Archives of Razi Institute, Vol. 78, No. 1 (2023) 161-166

DOI: 10.22092/ARI.2022.358492.2232

Original Article

Molecular Study of *ACE2* Gene Polymorphism of COVID-19 Infection among the Kurdish Population in Kurdistan Region- Iraq

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Received 16 April 2022; Accepted 7 May 2022
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Abstract

Coronavirus Disease 2019 (COVID-19) is a current pandemic infection of the human respiratory system, which is caused by which caused by Sever Acute respiratory syndrome virus 2 (SARS-CoV-2). The infection was classified by World Health Organization (WHO) as a universal pandemic in February 2020; there have been 494.587.638 confirmed cases and 6.170.283 deaths. The present study investigated the molecular genetics of the Angiotensin Converting Enzyme 2 (*ACE2*) gene in correlation to COVID-19 patients in the Kurdish population. Eighty-six individuals were clinically diagnosed with COVID-19 and control groups. After the genomic DNA extraction these participants the target 1, 2 and 8 exons of the ACE2 gene were amplified using the PCR technique, and then the Sanger sequencing technique was performed to analyze genetic variants of the *ACE2* gene in 70 DNA samples of COVID-19 hospital patients at Emergency Hospital in Erbil city, Sarchnar Hospital in Sulaymaniyah city, Lalav Hospital in Duhok city and Wafa Hospital in Halabja city from Kurdistan Region of Iraq. The current study was designed into two groups control group and a patient group. The patient group was divided into two subgroups, severe and mild patients of different ages and genders. As a result, there were no mutations at the positions 1, 2 and 8 exons sequences, while single nucleotide polymorphisms (SNPs) were detected and identified three different types of mutation at intron position: twenty-six of c.12405 del T, two of c.12407 T>G, and two of c.12406 G>A in a total 86 participants. This result shows that genetic difference does not impact the COVID-19 infection severity among the Kurdish population regarding *ACE2* gene polymorphism

Keywords: COVID-19, *ACE2* gene, SNP of *ACE2* gene and the X chromosome

1. Introduction

The family of *Coronaviridae* are positive single-threaded RNA genome about 29.8 to 29.9 kbp in size; because of this feature, it is seen as the largest among all human RNA viruses (1). All coronaviruses are most distinctive and were described as zoonotic viruses; bats are considered the main pool of coronaviruses, and most of the coronaviruses emerged initially from bats (2). For more than 20 years, seven types of coronavirus have caused severe respiratory diseases that appeared among humans. One of them is the severe acute respiratory syndrome2 (SARS-CoV-2) that infects the lung and causes lung injury; it also causes the failure of several organs with negative myocardial stress and myocardial remodelling (3).

The recent new coronavirus disease was recorded in Wuhan province in China towards the end of December 2019 (4). Following that, the Coronavirus disease 2019 (COVID-19) was named by World Health Organization (WHO) as a global pandemic syndrome (5). Then COVID-19 disease spread to most of the globe in every country (6). Until the 8th of April 2022,

more than 494.587.638 cases were confirmed, and the number of deaths exceeded 6.170.283 cases. The virus spread rapidly, all continents filled with havoc because of the virus and healthcare sections were disrupted severely. The main point to be considered is that the critical source of spreading the disease is caused by direct contact and splash droplets, but also other sources are considerable, including the direct faecal-oral route (7). The virus causes a type of disease with a different number of symptoms which together is called coronavirus disease 2019 (8). Some symptoms, such as fever, short breathing, sore throat, headache, anosmia, and diarrhoea, are general symptoms of COVID-19 (9).

Coronavirus is an agent which leads SARS-COV-2 entering the cell by attaching spike (S) protein of the viral, which heads of coronavirus to the membrane's Angiotensin-Converting Enzyme 2 (ACE2) receptors host cell, which permits the virus to enter the human bodies (3). Furthermore, the ACE2 receptor is a carboxyl peptidase recognized as the significant coronavirus entrance receptor (10). The expression of the ACE2 receptors target in the epithelium brought out to the virus may also be related to maximizing the vulnerability to COVID-19 disease (11).

Many receptors, primarily type II pneumocystes, exist inside human lungs and can also be found throughout the body. The symptoms are directly related to the respiratory system (4). The *ACE2* gene is found on Chromosome Xp22.2 and has 18 Exons and 17 Introns (12). It codes for 805 amino acids, which exist on several organs' membrane cells, including the gastrointestinal system, lungs, kidneys, heart and brain (13). The ACE2 gene produces ACE2 receptors (13). The approach was designed based on the study support for SARS-CoV-1 interaction with *the ACE2* gene (14).

This study is considered the first research on patients with Coronavirus COVID-19 infection in the Kurdistan region and Iraq. Our research aims to investigate whether the course of the mild and severe in patients is expected by identifying the genotype of ACE2 receptor polymorphism among the Kurdish population. Further, to identify the mutation and new variants of the *ACE2* gene was performed by sanger sequence for determining the single nucleotide polymorphism in DNA binding sites of COVID-19 patients from particular sequences, including the exon 1, 2 and 8 in the *ACE2* gene.

