Research Article

Association of Kell, Duffy and Kidd Blood Group Genotypes with COVID-19 Severity.

Ali Maleki[1], Zahra Mahmoudi[2], Nastaran Mohammadi[3], Jamal Khoa Karam[1], Omar Hashem Ghafor[1], Varzer Ahmad[1], Parisa Feizollahi[3], Alireza Rezaiemanesh[4], Fakhredin Saba[1*]

[1]Department of Medical Laboratory Sciences, Kermanshah University of Medical Sciences, Kermanshah, Iran, [2]Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran, [3]Department of Immunology, Tarbiat Modares University, Tehran, Iran, [4]Department of Immunology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran

Corresponding author:
Fakhredin Saba, Department of Medical Laboratory Sciences, Kermanshah University of Medical Sciences, Kermanshah, Iran, E-mail: Fakhredin.Saba@kums.ac.ir
Received: 29-Nov-2024, Manuscript No. amhsr-24-153649;
Editor assigned: 02-Dec -2024, PreQC No. amhsr-24-153649 (PQ);
Reviewed: 16-Dec-2024, QC No. amhsr-24-153649;
Revised: 23-Dec-2024, Manuscript No: amhsr-24-153649 (R);
Published: 30-Dec-2024, DOI: 10.54608.annalsmedical.2024.S6

Abstract

Background: The novel Coronavirus disease 2019 (COVID-19) is one of the worst pandemics in recorded history and has greatly impacted the health, economy and social life of the entire world. Recent research has shown that the ABO blood group may influence the risk and severity of the SARSCOV-2 infection. Therefore, this study examines the association between severity to COVID-19 infection and the Kell, Duffy and Kidd blood groups/genotypes.

Methods: In this case-control study, the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method was used for determining the genotype of three blood groups (Kell, Duffy and, Kidd) in 100 patients with severe COVID-19 and 50 healthy controls.

Results: There were no significant differences in the genotype or allele distribution of Kell, Duffy and Kidd SNPs (rs8176058, rs12075 and rs1058396, respectively) between the severe COVID-19 and healthy control groups.

Conclusion: According to the result, different genotypes of Kell, Duffy and Kidd might not be risk factors for the severity of COVID-19.

Keywords: COVID-19; Kell; Duffy; Kidd; Blood group; Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP); Single Nucleotide Polymorphisms (SNPs).

Introduction

Novel coronavirus disease, 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is an infectious respiratory illness transmitted *via* small respiratory droplets [1]. COVID-19 was first detected in Wuhan, Hubei, China, in December, 2019. It rapidly spread worldwide, leading to a global pandemic [2].

COVID-19-related mortality is linked to an excessive release of pro-inflammatory cytokines, known as a cytokine storm. This overproduction can lead to Acute Respiratory Distress Syndrome (ARDS), multi-organ failure, coagulation issues and ultimately death [3].

Viruses for entering a cell and infecting it first attach to surface molecules, which can be proteins, carbohydrates, or lipids [4]. Carbohydrates on the surface of red blood cells determine blood group antigens [5]. Infectious agents often attach to cell surfaces *via* glycoconjugates and glycosylation, so variations in blood groups can affect host-pathogen interactions, leading to different susceptibility levels in individuals. Blood groups are also associated with susceptibility to non-infectious diseases like cardiovascular disorders, hematologic conditions, cognitive disorders, metabolic diseases and cancer [6,7].

The Kell system is a highly polymorphic antigen system in red blood cells, with at least 27 antigens. The *KEL* gene, located on chromosome 7q33, produces these antigens through Single Nucleotide Polymorphisms (SNPs). KELL1 and KELL2 antigens result from an SNP (C578T) in exon 6, with KELL2 being the most common and KELL1 the least frequent [8,9].

The Duffy blood group antigens (Fya, Fyb, Fy3, Fy5, Fy6) are codified *via* two codominant allelic forms designated FYA (FY*01) and FYB (FY*02) from atypical chemokine receptor 1 (*ACKR1*) gene, previously called Duffy antigen receptor for chemokines (DARC) [10,11]. FY*A and FY*B differ *via* a single c.125G>A nucleotide polymorphism in their coding sequence in exon 2 [11]. This blood group has a significant effect on the immune response. As previously stated, severe cases of COVID-19 are caused by a hyperinflammatory immune response, Duffy blood group antigens are pro-inflammatory chemokine receptors that bind with high affinity to a wide range of CXC and CC inflammatory chemokines [12].

