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REVIEW ARTICLE

Gene and Alleles of TYK2 (rs11085727) and TYK2 (rs34536443) polymorphisms in COVID-19 Patients using ARMS-PCR.

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Abstract:

Due to The clinical presentation of COVID-19 varies greatly from case to case, with host characteristics playing a pivotal role. The immune system is a pivotal function in establishing the course of SARS-COV-2 infection. Severe instances of COVID-19 and the resulting death are caused less by the virus itself than by the destructive, unrestrained immune response. Among the host determinants for poor disease prognosis is probably genetic vulnerability to dysregulated immune response. The objective of this work is to examine some of the host genetic variables that are being studied in relation to the severity of COVID-19. The complex relationship between autoimmunity and the pathophysiology of COVID-19 was examined. Patients with COVID-19 were recruited from AL-Dewaniyah Teaching Hospital and AL-Hussain Teaching Hospital in Karbala city. All patients had been diagnosed with the virus using real-time polymerase chain reaction. A total of 105 blood samples (35 from the mild group, 35 from the severe group, and 35 from the controlled group) were obtained in EDTA tubes. Orderly to investigate the correlation between the progression of disease and variations in specific regions of host DNA (TYK2), DNA extraction, and genetic analysis (ARMS-PCR). The findings of the ARMS-PCR assay showed insignificant differences between the severity of COVID-19 in the present study. Genotype distributions were shown for both TYK2 (rs11085727) and TYK2 (rs34536443) polymorphisms. There was an insignificant difference between the observed and expected distribution of healthy by TYK2 (rs11085727) and TYK2 (rs34536443) genotypes. According to the results of ARMS-PCR, there was an insignificant difference between the severity and its mildness in the current study patients.

Keywords:TYK2 (rs34536443), TYK2 (rs11085727), COVID-19, polymorphism, ARMS-PCR.

Introduction

The coronavirus-2 strain that causes severe acute respiratory syndrome (SARS) has affected millions worldwide. In addition to SARS, the family Coronaviridae comprises four seasonal coronaviruses that typically only cause moderate symptoms [1]. SARS-CoV-2 has been shown to infect a number of mammal species besides humans in the wild, and numerous additional species have been found to be vulnerable to SARS-CoV-2 [2]. The symptoms of a sickness caused by COVID-19 can range from completely asymptomatic to severe, even lethal. Most people complain of a high temperature, a persistent cough, a headache, fatigue, difficulty breathing, a diminished sense of smell, and a diminished sense of taste. An estimated one-third of HIV carriers are completely unaware that they have the infection, according to the findings of several studies [3-5]. Other variables include age, being male, and the presence of comorbidities such as COPD, CVD, hypertension, diabetes, obesity, and cancer. Some host genetic features have also been hypothesized to raise the probability of a severe case of SARS-CoV-2 or COVID-19 infection [5]. Although coronaviruses can infect many systems in the human body, they most frequently attack the respiratory system. Examples of viruses that could be dangerous to public health include the coronaviruses that cause severe ARS and MERS-CoV. By the end of 2019, many people had sought medical attention for mysterious pneumonia. Wuhan, Hubei Province, China, is home to a massive wholesale market where the ton sells fresh seafood and wet animals. Due to the high replication rate, the 2019 novel coronavirus could cause an outbreak [1]. Docking studies are also necessary to understand the interaction efficiency of the novel variants with the host receptor molecule and proteolytic enzymes and thus infer the mechanistic basis of enhanced viral replication and higher transmissibility observed with these variants [6]. However, most COVID-19 association studies were conducted in Caucasian groups. Insufficient research has been conducted on the genetics of COVID-19 hosts from Iraq. So, the present study was designed to identify the associations between the development of disease and variations in specific host DNA regions (TYK2).

Materials and methods

Patients and Control: Patients with COVID-19 were recruited from AL-Dewaniyah and Karbala, AL-Hussaini Teaching Hospi-

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tals for a case-control study based on the assemblage of blood samples confirmed by qPCR. We classified patients with mild disease (n=35), severe disease (n=35), and a controlled group (n=35) based on the severity of COVID-19 disease.

