Trop J Nat Prod Res, February 2023; 7(2):2346-2351 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Genetic Association of Angiotensin-converting enzyme 2 ACE-2 (rs2285666) Polymorphism with the Susceptibility of COVID-19 Disease in Iraqi Patients

Risala H. Allami[1]*, Athraa H. Hassoon[2], Yasir M. Abdulateef [3], Ali A. Ghani[4], Shahad Jamal Al-Falahi[5]

[1]*Department of Medical and Molecular Biotechnology, College of Biotechnology, Al-Nahrain University, Iraq*
[2]*Department of Biology, College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad, Iraq*
[3]*Department of Microbiology - Medical College -Anbar University-Iraq*
[4]*Al Kunooze University College- Department of Medical Laboratory Techniques -Basrah, Iraq*
[5]*Pedodontics and Prevention Department, College of Dentistry, Al-Farahidi University*

ARTICLE INFO

Article history:
Received 02 December 2022
Revised 27 January 2023
Accepted 02 February 2023
Published online 01 March 2023

ABSTRACT

Significant risks to human health are posed by the 2019 coronavirus illness (COVID-19). SARS coronavirus type 2 receptor, also known as the major enzyme in the renin-angiotensin system (RAS), angiotensin-converting enzyme 2 (ACE-2), connects COVID-19 and RAS. This study was conducted with the intention of determining whether or not RAS gene polymorphisms and ACE-2 (G8790A) play a part in the process of predicting susceptibility to infection with COVID-19. In this study 127 participants, 67 of whom were deemed by a physician to be in a severe state of illness, and 60 of whom were categorized as "healthy controls" .The genetic study included an extraction of genomic DNA from blood samples of each covid 19 patients and healthy controls, then amplification the site of SNP (rs2285666) Within the ACE2 gene by using specific primers, sequencing PCR products, and genotyping to detect the role of the ACE-2 gene (rs2285666) in the incidence of COVID-19. ACE-2 (rs2285666) is statistically associated to COVID-19. The COVID-19 group had 65.67 %of individuals with the wild-type homozygous genotype (GG) and 20% in the control group, while the control group had 63.33% of individuals with the mutant genotype (AA). Consequently, the wild-type homozygous (GG) and allele (G) may be considered a risk factor (etiological fraction E. F) for COVID-19 in Iraqi patients, whereas the mutant homozygous (AA) and allele (A) may be considered a protective factor (preventive fraction). The findings of the present study reveal that carriers of the GG genotype of ACE2 (rs2285666) are substantially more susceptible to COVID-19.

Keywords: SARS-CoV-2; Coronavirus; Angiotensin receptors; Single Nucleotide Polymorphism

Introduction

The group of viruses known as coronaviruses causes a number of illnesses, including those that affect the respiratory and digestive systems. It is a member of the viral family and has the potential to infect both mammalian and avian hosts, causing sickness in both. The virus is an unique coronavirus that has been referred to in the past as "Corona Virus Disease 2019" It has the potential to produce a pandemic that affects people all over the world (2019-nC).[1] This infection can produce a disorder that was formerly referred to as severe acute respiratory SARS-CoV-2, but is now more often known as coronavirus illness 2019 (COVID-19). Because of this, the renin-angiotensin system, more commonly called the RAS, is being looked at as a possible therapy target for COVID-19.[2] Both are linked to the protein angiotensin-converting enzyme 2 receptor, or ACE-2 for short. ACE-2 is short for angiotensin converting enzyme 2, a type of receptor. Additionally, ACE-2 is used by the SARS-causing coronavirus as a functional receptor in the respiratory tract, where it promotes infection and cell fusion. [3,4] Lung alveolar epithelial cells may express the ACE-2 enzyme, which has role in the retinoic acid signaling pathway.