The Kidd blood system is composed of three antigens called JK1, JK2 (or Jka and Jkb) and JK3, which are encoded by the *Slc14a1* gene on chromosome 18q12. Three SNPs serve as the definition for the Jka/Jkb polymorphism [13]. The Jka/Jkb polymorphism is described by a transition (G838A) that results in an Asp280Asn amino acid substitution in exon 9 while the other two SNPs do not affect the amino acid sequence [14]. Since

How to Cite this Article: Saba F. Association of Kell, Duffy and Kidd Blood Group Genotypes with COVID-19 Severity. Ann Med Health Sci Res. 2024; S6: 1-6.

Kell, Kidd and Duffy are among the most important minor blood groups, which are more frequent than others and a few studies have assessed the relationship between the severity of COVID-19 and minor blood groups/genotypes [15]. Therefore, we decided to investigate the association between the severity of COVID-19 infection and the Kell, Duffy and Kidd blood groups/genotypes.

Material and Methods

Participants

Venous blood samples were collected from 100 severely infected patients with COVID-19 and 50 healthy persons as controls at the Imam Reza Hospital in Kermanshah, Iran. SARA-COV-2 infection was confirmed by nasopharyngeal Reverse-Transcription Polymerase Chain Reaction (RT-PCR) test for COVID-19.

Deoxyribonucleic acid (DNA) extraction

The genomic Deoxyribonucleic acid (DNA) was extracted from the whole blood of patients with COVID-19 and healthy control subjects according to the DNA extraction kit instructions (Favorgen, Taiwan). The quality and concentration of the extracted DNA were evaluated by the Nanodrop 2000 spectrophotometer ThermoScientific (Thermo company, USA). Then the DNA was stored at −20°C until further molecular tests.

PCR-RFLP for Kell, Duffy and Kidd genotyping

PCR was performed to amplify the *Kell, Duffy* and *Kidd* genes using gene-specific primers listed in Table 1. The total volume of PCR was 20 µL containing 10 µL 2X PCR Master Mix (Sinaclon, Tehran, Iran), 7.5 µL DNAse free distilled water, 1 µL of each primer and 0.5 µL genomic DNA. The amplification program of mentioned genes using an iCycler C1000 (Bio-Rad Life Sciences, Hercules, CA, USA) was as follows: An initial denaturation step at 95°C for 3 minutes then 45 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 1 minute. Also, a final extension step was at 72°C for 2 minutes. The specificity of amplified products of the three mentioned genes was evaluated by electrophoresis on a 2% agarose gel stained with Green Viewer (Parstous, Mashhad, Iran).

Table 1: The Characteristics of Primers and restriction enzymes.

Gene	SNP locus	Primer sequences	Product size	Restriction enzyme	Fragment size
Kell	rs1058396 c.578C>T	F: ATCAGGACCTTGGGAGAAGGCA R: CCTCACCTGGATGACTGGTGTG	322 bp	BsmI	C allele: 322 bp T allele: 224 bp+98 bp
Duffy	rs12075 c.125G>A	F: CTCCCCCTCAACTGAGAACTCAAG R: AGAGCTGCCAGCGGAAGAG	248 bp	BshNI	G allele: 248 bp A allele: 153 bp+95 bp
Kidd	rs1058396 c.838A>G	F:GGCATCTTCTGTGCTCCAGATC R: CGCCATGAACATTCCTCCC	256 bp	BseLI	A allele: 256 bp G allele: 181 bp+75 bp

Enzymatic digestion was performed for every three genetic variants in *Kell, Duffy* and *Kidd* genes using BsmI, BshNI and BseLI restriction enzymes, respectively (Fermentase, Thermo Fisher Scientific, USA). PCR products were digested overnight at 37°C in a final volume of 30 µl containing 10 µL of amplified product and adequate enzyme in 10X buffer according to the manufacturer's instructions. Then electrophoresis was performed to visualize the products of enzymatic digestion on 2% agarose gel.

The rs8176058 (c.578 C>T) in the *Kell* gene, the length of the PCR product with specific gene primer was 322bp that was digested by BsmI restriction enzyme (Fermentase, Thermo Fisher Scientific, USA). The C allele (k antigen) isn't cut by this enzyme but the T allele (K antigen) is cuttable and produces two fragments (224 bp and 98 bp). The homozygote CC (K2/K2 genotype) was shown with one band on gel with 322bp length; the heterozygote CT (K1/K2 genotype) was identified if three fragments with 322 bp, 224 bp and 98 bp were generated and the homozygote TT (K1/K1genotype) was detected with appearing two fragments with 224 bp and 98 bp length on the agarose gel.