Collection of Samples: All patients' specimens were collected under biosafety precautions and handled in a BSL2 biosafety hood practice [7]. Two types of samples were collected from both COVID-19 patients and the healthy control group, as detailed in the following steps: (Two ml of blood in EDTA tubes for the molecular study were stored at -20ºC until use).

The Primers: The Tetra-ARMS-PCR designed in NCBI-SNP and PRIMER1: primer design for tetra-primer ARMS-PCR. These primers were provided by Scientific Researcher Co. Ltd., Iraq, as in the following tables: Primers TYK2 rs11085727 C/T gene polymorphism, forward inner (T): CTGAGGTCAGGAGTTTGAGACCATCT (205 bp); reverse inner C: GGGTTTCACTGTGTTGGCGAG (231 bp); forward outer: AGCCATGATCGCCTCTGAAT; reverse outer: TCTTGTTGCACAGGCTGGAGTG (389 bp). Primers TYK2rs34536443 G/C-gene polymorphism, forward inner (G): CCTGGCTCTCACCGTGGGCGG (178 bp); reverse inner (C): GACGCACTGTGACTCCAGCCAGACCG (122 bp); forward outer: ACAGGCTTGAGCCACCGCGCCT; reverse outer: AGCCCTCAGTGCAGCCCCCGT (253 bp).

Statistical Analysis

The data were entered into a computerized database and checked for inaccuracies using a range and biological data cleaning approach after consulting with a statistician. We analyzed all the data using the Statistical Package for the Social Sciences (SPSS) V20 and Microsoft Excel 2021. The chi-squared test was used to determine the statistical significance of the relationships between the odds ratio (OR) and 95% CI. Bilateral probability was used to evaluate all of the statistics, and a significant estimate was defined as one with a P value of 0.05 or lower.

Results

The Detection of TYK2 (rs11085727) Polymorphism

Using the ARMS-PCR method, the TYK2 (rs11085727) polymorphism occurrence was determined. CC, CT, and TT were the three possible genotypes at this locus. Only the C allele was amplified at a size of 231 bp in the wild-type homozygote genotype. Homozygotes for the mutant type revealed just the T allele amplified at 205 bp. Fig. 1 shows that amplification of the C and T alleles occurred at 231 and 205 bp in the heterozygote genotype, respectively. Table 1 displays the results of using this equation on the occurrence disseminations of the CC, CT, and TT genotypes of the TYK2 (rs11085727) healthy group. Twenty of the healthy participants out of 35 carried the wild-type CC allele, while ten carried the heterozygous CT allele, and five carried the mutant TT allele. The hypothesized and actual disseminations of TYK2 (rs11085727) genotypes among healthy participants were insignificantly different.

Table (1): Hardy Weinberg equation

Genotypes	Observed	Expected	χ^2	P
Homozygote reference CC	20	17.9	3.161	0.206 ¥ NS
Heterozygote CT	10	14.3		
Homozygote variant TT	5	2.9		

The Genotypic and Alleles Analysis for The Studied Gene in Patients and Healthy Participates.

The dissemination of patients with COVID-19 and healthy subjects according to TYK2 (rs11085727)) genotypes and alleles was shown in table (2).

Table (2): TYK2 (rs11085727) POLY genotype occurrence in patients with COVID-19 and healthy.

TYK2 (rs11085727)	Severe, n = 35	Mild, n = 35	Healthy, n = 35	P value	
Genotype occurrence					
TT	7 (20.0%)	11(31.4%)	5(14.3%)	Severe vs healthy	0.732
C/T	11(31.4%)	13(37.2%)	10(28.6%)	Mild vs healthy	0.072
CC	17(48.6%)	11(31.4%)	20(57.1%)		
Allele occurrence					
T	25(35.7%)	35(50.0%)	20(28.%)		
C	45(64.3%)	35(50.0%)	50(71.4%)		
Related	P value	OR	95 % CI		
Severe vs healthy	0.365	1.38	0.68-2.83		
Mild vs healthy	0.009	2.5	1.24-5.02		

The Association among Severity of the Patients with COVID-19 The Results of ARMS-PCR

The association between the severity of patients and the results of ARMS-PCR indicated that there was insignificant variance between severe patient cases and mild cases according to the results of ARMS-PCR.