*Corresponding author. E mail: ririallami.bio@gmail.com
Tel: 00964 7730827772

Citation: Allami RH, Hassoon AH, Abdulateef YM, Ghani AA. Genetic Association of Angiotensin-converting enzyme 2 ACE-2 (rs2285666) Polymorphism with the Susceptibility of COVID-19 Disease in Iraqi Patients. Trop J Nat Prod Res. 2023; 7(2):2346-2351 http://.www.doi.org/10.26538/tjnpr/v7i2.7

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

The entry of virus into the lungs occurs through these cells.[5] In order to accomplish this objective, ACE-2 acts as a receptor for the COVID-19 virus, which paves the way for the virus to enter cells and spread throughout the body. The ACE-2 protein acts as a receptor for the SARS-CoV-2 virus, which paves the way for the virus to infect host cells.[6] There have been around 140 single nucleotide polymorphism (SNP) sites discovered in the human genome that are related with the ACE-2 gene. These loci can be found across the human genome. This gene has several variations.[7] The ACE-2 gene takes up a total of 21 kilobytes of space and may be found on chromosome 17q23.3. It consists of 26 exons and 25 introns in its make-up. Exon 26 is responsible for encoding the membrane-anchoring domain of the ACE protein, and this domain is necessary for the ACE protein to carry out its activity. The presence of the variant G8790A (rs2285666) on chromosome Xp22 in intron 3 of the ACE-2 gene demonstrates that this variant has the potential to change mRNA splicing and, as a consequence, ACE-2 gene expression. On the ACE-2 gene, this SNP was found to be a functional variant, making it one of the SNPs that were discovered there.[8]

The current study hypothesized that there is association between genetic polymorphism ACE2 (rs2285666) with the susceptibility of Coronaviruses in Iraqi patients and risk of SARS-CoV-2 infection.

Materials and Methods

Study groups

At the Al-Kindi hospital in Iraq, 67 different patients were being treated at the time when blood samples were collected from those patients. The investigation was carried out during the months of February and May of the year 2021, which was the same year. Between those two months

was the window of opportunity for doing the research. There were people in the group ranging in age from 25 all the way up to 70 years old. As controls for the experiment, there were a total of sixty people involved. These people ranged in age from 28 to 70 years old and were all regarded to be in good health. We obtained patient names, ages, heights, weights, body mass indexes, medications, and chronic conditions from the hospital, as well as demographic data such as heights and weights (T2DM and Hypertension).

Ethical approval
All of the procedures were carried out in accordance with what the research committee of either the institution or the country had declared to be acceptable methods of treatment, recording, and reporting of results. The approval code for this study is NMB-A345, and it has been assigned.

Extraction of DNA from blood samples
Whole blood samples were taken from both patients and controls, and then processed with the Presto TM Mini gDNA Kit in order to extract the individuals' respective DNA (Geneaid). A NanoDrop spectrophotometer was utilized for the aim of determining the DNA's concentration as well as the amount of purity it possessed. Gel electrophoresis was ultimately chosen since it was the most effective method for verifying the DNA's genuineness.

Identification of single nucleotide polymorphisms
In order to determine the identity of ACE-2, we performed amplification of the flanking areas by means of polymerase chain reaction (PCR), in conjunction with direct sequencing (rs2285666). The Bioneer Company in Korea was in charge of carrying out the DNA sequencing, and they used purified PCR products as the starting material for their analysis. Table 1 contains a list of the primer sequences that were utilized in this investigation, as well as the amplification conditions that were utilized.

Statistical analysis
In order to carry out the statistical analysis on the collected data, the software program known as SPSS 20.0 was utilized (IBM SPSS Statistics, SPSS Inc., Chicago, Illinois, USA).

Table 1: The sequences of the primers

Polymorphism	Primer sequence (5′-3′)	TA (°C)	Reference
G8790A (rs2285666)	F-TTCTCCCTGCTCCTATACTACCG	60	9
	R-TTCATTCATGTCCTTGCCCTTA		

Results and Discussion

PCR and DNA sequencing were used in the investigation of patient samples from Iraqi individuals in order to determine whether or not there was a correlation between ACE-2 gene polymorphism (rs2285666) and COVID-19 susceptibility. The goal of the investigation was to determine whether or not there was a correlation between the two.

