26.96	
LDH activity, (U/l)	Moderate	395.78 ± 240.34	**<0.001**	Severe	432.9 ± 211.86	**<0.001**
	Control	132.93 ± 37.95		Control	140.98 ± 39.41	
CRP, (mg/dl)	Moderate	9.56 ± 3.26	**<0.001**	Severe	15.02 ± 23.53	**<0.001**
	Control	0.34 ± 0.15		Control	0.36 ± 0.13	
ACE-2 activity, (ng/ml)	Moderate	3.14 ± 0.83	**0.001**	Severe	3.74 ± 0.85	0.345
	Control	2.55 ± 0.62		Control	3.66 ± 0.64	
ALT activity, (U/l)	Moderate	60.81 ± 19.25	**<0.001**	Severe	101.11 ± 266.69	**<0.001**
	Control	29.56 ± 11.71		Control	26.54 ± 9.7	
AST activity, (U/l)	Moderate	41.28 ± 12.32	**<0.001**	Severe	51.6 ± 53.43	**<0.001**
	Control	22.77 ± 8.33		Control	23.54 ± 8.78	
ALP activity, (U/l)	Moderate	89.87 ± 30.73	0.063	Severe	109.48 ± 39.34	**<0.001**
	Control	77.12 ± 23.52		Control	73.66 ± 24.41	

LDH: Lactate dehydrogenase; CRP: C- Reactive protein; ACE-2: Angiotensin Converting Enzyme; ALT: Alanine Aminotransferase; ALP: Alkaline phosphatase; AST: Aspartate Aminotransferase; S.D: Standard Deviation; DD: Deletion/Deletion; ID: Insertion/Deletion.

Table 8 show the comparison of biochemical in control group according to allele. In this table all parameters in control group are non-significant according to the allele DD and ID expect ACE-2 is significant.

Discussion

As indicated previously, age and BMI are non-significantly in moderate Covid-19 group according to the (ID) and (II) allele as shown in Table 2, whereas age is significant between severe and control groups according to the deletion/deletion allele (DD), as shown in Table 3 but BMI was non-significantly. The DD allele found in severe and control group but was absent in moderate group.

The ACE-2 receptor gene polymorphism allele (DD) in severe Covid-19 was significantly associated with hypertension and T2DM as compared with control groups $\boldsymbol{P < 0.001}$, but the (ID) and (II) alleles in moderate Covid-19 group was association non-significantly, $P = 0.720$ and $P = 0.450$ respectively as shown in Table 4. The total 113 cases of Covid-19 patients were selected with demographic and clinical characteristics. We observed that high frequency of hypertensive patients was (54.3%) as compared to diabetic (34.8%) as shown in Table 4. In contrast, de Abajo et al., found higher prevalence of diabetes in 1339 Covid-19 cases as compared with 13,390 matched controls (27.2% vs 20.3%; crude odds ratio, OR, 1.50).[29] Similarity, Singh et al., pooled different studies (N = 2209) and found higher percentage of hypertension (21%) as compared to diabetes (11%) and CVD (7%).[30] In contrast, a meta-analysis of Covid-19 patients (N = 1576 patients) reported percentage of different comorbid conditions such as hypertension (17%) > diabetes (8%).[31] To date, few studies have been published that investigate the relationship between ACE-2 gene polymorphism and Covid-19 severity, but we are still lacking definite results.[17,32–34] In present study, we observed that individual with 'DD' genotype showed significantly 3.69-fold higher risk of Covid-19 severity. Similarly, other studies found that ACE-1 D/D-genotype showed association with Covid-19 related mortality.[35,36] Gomez et al. found D allele was significantly associated with hypoxemic as compared to non-hypoxemic patients; however, 'DD' genotype individuals did not show any association with Covid-19 infection.[37]

Table 8. **Comparison of biochemical in control group according to allele**

Parameters	Group	Mean ± SD	*P*-value
Ferritin, (ng/ml)	DD allele	70.08 ± 26.96	0.581
	ID allele	81.36 ± 47.31	
LDH activity, (U/l)	DD allele	140.98 ± 39.41	0.441
	ID allele	132.93 ± 37.95	
CRP, (mg/dl)	DD allele	0.36 ± 0.13	0.278
	ID allele	0.34 ± 0.15	
ACE-2 activity, (ng/ml)	DD allele	3.66 ± 0.64	**<0.001**
	ID allele	2.55 ± 0.62	
ALT activity, (U/l)	DD allele	26.54 ± 9.7	0.305
	ID allele	29.56 ± 11.71	
AST activity, (U/l)	DD allele	23.54 ± 8.78	0.994
	ID allele	22.77 ± 8.33	
ALP activity, (U/l)	DD allele	73.66 ± 24.41	0.420
	ID allele	77.12 ± 23.52	

LDH, Lactate dehydrogenase; CRP, C- Reactive protein; ACE-2, Angiotensin Converting Enzyme-2; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; AST, Aspartate aminotransferase; SD, Standard Deviation; DD, Deletion/Deletion; ID, Insertion/Deletion.