2. Materials and Methods

2.1. Collection of Blood Samples

For this research, we registered 86 participants, a group with 70 patients diagnosed with COVID-19 and a group with 16 healthy participants, a control group in the Kurdish population. The patients were hospitalized at hospitals in the Kurdistan region, with around (12.8%) patients at Emergency Hospital in Erbil city, (31.4%) at Sarchnar Hospital in Sulaymaniyah city, (21.5%) at Lalav Hospital in Duhok and (34.5%) at Wafa hospital, Halabja city. All the participants in the current study were affected by COVID-19 following the positive outcomes via the swabs in the naso-oropharyngeal. They were enrolled on the relevant units for proper caring and checking. The patients were clinically confirmed and divided into 2 groups according to the severity and mildness of the infection as presented by the hospitalization results.

2.1.1. Severe Group

Forty-three patients aged between 32-99 years old. This group is defined as patients with respiratory system failure, needing intensive ventilating and staying in the Intensive Care Unit- ICU at the hospitals in Kurdistan.

2.1.2. Mild Group

Twenty-seven patients aged between 42-92 years old. This group is defined as the patients showing few symptoms, respiratory impairment but not requiring ventilating and Bi-level Positive Airway Pressure-BiPAP or Continuous Positive Airway Pressure-CPAP.

2.1.3. Control Group

Sixteen healthy individuals were randomly chosen between 19-42 years old, from both genders, to be matched with the patient population and were randomly taken as a control group.

From the veins of the 86 participants, about three millilitres of blood was taken from each person. According to the manufacturer's protocols, a genomic DNA extraction tool AddBio, KOREA, was utilized for extraction. The extraction of the genomic DNA in each blood sample was done in an (EDTA) anti-coagulant tube, and they were conducted in (Biolab Center, Sulaymaniyah - Kurdistan region -Iraq).

2.2. Preparations of the Primers

A set of primers for *ACE2* gene exons 1, 2, and 8 were designed that are found within these exons. All primers existing in table 1 were manufactured by hand with (Primer -Blast online software) . The primers were gained through the *ACE2* genomic sequence on GenBank under the accession number: [NM-000180 .4].

2.3. Sanger Sequencing

In the PCR product, mutations in the *ACE2* gene analysis were conducted by direct Sanger sequencing of three exons. Every exon was amplified by using primers. Standard Sanger sequencing was done in two centres for results qualifications. The first centre was Microgen, Korea, facilitated by (Biolab Center, Sulaymaniyah - Kurdistan region –Iraq). The second centre was (Practical Biosystems, Hitachi High Technologies, Tokyo/ Japan) in Iran, assisted by (IMMUNOGENE CENTER" Erbil _ Kurdistan region / Iraq). The Sequences result was investigated using Geneious Prime software to detect the recognized, unidentified, or new mutations.

Table 1. PCR Primer sets for Amplifications of coding Sequence of the *ACE2* gene

Amplicon	Forward Primer (5′_3′)	Reverse Primer (5′_3′)
Exon1	5′ CTGTCCTCTCCAGGATGAACT 3′ (21 bp)	5′GGAGGCAAACATCCAATCTCAC-3′ (22 bp)
Exon 2	5′ TGACCTTCAGCGGAGTAGAG 3′ (20 bp)	5′CCTTACCTAGGCATAGAGAGAGATT 3′(25 bp)
Exon 8	5′AGGAGGCCGAGAAGTTCTTT3′ (20 bp)	5′CTGTCCTCTCCAGGATGAACT 3′(21 bp)

3. Results and Discussion

3.1. Clinical Background of Patients

Eighty-six participants (severe patients group: 43, mild patients group; 27 and control group: 16 normal healthy individuals). All participants were tested by Molecular investigations (such as the RT-PCR test) from COVID-19 laboratory Centers of the Provinces in the Kurdistan region (Erbil, Sulaymaniyah, Duhok, and Halabja). The disease was diagnosed as COVID-19.

3.2. Molecular Biology Approach of ACE2 Gene

The genomic DNA was extracted from all positive participant groups for the COVID-19 test and the control group. The quantity of DNA was measured using a nano-drop spectrophotometer (Thermo Fisher/ USA). Confirmation and analysis of the mutation appearing in the code region and adjoining intron region of *the ACE2* gene were conducted involving exons 1, 2, and 8, with introns located among those exons.

3.3. PCR Amplification of Exons 1, 2, and 8:

PCR was done for all three amplicons to amplify each sequence target, and the results were obtained as expected in their exact sizes on agarose gel electrophoresis, as shown in figure 1.