Demographic and clinical characteristics of participants
In the current case-control analysis, we compared 67 persons with severe COVID-19 infection to 60 people who appeared to be healthy controls. This comparison was made using a case-control design. The clinical presentation of the patient, as well as the results of a positive PCR test, established the diagnosis of COVID-19 in accordance with WHO criteria. In Table 2, we can see some of the anthropometric information, such as BMI and age, of both COVID-19 patients and healthy controls. The controls were individuals who did not have the disease. According to the findings of the study, which showed a ratio of 50.7% women to 49.2% males, there were approximately the same number of women as there were men among the total of 127 participants. Age, gender, race, obesity, hypertension, diabetes, and geographic location are only few of the many factors that are known to increase a person's likelihood of becoming infected with this coronavirus. Other risk factors include.[10] Even while there was not a significant correlation between gender and the frequency of COVID-19 disease; But, earlier study has shown that men had a significantly higher chance of acquiring severe COVID-19 disease.[11-13] In addition, samples were collected from participants ranging in age from 25 to 49 years old as well as from 50 years old all the way up to 78 years old. Those under the age of 30 have a significantly reduced risk of having COVID-19, whereas patients above the age of 30 have a significantly increased risk. When compared to people in their later years, adults who are in the middle years of their lives have a lesser risk of having serious diseases.[14] According to the data collected, the vast majority of individuals diagnosed with COVID-19 are classified as either obese (46%) or overweight (34%), with the latter category coming in second.
In Iraqi patients, it was found that COVID-19 was linked to the development of diabetes mellitus, hypertension, and renal disease. Patients with COVID-19 had a higher prevalence of type 2 diabetes (60%) and hypertension (67%), both of which are more prevalent in the COVID-19 group than in the control group (33%). Patients with COVID-19 also had a higher prevalence of cardiovascular disease (80%). The effect that COVID-19 has on the lungs, the possible threat that it poses to the heart, and its propensity to create a surge in blood glucose levels are probably the cause of health concerns such disseminated intravascular coagulation and deep vein thrombosis[15] These data indicate that diabetic patients with COVID-19 had considerably greater rates of severe infection and case fatality than those without diabetes. COVID-19 people with type 2 diabetes mellitus (T2DM) are at increased risk for developing a life-threatening infection and for dying from their illness. Researchers believe that overactive renin-angiotensin systems may be at the root of the illness. Angiotensin-converting enzyme inhibitors, also known as ACEIs, and angiotensin receptor blockers, often known as ARBs, are two popular types of diabetic drugs that are prescribed to people who have type 2 diabetes (ARBs). There is a correlation between these two parameters and higher ACE-2 expression in tissues, which, in turn, enhances viral absorption and raises the risk of life-threatening infection in type 2 diabetics.[16] Multiple studies have shown that adults with proven SARSCOV-2 infection are more likely to die if they are older and have health problems like high blood pressure, diabetes, and heart disease.[17,18] According to the data, hypertension, diabetes, and COVID-19 were found to be prevalent in persons at rates of 21, 11, and 7%, respectively. The researchers found that patients with COVID-19 had a prevalence of 17, 8, and 5%, respectively, for hypertension, diabetes, and COVID-19 in a different study that was carried out by[19]. This study indicated that patients with COVID-19 had COVID-19. Diabetes was shown to be associated to 58.0% of those patients who had COVID-19, according to the findings of a study that involved 24 patients from the United States.[18]

Frequency and distribution of ACE-2 gene polymorphism and susceptibility to COVID-19
The purity of the extracted DNA ranged from 1.7 to 1.8, with 1.75 being the most common value, while the concentration of the DNA ranged from 12.5-170 ng/L, with 58.81 ng/L being the average value. The purity of the DNA that was isolated is illustrated in Figure 1. The statistical analysis of the genotype frequency distribution for the rs2285666 gene revealed the presence of two alleles, G and A. It also revealed the presence of three genotypes, with GG representing the wild-type genotype, GA representing the mutant heterozygote genotype, and AA representing the mutant homozygote genotype. The genotype and allelic distribution of the ACE-2 gene polymorphism are presented in detail in Tables 3 and 4, as well as Figures 2 and 3, respectively. According to Table 3 and Figure 2, the genotype (GG) was

