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The *rs233569* Polymorphism of the *ACE2* Gene in Iraqi Hospitalization Patients with SARS-COV2

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Abstract: During the year 2020, the emergence of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV2), inflicted considerable damage and devastation upon the global populace, resulting in the unfortunate demise of approximately 6.5 million individuals .This study was conducted from January 2022 till August 2022 on 70 hospitalized patients infected with COVID-19 in Dar Al Salam Field Hospital and Dar Al Shifa Hospital affiliated to the Medical City with age range of 19-60 years and 70 healthy subjects with the same age . Blood samples were collected from all participated for DNA extraction and detection the *rs233569* polymorphism of the ACE2 gene using Tetra Arms Technique. Primers were designed for the tetra amplification refractory mutation system polymerase chain reaction technique (tetra ARMS PCR) based on their corresponding sequence in the National Center for Biotechnology Information NCBI https://www.ncbi.nlm.nih.govpmc. The results of *rs233569* genotype revealed three genotypes (CC, CT, TT) frequency distribution in both SARS- COV2 patients and controls the homozygous genotype (TT) appeared with a significant increase ($0 \leq 0.05$) in SARS- COV2 patients compared with control. The homozygous genotype (CT) had also appeared with highly significant increase ($0 \leq 0.01$) in SARS- COV2patients compared with control. Resulting that heterozygote CT was a higher risk of SARS- COV2 patients comparing with genotype TT that estimated a significant increase. Allele C allele is more frequent in both in SARS- COV2 patients and control.

Keyword: SARS- COV2, ACE2gene, *rs233569* genotype

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Introduction

During the calendar year of 2020, the advent of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV2), responsible for the inception of COVID-19, led to significant repercussions and widespread devastation among the international population, culminating in the regrettable passing of an estimated 6.5 million individuals and steadily increasing on a global scale (1,2). SARS-CoV-2 is a member of the Coronaviridae family, known for its non-segmented single-stranded positive-sense RNA that contains genetic information for a spike protein (S-protein) and an envelope protein (E-protein) (3), the membrane glycoprotein, also referred to as the M-protein, as well as the nucleocapsid protein, known as the N-protein, have been documented in references(4,5). This particular virus is responsible for initiating respiratory infection in mammals, as stated in reference(6). The entry of a virus into the body is facilitated by several host molecules which serve as receptors, co-receptors, and co-factors. Among these, the Angiotensin-converting enzyme 2 (ACE2) is the most crucial host receptor

that assists in the entry of the virus (7). ACE2 is an enzyme belonging to the family of Dipeptidyl carboxypeptidases. Given that ACE2 displays differential expression patterns across various human organs and cell types, it stands to reason that this enzyme may exert regulatory control over crucial physiological processes pertaining to reproduction, cardiovascular activity, and renal function (8). The normal role of ACE2 is to catalyze the process of cleaving Angiotensin I to produce Angiotensin and converting Angiotensin II into Angiotensin (9)(10). The present study was designed to determination the rs233569 Polymorphism of the ACE2 Gene in Iraqi Hospitalization Patients with SARS-COV2.

Materials and methods

A case-control study was undertaken between January 2022 and August 2022, wherein written informed consent was acquired from all patients and the study was granted ethical approval by the committees responsible for ensuring ethical standards in research within the Department of Biology, the College of Science, University of Baghdad. The investigation was carried out on a cohort of 70 patients who were admitted to Dar Al Salam Field Hospital and Dar Al Shifa Hospital, both of which are affiliated with the Medical City. and diagnosed with COVID-19. The age range of the patients was from 19 to 60 years. The blood sample were collected and diagnosed as having according to WHO guidance (based on a positive RT-PCR test) and pneumonia (based on computed tomography imaging) patients were paneled of routine laboratory tests.

The extraction of genomic DNA from a blood

Sample obtained from the studied group was conducted using a DNA extraction kit (Favorgen, China). The DNA that was extracted was subsequently stored at a temperature of -20ºC or lower.

Measurement of DNA concentration and purity

The use of Nano drop spectrophotometers allowed for the determination of both the purity and concentration of the DNA that was extracted. The light emanating from a xenon flash lamp traversed the upper optic fiber, descended through the fluidic column, and was subsequently perceived by the internal spectrometer in the assay that relied on the DNA's absorbance at 260-280 nm. Initially, a quantity of 1-2 µl was transferred directly onto the measuring pedestal utilizing the micropipette. Subsequently, the sampling arm was lowered, and a spectrum measurement was initiated by employing the programmers situated on the computer(11).

Detection of the ACE2 gene (*rs233569*) in the human genome was conducted using the Tetra Arms Technique. Specific primers were employed to identify single nucleotide polymorphisms (SNPs) within the targeted gene through the tetra arm technique. Primers were designed for the tetra amplification refractory mutation system polymerase chain reaction technique (tetra ARMS PCR) based on their corresponding sequence in the National Center for Biotechnology Information NCBI The primer sequence and resulting product are listed as show in (Table 1).

Table (1): Sequence of Primers Used in The Present Study For *rs233569* ACE2 by using Tetra Arms Technique.

***rs233569* ACE2**	IF: GGTTTTGCTGTATTCAGTTAGATATTCTC	58	C allele: 193 T allele: 133 outer primers: 271
	IR: GCTTATGGTTTGTAAGCATTTAACAA		
	OF: TATCTTTAGGGTGGGTTTCTGAGTA		
	OR: GAAAACACATCAACACAGACCTTAA		

Results and discussion

Amplification of ACE2

The genomic DNA was amplified using the Tetra Arm PCR technology thought using specific primers for a normal and mutated DNA sequence. As a result, the product was detected as a band via gel electrophoresis. The SNP of ACE2 *rs233569* distinguished according to the size of the product (271, 193,133 bp) as show in (Table 2).