For rs12075 (c.125 G>A) in the *Duffy* gene, the length of PCR product with specific gene primer was 248 bp that was digested by BshNI restriction enzyme (Fermentase, Thermo Fisher Scientific, USA). The G allele (Fya antigen) isn't cut by this enzyme but the A allele (Fyb antigen) is cuttable and produces two fragments (153 bp and 95 bp). The homozygote GG (FYA/A genotype) was shown with one band on gel with 248 bp length; the heterozygote GA (FYA/B genotype) was identified in three fragments with 248 bp, 153 bp and 95 bp were generated and the homozygote AA (FYB/B genotype) was detected with appearing two fragments with 153 bp and 95 bp length on the agarose gel.

For rs1058396 (c.838 G>A) in the *Kidd* gene, the length of PCR product with specific gene primer was 256 bp that was digested by BseLI restriction enzyme (Fermentase, Thermo Fisher Scientific, USA). The G allele (Jka antigen) isn't cut by this enzyme but the A allele (Jkb antigen) is cuttable and produces two fragments (181 bp and 75 bp). The homozygote GG (JKA/A genotype) was shown with one band on gel with 256 bp length; the heterozygote GA (JKA/B genotype) was identified if three fragments with 256 bp, 181 bp and 75 bp were generated and the homozygote AA (JKB/B genotype) was detected with appearing two fragments with 181 bp and 75 bp length on the agarose gel.

Statistics

The statistical analysis was carried out using the SPSS statistical software v23 (SPSS, Chicago, IL, USA). Comparison of the genotype and allele frequencies of the rs8176058 (c.578C>T), rs12075 (c.125G>A) and rs1058396 (c.838G>A) SNPs between the severe COVID-19 and healthy control groups were analysed by Chi-square test. logistic regression analyses (Odds Ratios (ORs) and 95% Confidence Intervals (CIs)) were performed to estimate the relation between genotypes and COVID-19 severity. The chi-square test was used to evaluate the Hardy–Weinberg equilibrium for the three SNPs in the severe COVID-19 and control groups. A difference with a P value less than 0.05 is considered statistically significant (P<0.05).

Results

Demographic data

In this study, we examined 100 severe COVID-19 patients (51 women, 49 men) and 50 healthy subjects (18 women, 32 men) whose mean ages were 61.34 ± 15.35 and 61.76 ± 15.09, respectively. There was no significant difference between the two studied groups according to age (p=0.892) and gender (p=0.297).

Allele and genotype frequencies of Kell, Duffy and Kidd SNPs and their association with COVID-19 severity

The association between Kell, Duffy and Kidd SNPs and susceptibility to COVID-19 severity was distinguished by investigating three polymorphic regions, rs8176058 (c.578 C>T) in the *Kell* gene, rs12075 (c.125 G>A) in the *Duffy* gene and rs1058396 (c.838 G>A) in the *Kidd* gene. The distributions of genotypes, allele frequencies and different genetic models (dominant, additive and recessive) in severe COVID-19 patients and controls are presented in Table 2. Significant deviation from the Hardy–Weinberg equilibrium (HWE) was observed in different genotypes of the Duffy gene in the severe COVID-19 patient group and genotypes of the Kell gene in healthy controls (P<0.05). No statistically significant differences were shown in allele and genotype distributions or different genetic models (dominant, additive and recessive) between patients with severe COVID-19 and healthy participants.

Table 2: Distribution of allele and genotype frequencies of Kell, Duffy and Kidd genes polymorphisms in patients with COVID-19 and healthy controls.

SNP	Groups		P-value	OR (95% CI)
	Severe (N=100) n (%)	Control (N= 50) n (%)		
rs8176058 c.578 C>T				
Allele frequency				
C	197 (98.5%)	98 (98%)	-	Reference
T	3 (1.5%)	2 (2%)	0.741	1.358 (0.255–8.681)