found in 65.67% of the COVID-19 group while only 20% of the control group had it. On the other hand, the mutant homozygous (AA) was found in 11.9% of the COVID-19 group while 63.3% of the control group had it. There is evidence to suggest that ACE-2 (rs2285666) and COVID-19 are related to one another. This link is corroborated by statistics. In addition, the wild-type homozygous (GG) and allele (G) may be considered a risk factor (etiological fraction E. F) for COVID-19 in Iraqi patients, whereas the mutant homozygous (AA) and allele (A) may be considered a protective factor. This may be due to the fact that the wild-type homozygous (GG) and allele (G) have a higher frequency of the disease. This is due to the fact that both the wild-type homozygous and the allele (G) are linked to an increased risk of acquiring COVID-19 (preventive fraction).

In the current investigation, it was discovered that the wild-type genotype and the G allele were considerably connected with the prevalence of SARS-CoV-2 infection as well as the risk of contracting the virus. These findings are consistent with those discovered in earlier research carried out on populations of both Indian and Caucasian ancestry.[20,21] This study's findings are congruent with those of a previous one that linked ACE2 (rs2285666) to the rate of COVID-19 infection.[22] According to Table 4 and Figure 3, the percentage of patients with COVID-19 who had a G allele frequency was significantly higher (73.88%) than that of controls (51.67%). According to the findings, the G allele, which was linked to a more severe form of COVID-19 disease, represented the etiological part of the disease (EF). On the other hand, it was shown that the A allele, which was more common in healthy controls (48.33%) than in COVID-19 patients (26.12%), represented the protective EF gene.

Table 2: Anthropometric indices of COVID-19 patients and control groups.

Variables		Covid 19 Patients (n=67)	Control (n=60)
Gender	Male	34 (50.75%)	36 (60%)
	Female	33 (49.25%)	24 (40%)
Age	(25-49)	28 (42%)	38 (63%)
(Years)	(50- >70)	39 (58%)	22 (37%)
BMI	Normal	13 (20%)	23 (38%)
(Kg/m^2)	(18.5-24.9)		
	Overweight	23 (34%)	21 (35%)
	(25-30)		
	Obese	31 (46%)	16 (27%)
	(>30)		
T2DM	Yes	40 (60%)	20 (33%)
	No	27 (40%)	40 (67%)
Hypertension	Yes	45 (67%)	22 (37%)
	No	22 (33%)	38 (63%)

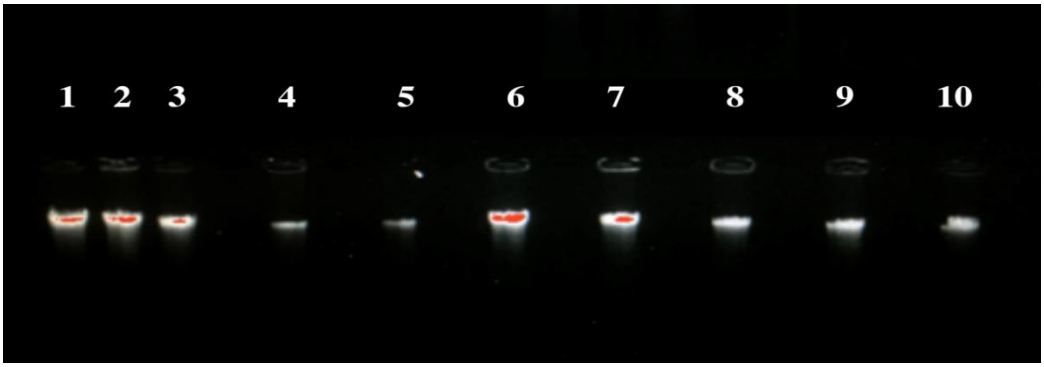

Figure 1: Electrophoresis of human genomic DNA on agarose gel (1%) at (70V/cm) for 30 min.