Table (3): Association among severity of patients with COVID-19 the results of ARMS-PCR (TYK2 (rs11085727 polymorphism).

TYK2 (rs11085727(		Severity		*p-value	**OR
		Severe(35)	Mild(35)		
Genotypes	TT	7 (20.0%)	11(31.4 %)	0.148	0.411
	CT	11(31.4%)	13(37.2 %)	0.284	0.547
	CC	17(48.6%)	11(31.4%)	Reference	

Allele	T	25(35.7%)	35(50.0%)	0.087	0.555
	C	45(64.3%)	35(50.0%)		1.8

The Detection of TYK2 (rs34536443) Polymorphism

Using the ARMS-PCR method, the TYK2 (rs34536443) polymorphism occurrence is determined. GG, GC, and CC were the three possible genotypes at this locus. The wild-type homozygote genotype amplifies only the G allele at a size of 187 bp. Homozygotes for the mutant type revealed only the C allele, amplified at 122 bp. Fig. 2 shows that the heterozygote genotype amplified both the G and C alleles to a size of 253 bp. The genotypes were dispersed across all groups. Table 4 displays the results of applying the aforementioned equation to the occurrence disseminations of the GG, GC, and CC genotypes of TYK2 (rs34536443) within the healthy group. The results indicate that among the 35 healthy subjects, 18 had the homozygous wild GG genotype, 10 had the heterozygous GC genotype, and 7 had the homozygous mutant CC genotype. We found that the distribution of TYK2 (rs34536443) genotypes among healthy subjects did not significantly deviate from our predictions.

Table (4): Hardy Weinberg equation

Genotypes	Observed	Expected	χ^2	P
Homozygote reference GG	18	15.1	4.695	0.096 ¥ NS
Heterozygote GC	10	15.8		
Homozygote variant CC	7	2.9		

The Genotypic and Alleles Analysis for The Studied Gene in patients and Healthy Participants

The dissemination of patients and healthy subjects according to TYK2 (rs34536443) genotypes and alleles was shown in table (5).

Table (5): TYK2 (rs34536443) POLY genotype occurrence in patients with COVID-19 and healthy

TYK2 (rs34536443)	Severe, n = 35	Mild, n = 35	Healthy, n = 35	P value	
	Genotype occurrence				
CC	9(25.7%)	8(22.9%)	7(20.0%)	Severe vs healthy	0.485
G/C	12(34.3%)	12(34.3%)	10(28.6%)	Mild vs healthy	0.771
GG	14(40.0%)	15(42.8%)	18(51.4%)		
	Allele occurrence				
C	30(42.9%)	28(40.0%)	24(34.3%)		
G	40(57.1%)	42(60.0%)	46(65.7%)		
	Related	P value	OR	95 % CI	
	Severe vs healthy	0.299	1.43	0.72-2.84	
	Mild vs healthy	0.484	1.28	0.64-2.54	

The Association among The severity of The Patients with COVID-19 and The Results of ARMS-PCR

The association among severity of the patients, results of ARMS-PCR indicated that there was insignificant variance among the severe patients and mild cases according to the results of ARMS-PCR.

Table (6): Association among severity of patients with COVID-19 the results of ARMS-PCR (TYK2 rs34536443 polymorphism).