Some studies have reported an association of the DD genotype of ACE-2 polymorphism and higher circulating levels and activity of ACE-2,[38] which could explain the higher susceptibility to develop severe forms of the disease in patients with the DD genotype, in addition to hypertension and T2DM. SARS-CoV-2 sequesters ACE-2 to invade cells, decreasing the bioavailability of ACE-2 which entails a reduction in the degradation of ANG II and an exacerbation of the damaging effects of ANG II.[39] Thus, the lung injury and inflammation caused by the reduced ACE-2 levels due to the viral infection, and also by the hypertension and diabetes may be worsened by ACE-2 genotypes that further increase ACE-2 levels, and hence ANG II levels, such as the DD genotype of ACE-2 analyzed. In our study we confirmed that ACE-2 levels are associated with the DD genotype of both polymorphisms, which in turn is associated with greater severity of the disease in hypertensive and T2DM. Although according to our data we could not affirm a direct association with Covid-19 severity.

Covid-19 is a highly contagious disease characterized by high mortality, especially for patients with severe comorbidities such as diabetes, hypertension, CVD and CKD.[9] The documented history of diabetes has been stated to be an independent indicator of morbidity and death in SARS patients.[40,41] Diabetes hyperglycemia is suspected to cause immune response dysfunction, which fails to regulate the spread of invasive pathogens. Therefore, diabetic individuals are considered to be more prone to infections and incidence of infectious diseases and it will increase associated comorbidities.[42] A study was released by the Chinese Centre for Disease Control and Prevention, which showed elevated mortality rate in people with diabetes (2.3%, total and 7.3%, diabetes patients) study was performed in 72,314 cases of Covid-19.[43]

Blood pressure homeostasis maintained by renin–angiotensin system (RAS).[44,45] RAS system modulated by ACE-1 and ACE-2, angiotensin I is converted into angiotensin II (ATII) by ACE1 and degraded bioactive bradykinin.[46] Insertion/deletion (I/D) polymorphism has been correlated with levels of circulating and tissue ACE-1 and influences almost half of the variability of serum ACE levels in the general population. The 'D' allele of ACEI/D is associated with higher ACE activity.[47] This means individuals with DD genotype showed approximately twice ACE activity levels as compared to II genotype individuals.[48] The 'D' allele of ACE-1 gene is significantly associated with hypoxemic group as compared to non-hypoxemic group.[49] Recently, Delanghe et al. found that the prevalence of Covid-19 in 33 countries has been substantially associated with ACE1 I/D polymorphism.[50]

However, our results clearly revealed that polymorphisms of the ACE-2 gene are related to the risk of developing severe Covid-19 (ICU admission) in hypertension and diabetic patients. Our study confirms with previously reported findings of another ACE polymorphism on Covid-19 patients with hypertension[37] however; it did not corroborate the association with Covid-19 severity found by Gomez et al. and other authors for the ACE I/D polymorphism, regardless of comorbidities. Unlike these studies, we also found an additional association of D Allele with enhanced severity in hypertension and diabetic patients, and interestingly, we further found a higher prevalence of the DD, ID genotypes (all containing the D allele) among deceased ICU-patients, not described so far, confirming the deleterious effect of the D allele in Covid-19 outcomes. Precisely most of these patients were hypertensive.

In Table 7 all parameters (Ferritin, LDH, CRP, ACE-2, ALT and AST) are significant in moderate group according to the allele ID compared with control group expect ALP. All these parameters are significantly elevated in severe group according to the allele DD as compared with control group expect ACE-2, because D allele responsible for activity of ACE-2 and this allele found homogenous at form DD in severe and control group that carry DD genotype, so that non-significant between severe and control according to the allele DD. Genetic polymorphisms in ACE-2 indicate that the ACE I/D polymorphism, have been shown to affect ACE-2 activity levels and confer susceptibility to hypertension type 2 diabetes,[38] overweight nephropathy and certain cardiovascular and autoimmune diseases. More specifically, the DD genotype of the ACE I/D polymorphism has been associated to higher levels of serum ACE and higher levels of circulating IL-6 in patients with myocardial infarction. In contrast, the II genotype has been associated to lower circulating ACE levels. Since some of these processes have been reported to be involved in the pathogenesis of Covid-19, the DD genotype could predispose to complications of Covid-19 due to higher baseline ACE levels and its consequences.[37] Indeed, an association of the DD genotype of the ACE I/D polymorphism with severe Covid-19 has been reported in hypertensive males.[37] However, analyzing the I/D polymorphism is laborious and time-consuming and some authors have described a preferential amplification of the D allele. The ACE-2 gene polymorphisms are in complete linkage disequilibrium with the ACE I/D polymorphism, therefore they could be better prognostic markers.

Table 8 show all parameter are non-significant correlated because there were healthy peoples. ACE-2 was significantly to DD and ID alleles because the D allele is associated with higher ACE-2 activity. Mean ACE activity levels in DD carriers were approximately twice that in II genotype individuals. Therefore, it may be propose a hypothesis that ACE-2 gene polymorphism may play an important role in patients with Covid-19 who are susceptible to develop severe lung injury or ARDS.[48]

Conclusions

The severity of Covid-19 patients may depend on age, diabetes, hypertension and ACE-2 gene polymorphism. The observed results suggest that a prospective paradigm of DD genotype that has the potential help to explain the susceptibility of the host response to SARS-Cov-2 infection and involve in numerous pathological. Thus, ACE-2 I/D polymorphism may be act as a useful tool to predict the development of disease and may have an influence on the treatment outcomes against the Covid -19 to establish a population-based therapeutic development.

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Conflicts of Interest

None.

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