Both ACE and ACE-2 are responsible for a variety of different physiological processes in the body. When the ratio of ACE to ACE-2 increases, cross-models of ACE and ACE-2 genotypes have the potential to exacerbate COVID-19 by causing a RAS imbalance. This is because RAS has been linked to the development of a variety of diseases, including those that impact the cardiovascular system, the pulmonary system, and diabetes.[23-27] Numerous studies have been conducted on ACE-2, and as a result of those studies, a variety of SNPs that could be involved in COVID-19 have been discovered.[9,25-30] To the best of our knowledge, this work is the first publication on the frequency distribution of the rs2285666 genotype in the Iraqi population as well as its link to COVID-19. The country of Iraq served as the location for the research. On the other side, having a small sample size increases the likelihood that the conclusions of the study would be inconsistent and difficult to understand. An increase in the size of the sample will provide results that are easier to distinguish in terms of the effect that the ACE-2 gene has on COVID-19. The molecular approach was used to diagnose many pathogens, including : *Proteus vulgaris*,[31,32] *Clostridium perfringens*,[33] *Staphylococcus auras*,[34,35,36] *Brucella melitensis*,[37] Pseudomonas spp, [38] *Toxoplasma spp* [39,40] and SARS-Cov-2.[41,42] Also, Chronic Myeloid Leukemia,[43] Adenocarcinoma,[44,45] and another cancers [46],were considered severe genetic illnesses and diagnostic by PCR was suggested. On other hand, the CT chest patterns were consider in COVID-19 diagnosis in some local studies.[47,48]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 3: Genetic distribution of ACE-2 polymorphism in the COVID-19 against the healthy control groups

Gene ACE2	Genotype	Control (60) (%)	Patients (67) (%)	OR (95%CI)	P value
rs2285666	AA	(%63.33)38	(%11.9)**8**	3.3; 1.8–18.8	*0.000*
	P. F		%56.1		
	GA	(%16.67) 10	(%22.38)**15**	1.2; 0.55–5.0	0.565
	E. F		%1.5		
	GG	(%20)12	(%65.67)**44**	3.2; 1.8–18.5	
	E. F		%57.1		0.000**

OR: Odds ratio; CI: Confidence intervals; P.F: Preventive faction; E.F: Etiological faction;
*: Significant difference at P<0.05 probability level using test Fisher's test.

Table 4: Distribution of allelic frequency within the locus rs2285666 of ACE-2 gene between the patient and healthy groups

Gene ACE2	Allele	Control (60) (%)	Patients (67) (%)	OR (95% CI)	P- value
rs2285666	*G*	(% 51.67)31	(% 73.88)49	2.65)4.98-1.41	
	E.F		%46		** 0.002
	A	(% 48.33)29	(% 26.12)18	(0.71-0.20) 0.38	
	P.F		% 30.1		

OR: Odds ratio; CI: Confidence intervals; P.F: Preventive faction; E.F: Etiological faction;
*: Significant difference at P<0.05 probability level using test Fisher's test.

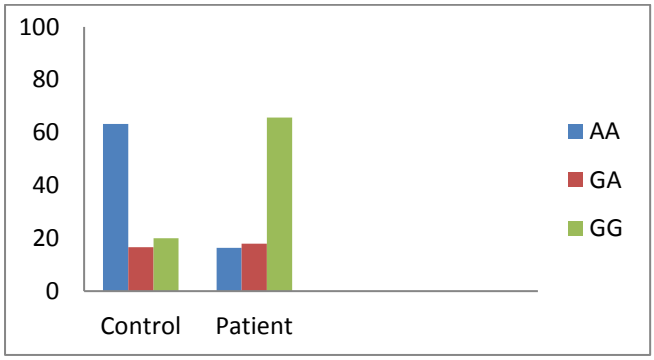

Figure 2: Comparison between the distributions of genotype within the locus rs2285666 of ACE-2 gene between patient and healthy groups.