TYK2 (rs34536443)		Severity		*p-value	**OR
		Severe (35)	Mild (35)		
Genotypes	CC	9 (25.7%)	8(22.9%)	0.760	1.2
	GC	12(34.3%)	12(34.3%)	0.900	1.07
	GG	14(40.0%)	15(42.8%)	Reference	
Allele	C	30(42.9%)	28(40.0%)	0.731	1.13
	G	40(57.1%)	42(60.0%)		0.888

Discussion

van Blokland and Lanting [8] identified an increased prevalence of polymorphisms in many COVID-19 candidate genes, such as TYK2, among individuals diagnosed with COVID-19. The variation in the severity of COVID-19 symptoms among the individuals can be elucidated by disparities in their TYK2 levels. This study elucidates the genetic polymorphisms within patients' genes associated with the severity or fatality of COVID-19 within a specific population from Iraq. The clinical results and death rates of patients who were diagnosed with COVID-19 are shown to be more severe and elevated, particularly in those who possessed certain polymorphisms in the TYK2 gene, which were regulated by sex and ethnicity. A confluence of polymorphisms influences the impact of COVID-19 on severity and mortality rates. The initial immune response against viral infections depends on the functioning of TYK2 proteins for transmitting signals through the pathway [9, 10]. These polymorphisms risk susceptibility to COVID-19 infection, as many infected individuals exhibited variant alleles. One of the two TYK2 variants (rs11085727 or rs34536443) exhibited a significant disparity in the manifestation of critical illness, distinguishing between individuals with asymptomatic or mild cases and those with severe or fatal cases. Interestingly, the two investigations described in the previous statement did not identify any polymorphisms in the IFNAR2 or TYK2 genes. The HGI analysis reveals a significant association between TYK2 and the progression to advanced stages of COVID-19 and the development of critical disease. The occurrence of hospitalization as a result of COVID-19 is associated with the presence of the TYK2 mutation rs34536443 and the ICAM5/TYK2 variant rs11085727, both of which have been linked to the manifestation of severe disease. The observed connection between the increased TYK2 expression and disease severity indicated the potential causal role of TYK2 in severe COVID-19. Theoretical speculation demonstrated that

elevated TYK2 activity can pose a risk for developing chronic inflammatory diseases. However, this hypothesis contradicts the prevailing knowledge that TYK2 serves a role in COVID-19, particularly in the initial stages of the illness when robust IFN1 responses are crucial [11]. According to Nhung and Ton [9], a slight alteration in the rs2236757 gene, which is responsible for encoding a membrane protein of type-I, can potentially reduce the functionality of TYK2 and impede the transmission of signals related to type IFN. This can lead to an increased ability for viral reproduction. Most susceptibility variants have been discovered in genes related to immunology and antiviral functions, such as CCR2, TYK2, and IFNAR2 [12]. These genes have been associated with the critical manifestation of COVID-19, along with genes implicated in the entry of SARS-CoV-2 into host cells. Furthermore, the interaction between interferon (IFN) and its receptor subunit IFNR2 induces a physical connection between these receptors, initiating signaling pathways involving CCR2 and TYK2 [13]. The findings of this study established a correlation between the severities of COVID-19 and genetic differences in the IFNAR2, TYK2, and CCR2 genes. There was a consistent association between genes implicated in the immune system and the severity of COVID-19 across several studies, even though these genes cannot serve as biomarkers in all research [9, 10, 14-16].

Conclusion

There was no deviation in all the study groups regarding the distribution of TYK2 (rs11085727) and TYK2 (rs34536443) polymorphism, and the present results showed that there was an insignificant difference between severe and mild patients according to ARMS-PCR.

Ethics Attention

The Ministry of Health in Iraq's ethics committee approved and directed this training. Ethical approval ensures that the research adheres to established ethical principles and guidelines.
Conflict of interest: No known struggle of awareness is linked with this publication.

Funding: This research did not obtain any monetary provision or grant from public agencies, commercial entities, or not-for-profit organizations.
Availability of data and materials: The information used and/or investigated throughout this training is accessible from the consistent authors on evenhanded demand.
Consent for publication: Not appropriate.
Competing interest: The authors avowed that they take no opposing attention.

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