Figure 1. Agarose gel electrophoresis for amplified PCR product of *ACE2* gene (which contains target sequences on exons 1, 2 and 8). The band were fractionated by electrophoresis and visualized by U.V. after staining with a safe stain. Ladder 1300 bp, Lane: 1-2 severe patients, 3-4 mild patients (Experiment Group) and 5-6 Control groups of the present study. All Samples showed a band between 300-499 bp, NC: Negative Control

3.4. Sequencing Analysis and Bioinformatics

The regions were analyzed through direct sequence analysis from two groups (patients and controls). In 28 out of 86 participants, variants in the *ACE2* gene were identified. As a result, all the sequences from exon 1 and exon 8 were precisely similar to the RefSeqGen sequence in the GenBank database, but in exon 2, from a total of examined participants, 30 mutations were identified in introns positions. SNPs were detected at positions of the intron, c.12405 del T, which indicates 26 samples from 5 healthy individuals in the control group, 10 mild patients, and 11 severe patients. Detected four deletions at positions of the intron, which c.12407 T>G indicated in two samples, 1 from a healthy Individual in the control group, 1 from severe patients, and c.12408 G>A, which indicated in two samples, 1 from a healthy Individuals in the control group, and 1 from severe patients. As shown in figure 2.

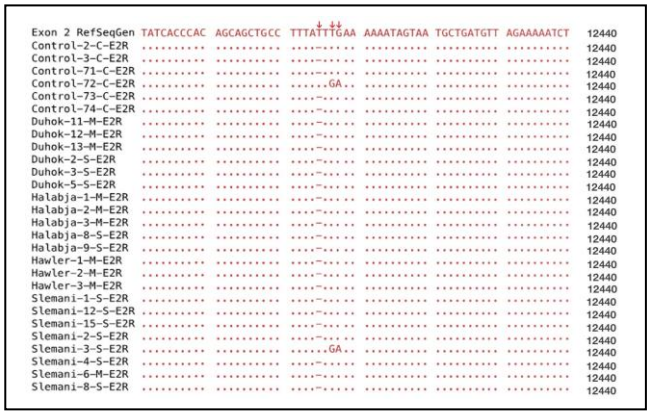

Figure 2. Multiple sequence alignment for amplicon 2 final sequences from 28 samples which contain the SNPs in the intron number two of *the ACE2* gene. The SNPs were clearly shown at positions 12405, 12407, and 12408, which indicates a deletion in 26 samples and a substitution in two samples

All mutations detected do not affect the protein coding of the *ACE2* gene. This result concluded that genetic difference does not impact COVID-19 severity among the Kurdish nation for the *ACE2* gene. This outcome confirmed that the changes in the Introns of *the ACE2* gene did not affect on COVID-19 disease the severity.

3.5. Correlational Outcomes

This research study is considered the first molecular genetics study of COVID-19 patients on *the ACE2* gene of the SARS-CoV-2 receptor. The exons (1, 2, and 8) of the *ACE2* gene were analyzed for the Kurdish Population individuals in the Iraqi Kurdistan region. The analysis was based on an investigation of the infection severity for COVID-19 disease per control, mild and severe groups.

After utilizing the Sanger Sequencing technique, it was noticed that there was no significant variation in the exons sequences for the participants (control, mild and severe groups) in all the participants' DNA samples. As a complementary result in the outcomes of this study, three different mutations of synonymous SNPs were detected in intron number two at positions of c.12405 del T, c.12407 T>G, and c.12408 G>A. All the mutations found do not influence the *ACE2* gene's coding production.

This outcome confirmed that the changes in the *ACE2* gene Do not have any effect on COVID-19 infection severity. However, the availability of the mutation in most samples per different clinical groups might be a race-related sequence that can be available in the Kurdish nation. Other studies also detected that the differences in the introns did not affect the severity of (COVID-19) disease (15).

The difference in COVID-19 infection severity was not related to the different genetic variants of the *ACE2* gene; this study's results match a previous study in a European country (15). Moreover, the outcomes of other research studies designated the same outcome that differences in the exons do not affect the levels of infecting of COVID-19 infection severity (16).

Taking altogether and based on the other scientific evidence in this study, the outcomes can be summarized as the following points:

1. This is the first correlational study between the human molecular genetics of the ACE2 gene and the severity of COVID-19 disease among the Kurdish population.

2. Our findings show no significant evidence of a consistent connection between ACE2 genetic variations and COVID-19 liability.

The specific mutations detected according to the molecular background investigations of the *ACE2* gene receptor in COVID-19 patients is considered race-related genotype in the Kurdish population.

Further studies are recommended to be done:

1. Since the COVID-19 disease is a novel emerging epidemic globally and there are many hidden and undiscovered aspects of the COVID-19 infection, this study suggests investigating the genetic factors of the COVID-19 disease based on targeting other genes.

2. This study recommends that other researchers in this field work on the other exons and introns which were not included in the analysis of our study.

Authors' Contribution

Study concept and design: H. R. M. and G. O. O.
Acquisition of data: H. R. M. and G. O. O.
Analysis and interpretation of data: H. R. M. and G. O. O.
Drafting of the manuscript: H. R. M. and G. O. O.
Critical revision of the manuscript for important intellectual content: H. R. M. and G. O. O.
Statistical analysis: H. R. M. and G. O. O.
Administrative, technical, and material support: H. R. M. and G. O. O.

Ethics

The human study was approved by the ethics committee at the Salahaddin University, Erbil, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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