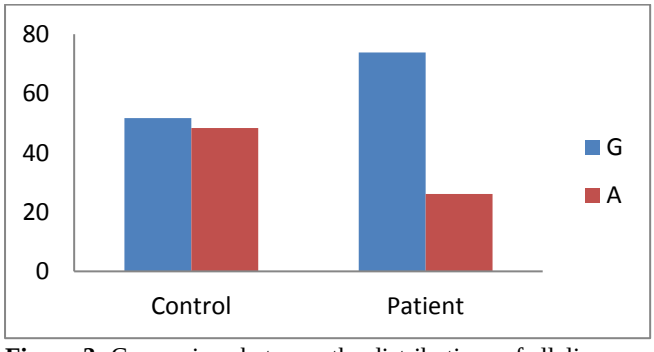

Figure 3: Comparison between the distributions of allelic frequency within the locus rs2285666 of the ACE-2 gene between the patient and healthy groups.

Conclusion

Based on the results of this study, the ACE-2 receptor SNP variant called rs2285666 is a factor that makes Iraqi patients more likely to get COVID-19. These results support the idea that having a copy of the ACE-2 gene makes it more likely that someone will get COVID-19.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Villaquirán Hurtado AF, Ramos OA, Jácome SJ, Meza MD. Actividad física y ejercicio en tiempos de COVID-19. Ces Medicina. 2020; 34:51-58.
2. Vaduganathan M, Vardeny O, Michel T, McMurray JJ, Pfeffer MA, Solomon SD. Renin–angiotensin–aldosterone system inhibitors in patients with Covid-19. N Engl J Med. 2020; 382(17):1653-1659.
3. Wu J, Deng W, Li S, Yang X. Advances in research on ACE2 as a receptor for 2019-nCoV. Cell Mol Life Sci. 2021; 78(2):531-544.
4. Costa LB, Perez LG, Palmeira VA, Macedo e Cordeiro T, Ribeiro VT, Lanza K, Simoes e Silva AC. Insights on SARS-CoV-2 molecular interactions with the renin-angiotensin system. Front Cell Dev Biol. 2020; 8:559841.
5. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis GV, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol. 2004; 203(2):631-637.
6. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, Choe H. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003; 426(6965):450-454.