Genotype frequency				
CC	97 (97%)	48 (96%)	-	Reference
CT	3 (3%)	1 (2%)	0.734	0.674 (0.068–6.648)
TT	0 (0%)	1 (2%)	0.158	0.331 (0.263–0.417)
Dominant model				
CC	97 (97%)	48 (96%)	-	Reference
TT+CT	3 (3%)	2 (4%)	0.748	0.742 (0.120–4.592)
Additive model				
CT	3 (3%)	1 (2%)	-	Reference
CC+TT	97 (97%)	49 (98%)	0.72	1.515 (0.154–14.92)
Recessive model				
TT	0 (0%)	1 (2%)	-	Reference
CC+CT	100 (100%)	98 (98%)	0.15	3.04 (2.418–3.825)
HWE	0.87	0		
rs12075 c.125 G>A				
Allele frequency				
G	134 (67%)	71 (71%)	-	Reference
A	66 (33%)	29 (29%)	0.483	1.206 (0.715–2.034)
Genotype frequency				
GG	52 (52%)	27 (54%)	-	Reference
GA	30 (30%)	17 (34%)	0.82	1.091 (0.513–2.322)
AA	18 (18%)	6 (12%)	0.399	0.642 (0.228–1.806)
Dominant model				
GG	52 (52%)	27 (54%)	-	Reference
AA+GA	48 (48%)	23 (46%)	0.817	1.084 (0.549–2.140)
Additive model				
GA	30 (30%)	17 (34%)	-	Reference
GG+AA	70 (70%)	33 (66%)	0.619	0.835 (0.341–1.717)
Recessive model				
AA	18 (18%)	6 (12%)	-	Reference
GG+GT	82 (82%)	54 (88%)	0.17	1.976 (0.737–5.2940)
HWE	0	0.21		
rs1058396 c.838 G>A				

Allele frequency				
G	102 (51.3%)	50 (50%)	-	Reference
A	97 (48.7%)	50 (50%)	0.838	0.951 (0.588–1.538)
Genotype frequency				
GG	31 (31%)	13 (26%)	-	Reference
AG	41 (41%)	24 (48%)	0.425	1.396 (0.614–3.171)
AA	28 (28%)	13 (26%)	0.829	1.107 (0.440–2.787)
Dominant model				
GG	31 (31%)	13 (26%)	-	Reference
AA+AG	69 (69%)	37 (74%)	0.526	0.782 (0.365–1.6740)
Additive model				
AG	41 (41%)	24 (48%)	-	Reference
AA+GG	59 (59%)	26 (52%)	0.415	0.753 (0.380-1.491)
Recessive model				
AA	28 (28%)	13 (26%)	-	Reference
GG+AG	72 (72%)	37 (74%)	0.796	1.107 (0.440–2.7870)
HWE	0.07	0.77		

Discussion

In the current study, the association between COVID-19 severity and Kell, Duffy and Kidd SNPs (rs8176058, rs12075 and rs1058396, respectively) was investigated between two groups, including severe COVID-19 patients and controls. According to our data, there are no significant differences in allele and genotype distributions or different genetic models (dominant, additive and recessive) between the two studied groups.

According to the International Society of Blood Transfusion (ISBT), 43 blood group systems contain 345 antigens for human red blood cells, the most prominent of which are the ABO, RH, Kell, Kidd and Duffy blood group systems. It is well known that blood types and vulnerability to various infections are related. Blood group antigens, as cell-surface glycoproteins, are mostly found on erythrocytes and may affect people's susceptibility to infectious illnesses. These antigens may impact infections directly by serving as receptors or co-receptors for microorganisms or indirectly through anti-blood group antibodies, which may be induced by bacteria and viruses with envelopes that mimic blood groups. Numerous studies have been done during the present pandemic to examine the connection between ABO blood types and SARS-COV-2 infection severity and mortality. According to the findings, blood group O, which seems to have a protective effect, is at a lower risk for contracting COVID-19 than blood groups A, B and AB. In comparison individuals with blood group A exhibit a heightened susceptibility to contracting COVID-19 and are more likely to develop severe symptoms.

Similarly, The existence of the Duffy antigen, which is the key entry point for the parasite's entrance to erythrocytes, is necessary for the infection of human erythrocytes with *Plasmodium vivax*. Duffy-negative people are usually resistant to infection by *P. vivax* infection; however, recent research from several malaria-endemic countries, particularly Western Africa, has demonstrated that *Plasmodium vivax* can cause clinical malaria in Duffy-negative people.

The DARC may increase the risk of Human Immunodeficiency Virus (HIV) *via* facilitating trans-infection of HIV-1 and by influencing chemokine-HIV interactions as well as chemokine-induced inflammation. Previous research has indicated a relationship between DARC-null-related neutropenia and an increased risk of HIV infection and transmission from mother to child. However, following infection, data suggest that the DARC-null state is related to slower illness progression. It was demonstrated that there is a link between the c.-67T>C variation of the ACKR1 gene (FY*02N.01) and the necessity for COVID-19 patients to be hospitalized. African Americans with COVID-19 infections may have a worse prognosis due to a reduction in Duffy receptors in erythrocytes caused by FY*02N.01 [12].