Table (2): Genotype and Allele Frequency (%) Of (*Rs233569*) Polymorphism Genotype Frequency (%) *rs233569* .

Genotype	Control n=30	Patients n=50	P-value	Chi-square	Odds Ratio	95% Cl
CC	28 (93%)	26 (52%)	----	----	1.0	----
CT	2 (7%)	19 (38%)	0.0008 **	11.29	10.23	2.343 to 46.68
TT	0 (0%)	5 (10%)	0.0263 *	4.934	11.8302	0.6235 to 224.4573
Chi-square	0.03564 NS	0.2982 NS				
***p* Value**	0.9823	0.8615				
Allele frequency (%)						
Allele	**n=60**	**n=100**	**P-value**	**Chi-square**	**Odds Ratio**	**95% Cl**
C	0.97 (58)	0.71 (71)	<0.0001 **	15.81	11.85	3.025 to 51.64
T	0.03 (2)	0.29 (29)				

Genotype and allele frequency distributions of ACE2 receptor snp

The *rs233569* genotype was established by using the tetra arms – PCR technology among SARS- CO2 patients and control samples. Findings illustrated the statistical result of *rs233569* genotype that revealed three genotypes (CC, CT, TT) frequency distribution in both SARS- CO2 patients and controls. (38%) in SARS-COV2 patients compared with control (7%) as show in table (3) and figures (1and 2), odds ratio was (11.8302) and 95% Cl (0.6235 to 224.4573) at P-value (0.0263) for TT and odds ratio for CT was (10.23) and 95% Cl (2.343 to 46.68) at P-value (0.0008). Resulting that heterozygote CT was a higher risk of SARS- COV2 patients comparing with genotype TT that estimated a significant increase. Allele C allele are more frequent in both in SARS- COV2 patients and control and it have odds ratio (11.85) and 95% Cl (3.025 to 51.64) at P-value (0<0.0001) as shown in (Table 3).

Table (3): Numbers and Percentage Frequencies (Observed and Expected) Of Ace2 Receptor Gene (*Rs233569* Snp) Genotype and Their Hardy-Weinberg Equilibrium (Hwe) In Patients and Controls.

		Control		*p*-value	Patients		*p*-value
		Observed	Expected		Observed	Expected	
rs4532	**CC**	28	28 (100%)	>0.9999 NS	26	25 (50%)	0.8910 NS
	CT	2	2 (0%)		19	21 (42%)	
	TT	0	0 (0%)		5	4 (8%)	

NS=non-significant, * significant at p value ≤ 0.05, ** significant at p value ≤ 0.01.

Recent reports have indicated that ACE2 plays a crucial role in facilitating the entry of SARS-CoV-2 into cellular structures. Furthermore, recent investigations employing single-cell RNA studies have provided evidence demonstrating the expression of ACE2 in human lung cells (12). The predominant population of cells that express ACE2 consists of alveolar type 2 cells, while alveolar type 1 cells, airway epithelial cells, fibroblasts, endothelial cells, and macrophages also express ACE2 albeit at a relatively low and variable ratio in different individuals. The expression and distribution of the ACE2 receptor may, therefore, provide a rationale for the route of infection and the primary localization at the alveolar level. The clinical variability may be partially attributed to the varying density of ACE2 receptors in the upper respiratory tract among individuals, the spectrum of patients affected by the virus encompasses individuals who show no symptoms or minimal symptoms to those who are severely affected. However, it is important to note that this variability cannot be solely attributed to viral load. Moreover, recent studies have not identified any substantial differences in viral loads between symptomatic and asymptomatic patients when analyzing nasal swabs (13)(20). (14) conducted an analysis on the whole-exome sequencing data obtained from a total of 6930 Italian control individuals, which were collected from five different research centers. The primary aim of this analysis was to identify any potential variations in the ACE2 gene. Previous studies have established that the SARS-CoV-2 virus relies on the angiotensin converting enzyme 2 (ACE2) as a receptor for host cell binding and internalization. Consequently, it is plausible to hypothesize that specific genetic backgrounds may contribute to an individual's susceptibility and/or severity of the disease. In order to explore this possibility, the researchers utilized the Network of Italian Genomes (NIG) to gather the necessary data.in our present study the homozygous genotype (TT) appeared with a significant increase 0≤ 0.05 (10%) in SARS- CO2 patients compared with control (0%) with odds ratio (11.8302) and 95% Cl (0.6235 to 224.4573) at P-value (0.0263). The homozygous genotype (CT)had also appeared with highly significant increase 0≤ 0.01 (38%) in SARS- CO2 patients compared with control (7%) with odds ratio (10.23) and 95% Cl (2.343 to 46.68) at P-value (0.0008). resulting that heterozygote CT was a higher risk of SARS- CO2 patients comparing with genotype TT that estimated a significant increase. Allele C allele are more frequent in both in SARS- CO2 patients and control and it have odds ratio (11.85) and 95% Cl (3.025 to 51.64) at P-value (0<0.0001). this result was agree with(15). they reported the wild genotype and the C allele was substantially related with the prevalence and risk of SARS-CoV-2 infection in our investigation, this result indicted significant increase found in genotypes of *Rs 233569* associated with accessibility to SARS-CoV-2 as a risk factor this result was dis agree with (16). they were suggested that no relationship was found between ACE-2 receptor gene intron variants and COVID-19 severity (17, 18, 19).

Conclusion

In current study, we have reached a conclusion regarding the determination of the rs233569 Polymorphism within the ACE2 gene, which holds significant implications for diagnosing patients affected by SARS-COV2.

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