7. 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(19):2654-2659.
8. 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; 5:227.
9. Firouzabadi N, Farshadfar P, Haghnegahdar M, Alavi-Shoushtari A, Ghanbarinejad V. Impact of ACE2 genetic variant on antidepressant efficacy of SSRIs. Acta Neuropsychiatr. 2022; 34(1):30-66.
10. Kim L, Garg S, O'Halloran A, Whitaker M, Pham H, Anderson EJ, Armistead I, Bennett NM, Billing L, Como-Sabetti K, Hill M. Risk factors for intensive care unit admission and in-hospital mortality among hospitalized adults identified through the US coronavirus disease 2019 (COVID-19)-associated hospitalization surveillance network (COVID-NET). Clin Infect Dis. 2021; 72(9):e206-214.
11. Gómez J, Albaiceta GM, García-Clemente M, López-Larrea C, Amado-Rodríguez L, Lopez-Alonso I, Hermida T, Enriquez AI, Herrero P, Melón S, Alvarez-Argüelles ME. Angiotensin-converting enzymes (ACE, ACE2) gene variants and COVID-19 outcome. Gene. 2020; 762:145102.
12. Killerby ME, Link-Gelles R, Haight SC, Schrodt CA, England L, Gomes DJ, Shamout M, Pettrone K, O'Laughlin K, Kimball A, Blau EF. Characteristics associated with hospitalization among patients with COVID-19—Metropolitan Atlanta, Georgia, March–April 2020. Morb Mortal Wkly. 2020; 69(25):790-794.
13. Klein SL, Dhakal S, Ursin RL, Deshpande S, Sandberg K, Mauvais-Jarvis F. Biological sex impacts COVID-19 outcomes. PLoS pathogens. 2020; 16(6):e1008570.
14. Liu YC, Kuo RL,Shih SR. COVID-19: The first documented coronavirus pandemic in history. Biomed j. 2020; 43(4):328-333.
15. Liu Y, Yang Y, Zhang C, Huang F, Wang F, Yuan J, Wang Z, Li J, Li J, Feng C, Zhang Z. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. Sci China Life Sci. 2020; 63(3):364-374.
16. Holt A, Mizrak I, Lamberts M, Madsen PL. Influence of inhibitors of the renin–angiotensin system on risk of acute respiratory distress syndrome in Danish hospitalized COVID-19 patients. J Hypertens; 2020; 38(8): 1612-1613.
17. Zhou C, Chen Y, Ji Y, He X, Xue, D. Increased serum levels of hepcidin and ferritin are associated with severity of COVID-19. Med Sci Monit, 2020; 26: e926178.
18. Singh A. , Gupta R, Ghosh A, & Misra, A. Diabetes in COVID-19: Prevalence, pathophysiology, prognosis and practical considerations. Diabetes & Metabolic Syndrome: Clin. Res. 2020; 14(4), 303-310.
19. Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo, Q, Zhou, Y. Prevalence of comorbidities in the novel Wuhan coronavirus (COVID-19) infection: a systematic review and meta-analysis. Int J Infect Dis, 2020; 10 (10.1016).
20. Srivastava A, Bandopadhyay A, Das D, Pandey RK, Singh V, Khanam N, Srivastava N, Singh PP, Dubey PK, Pathak A, Gupta P. Genetic association of ACE2 rs2285666 polymorphism with COVID-19 spatial distribution in India. Front Neurol. 2020; 11:1163.
21. Möhlendick B, Schönfelder K, Breuckmann K, Elsner C, Babel N, Balfanz P, Dahl E, Dreher M, Fistera D, Herbstreit F, Hölzer B. ACE2 polymorphism and susceptibility for SARS-CoV-2 infection and severity of COVID-19. Pharmacogenet. Genomics. 2021;31(8):165-171.
22. 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(1):1-9.
23. Ferreira AJ, Murça TM, Fraga-Silva RA, Castro CH, Raizada MK, Santos RA. New cardiovascular and pulmonary therapeutic strategies based on the Angiotensin-converting enzyme 2/angiotensin-(1–7)/mas receptor axis. Int J Hypertens 2012; 2012:5.
24. Tan WS, Liao W, Zhou S, Mei D, Wong WS. Targeting the renin–angiotensin system as novel therapeutic strategy for pulmonary diseases. Curr Opin Pharmacol. 2018; 40:9–17.
25. Srivastava P, Badhwar S, Chandran DS, Jaryal AK, Jyotsna VP, Deepak KK. Imbalance between Angiotensin II-Angiotensin (1-7) systems is associated with vascular endothelial dysfunction and inflammation in type 2 diabetes with newly diagnosed hypertension. Diabetes & Metabolic Syndrome: Clin Res. 2019; 13(3):2061-2068.
26. Cook JR, Ausiello J. Functional ACE2 deficiency leading to angiotensin imbalance in the pathophysiology of COVID-19. Rev Endocr Metab Disord. 2022; 23(2):151-70.
27. Lanza K, Perez LG, Costa LB, Cordeiro TM, Palmeira VA, Ribeiro VT, Simoes e Silva AC. Covid-19: the renin–angiotensin system imbalance hypothesis. Clin sci. 2020; 134(11):1259-1264.
28. Gemmati D, Tisato V. Genetic hypothesis and pharmacogenetics side of renin-angiotensin-system in COVID-19. Genes. 2020; 11(9):1044.
29. Alimoradi N, Firouzabadi N. Impact of Genetics on Predisposition and Prognosis of COVID-19. Trends Pharmacol Sci. 2021; 7(2):73-80.
30. 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.
31. Abdul-Gani MN, Laftaah BA. Purification and characterization of chondroitinase ABC from Proteus vulgaris, an Iraqi clinically isolate. Curr Sci. 2017:2134-2140.
32. Kadhim AL-Imam MJ, AL-Rubaii BA. The influence of some amino acids, vitamins and anti-inflammatory drugs on activity of chondroitinase produced by Proteus vulgaris caused urinary tract infection. Iraqi J Sci., 2016; 57 (4A):2412-2421.
33. Hashim ST, Fakhry SS, Rasoul LM, Saleh TH, Alrubaii BA. Genotyping toxins of *Clostridium perfringens* strains of rabbit and other animal origins. Trop J Nat Prod Res. 2021; 5(4):613-616.
34. Fakhry SS, Hammed ZN, Bakir WA-E, ALRubaii BAL. Identification of methicillin-resistant strains of *Staphylococcus aureus* isolated from humans and food sources by use mecA 1 and mecA 2 genes in Pulsed-field gel electrophoresis technique. Bionatura. 2022;7(2):44. doi: 10.21931/RB/2022.07.02.44.
35. Ali M, Al-Rubaii B. Study of the Effects of Audible Sounds and Magnetic Fields on *Staphylococcus aureus* Methicillin Resistance and mecA Gene Expression. Trop J Nat Prod Res. 2021; 5(5):825-830.
36. Abdulrazaq, R.A., Mahmood, W.S., Alwan, B., Saleh, T.H., Hashim, S.T., Al-Rubaii, B.A.L. Biological Study of protease produced by clinical isolates of *Staphylococcus aureus*. Res J Pharm Technol.2022; 15(12):5415-5420.
37. Abdulkaliq Awadh H, Hammed ZN, Hamzah SS, Saleh TH, AL-Rubaii BA. Molecular identification of intracellular survival related *Brucella melitensis* virulence factors. Biomedicine. 2022; 42(4):761-765.
38. Al-Rubii, B.A.L. Cloning LasB gene of *Pseudomonas aeruginosa* eiastase 10104-2aI in *E. coli* BL21 and *E.coli* DH5α and investigated their effect on the stripping of Vero cells. Pakistan J Biotechnol. 2017; 14(4):697-705.
39. Abdulla L, Ismael MK, Salih TA, Malik SN, Al-Rubaii BA. Genotyping and evaluation of interleukin-10 and soluble HLA-G in abortion due to toxoplasmosis and HSV-2