There is no evidence of Kell serving as a receptor for microorganism pathogens exists, although there are numerous

case reports of severe infection with bacteria, including *E. coli* O125, *Enterococcus faecalis, M. tuberculosis* and *Streptococcus faecium*, inducing cross-reactive antibodies with anti-Kell activity. Also, Bhandar et al. showed that Kell-negative people are more vulnerable to COVID-19 infection than Kell-positive people.

Conclusion

In conclusion, for the first time, we investigated the relationship between different genotypes of the Kell, Duffy and Kidd blood group and the severity of COVID-19, but we could not find a significant association between genotype or allele distribution of these blood groups with the severity of SARS-COV-2 infection.

Ethical committee consent

The protocol of the study was endorsed by the ethical committee of Kermanshah University of Medical Sciences (Ethical codes: IR.KUMS.REC.1400.524, IR.KUMS.REC.1400.409, IR.KUMS.REC.1400.410) for the use of human participants. All study subjects signed informed consent forms before sampling. All research was performed following the relevant guidelines and regulations of Kermanshah University of Medical Sciences.

Funding

This research has been supported by grants from Kermanshah University of Medical Sciences (KUMS); Grant No. 4000540, 4000549, 4010074.

References

1. Cascella M, Rajnik M, Aleem A, Dulebohn SC, Di Napoli R. Features, Evaluation and Treatment of Coronavirus (COVID-19). Statpearls. 2022.
2. Kusumoto T, Chubachi S, Namkoong H, Tanaka H, Lee H et al. Association between ABO blood group/genotype and COVID-19 in a Japanese population. Ann Hematol. 2023;102:3239-3249.
3. Ragab D, Salah Eldin H, Taeimah M, Khattab R, Salem R. The COVID-19 cytokine storm; what we know so far. Front Immunol. 2020;11:551898.
4. Dimitrov DS. Virus entry: Molecular mechanisms and biomedical applications. Nat Rev Microbiol. 2004;2(2):109-122.
5. Agrawal A, Tiwari AK, Mehta N, Bhattacharya P, Wankhede R, et al. ABO and Rh (D) group distribution and gene frequency; the first multicentric study in India. Asian J Transfus Sci. 2014;8:121-125.
6. Ewald DR, Sumner SC. Blood type biochemistry and human disease. Wiley Interdiscip Rev Syst Biol Med. 2016;8:517-535.
7. Umit T, Tiftik EN, Sakir U, Ozrur G, Tamer IK, et al. Relationship between ABO blood group and skin. Dermatol Online J. 2008;11:1-6.
8. Arnoni CP, Muniz JG, Paula TA, Person RD, Gazito D, et al. An easy and efficient strategy for KEL genotyping in a multiethnic population. Rev Bras Hematol Hemoter. 2013;35:99-102.
9. Kausar T, Fatima M, Noureen S, Javed S, Abdulsattar S, et al. Kell Blood Group System: A Systematic Review and Meta-Analysis.
10. Abou-Ali RK, Dhyani A, Terço AL, Toro DM, Gomes KS, et al. Impact of Duffy polymorphisms on parasite density in Brazilian Amazonian patients infected by *Plasmodium vivax*. Malar J. 2019;18:1-9.
11. Horuk R. The Duffy antigen receptor for chemokines DARC/ACKR1. Front Immunol. 2015;6:279.
12. Conrado MC, Dezan MR, Oliveira VB, Ziza KC, Fanciscani T, et al. Association between FY*02N.01 and the severity of COVID-19: Initial observations. Hematol Transfus Cell Ther. 2022;44:213-217.
13. Lawicki S, Covin RB, Powers AA. The Kidd (JK) blood group system. Transfus Med Rev. 2017;31:165-172.
14. Vorholt SM, Lenz V, Just B, Enczmann J, Fischer JC, et al. High-throughput next-generation sequencing of the Kidd blood group: unexpected antigen expression properties of four alleles and detection of novel variants. Transfus Med Hemother. 2023;50:51-65.
15. Dimri U, Kumar S, Kapoor U, Jagani R. Major and minor blood group phenotyping and database generation for recruits: A pilot study. Med J Armed Forces India. 2021;77(4):466-473.