infections. Ann Parasitol., 68(2):385–390.
40. Jiad AL, Ismael MK, Muhsin SS, Al-Rubaii BA. ND2 Gene Sequencing of Sub fertile Patients Recovered from COVID-19 in Association with Toxoplasmosis. Bionatura. 2022; 7(3):1-4.
41. Rasoul, L.M., Marhoon, A.A., Albaayit, S.F.A., Ali, R.W., Saleh, T.H., Al-Rubaii, B.A.L. Cytotoxic effect of cloned EGFP gene on NCI-H727 cell line via genetically engineered gene transfer system. Biomedicine. 2022; 42(5):938-942.
42. Rasoul LM, Nsaif MM, Al-Tameemi MT, Al-Rubaii BA. Estimation of primer efficiency in multiplex PCR for detecting SARS-Cov-2 variants. Bionatura, 2022; 7(3):48.
43. Ahmed AA, Khaleel KJ, Fadhel AA, Al-Rubaii BA. Chronic Myeloid Leukemia: A retrospective study of clinical and pathological features. Bionatura. 2022; 7(3):41.
44. Ali SM, Lafta BA, Al-Shammary AM, Salih HS. *In vivo* oncolytic activity of non-virulent newcastle disease virus Iraqi strain against mouse mammary adenocarcinoma. In AIP Conference Proceedings 2021; 2372 (1):030010.
45. Ali SM, Laftah BA, Al-Shammary AM, Salih HS. Study the role of bacterial neuraminidase against adenocarcinoma cells in vivo. In AIP Conference Proceedings 2021; 2372(1): 030009.
46. Salih HS, Al-Shammari AM, Laftaah BA. Intratumoral co-administration of oncolytic newcastle disease virus and bacterial hyaluronidase enhances virus potency in tumor models. J Glob Pharma Technol. 2018; 10(10):303-10.
47. Ahmed AW, Ahmed RN, Naif MM, Yahya MT, Maulood KS, Alchalabi GB, Mohammed AG. Chest CT findings and experience in 100 COVID19 patients in Mosul city, Iraq. Biomedicine. 2021 Dec 31; 41(4):793-798.
48. Suhail H, Abdulrahman D, Ahmed A. Fetal Biometry and Doppler Assessment of Pregnant Women with COVID-19. Int J Biomed. 2022; 12(4):554-559.