Effect of Toll-Like Receptor 7 Gene Polymorphism and ABO Blood Groups on the Severity of COVID-19 Patients

Zainab H. D. AL-Tamimi¹, Abdulsamie H. Alta’ee¹, Ahmed H. Jasim¹

¹College of Medicine, University of Babylon, Hillah, Babylon state, Iraq

Corresponding author: Zainab H. D. AL-Tamimi. College of Medicine, University of Babylon, Hillah, Babylon state, 51001, Iraq. Tel: +964-773-4986-127. E-mail: Zainabhatem2020@gmail.com, ORCID ID: http://www.orcid.org/0000-0000-0000-0000.

doi: 10.5455/aim.2022.30.191-195

ACTA INFORM MED. 2022 SEP; 30(3): 191-195

Received: JUL 15, 2022
Accepted: AUG 12, 2022

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ABSTRACT
Background: The most current threat to global health is the continuous spread of a respiratory disease known as COVID-19 Disease 2019 in recent years. COVID-19 was recognized in December 2019. It was quickly determined that a novel COVID-19 virus, which is structurally linked to the virus that causes the severe acute respiratory syndrome, was to cause (SARS). Objective: The aim of this study is to investigate the presence of effect between the rs179008 (A/T) SNP polymorphism in TLR7 gene and blood group on the severity of COVID-19. Methods: The study included 90 patients divided into three groups mild, moderate, severe, and experimental research work was conducted during the period of sample collection extended from November 2021 to February, PCR-RFLP technique was used to determine SNP rs179008 polymorphism in TLR7 in the blood. Results: A present study found non-significant differences between patient groups for TLR7 rs179008 (A, T) allele were (p=0.79152) for mild to moderate and severe, (p=0.84872) for mild and moderate and (p=0.58741) for mild and severe. When comparison (AA, AT, TT) genotypes in three groups found a significant difference between mild and moderate groups (p=0.036) for the AA genotype. Found (A blood group) more frequency than other groups but observes no significant difference between patients’ group. Conclusion: We conclude that the (AA) genotype for TLR7 rs179008 polymorphism was a risk factor and effect on severity of COVID-19 infection, so (AA) can consider an independent risk factor for development of COVID-19.

Keywords: TLR-7, ABO, COVID-19, rs179008, Genetic Polymorphism.

1. BACKGROUND
Coronavirus disease 2019 (COVID-19) is the third plague of this century, and it has been designated as the sixth international health issue. The World Health Organization has declared a global health emergency in 2020 (1, 2). The International Committee on Virus Taxonomy of Viruses named it severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (3). In 2019 December, A group of individuals with pneumonia of indefinite etiology was discovered in Wuhan, Hubei Province, China (4). The China CDC (the Chinese Center for Disease Control and Prevention) detected a new coronavirus from lower respiratory tract samples of pneumonia patients that will be collected on January 7, 2020, and released the genomic sequence on 11 January 2020. In less than a few months after the unknown pathogen was discovered, it had infected at least 114 countries, killing nearly 4,000 people (5).

It’s the seventh coronavirus to infect humans; the other four (229E, NL63, OC43, and HKU1) cause very moderate cold symptoms. SARS-CoV, MERS-CoV, and SARS-CoV-2, on the other hand, can cause life-threatening symptoms and even death (6). This is a worldwide emergency that requires the joint determinations of all humanity to prevent it (7). The most well-known viruses are divided into groups based on their phylogenies with viruses that have previously infected the same host, as well as their genotype (8), it was spherical or pleomorphic particles ranging in length from 80 to 160 nm the presence of single-stranded (positive-sense) RNA coupled with a nucleoprotein was...
found in a capsid containing matrix protein (9). SARS-CoV-2 has four major structural proteins: membrane (M) glycoprotein, spike (S) glycoprotein, small envelope (E) glycoprotein and nucleocapsid (N) protein, as well as a number of auxiliary proteins (10).

This viral can enter to human body through the receptors ACE2 type I integral membrane protein that has mono carboxypeptidase activity which are found in many organs such as the heart, lungs, kidneys, and gastrointestinal system, thus facilitating virus entry into target cells. Attachment of the S glycoprotein that is form homotrimer protruding from the virus surface to the receptor ACE2 in the host cell, is the first step in Covid-19 entering the host cell such as in type II pneumocystis in the lungs (11).

The most commonly reported symptoms are fever, cough and shortness of breath (12). Cough (with or without sputum), fatigue, discomfort, weakness, arthralgia or myalgia, chest tightness, extreme mucus creation with expectoration, hemoptysis, and dyspnea are some of the symptoms that might occur (13, 14). Gastrointestinal symptoms such as vomiting, diarrhea and abdominal pain are observed in some patients with COVID-19 (15).

Toll Like Receptor (TLR7) is PRRs located on intracellular organelles (16, 17), which produce antiviral immunity by recognizing single-stranded RNA (ss- RNA) from viruses and the consequent activation of pro-inflammatory pathways (18, 19). TLR7’s ability to inhibit virus replication has been established in MERS-CoV (20). As viral ssRNA binds to TLR7/8 upon entrance into the cell, increasing activation and antiviral immunity (21). TLR7 activation causes the adaptor molecule MyD88 to be recruited, subsequent in the release of pro-inflammatory cytokines and chemokines (22). IFNs of type I (IFN-alpha and IFN-beta) and III (IFN-alpha and IFN-beta) (IFN-lambda) (23).

2. OBJECTIVE

The aim of this study is to investigate the presence of effect between the rs179008 (A/T) SNP polymorphism in TLR7 gene and blood group on the severity of COVID-19.

3. MATERIAL AND METHODS

3.1. Sample Collection

This study was done at the laboratory of Chemistry and Biochemistry Department, College of Medicine University of Babylon. This study was designed as cross-sectional study, involved a total of 90 patients divided into three groups according to severity by physician mild, moderate and severe groups, all sample collection from November 2021 to February, Samples were collected from Baquba Teaching hospital and Muqdadiya General Hospital, Diyala Province, Iraq.

3.2. Biochemical analysis

Drawn five milliliters blood from venous and put into Sodium Citrate for D-dimer use and 2ml into EDTA tube for CBC, Blood group, genotype and other blood placed into gel tube then Centrifuge at (3000 X g) 10 min for separation serum, Blood for ABO blood group, genotype by PCR-RFLP using Zh08 amplification primer including forward 5’ GTTGCAAAGAGGCGAGGCA 3’, and reverse 3’CTGTGAGTCCACGATCACAS’, then procedure per according to the manufacturing instructions.

3.3. Determination the ABO blood Group

First, a glass side was prepared and marked with three circles after cleaning the slide and then Anti-A, Anti-B and Anti-D in the first, second and third circle respectively were added with the help of a dropper. By use pipette, three drops of the antigen were added on anti A, B, and D in glass slid. With the use of a toothpick, the blood sample was gently mixed and the outcome was observed after a minute.

4. RESULTS

4.1. Demographic characteristics of patients enrolled in this study

Table 1 showed the mean of age between the COVID-19 patients and severity of disease, where the age ranges from (1-85 years), 63 (70%) as females and 27(30%) as males. In addition, the we found 21 cases for mild symptoms at percentage (23.3%), 26 cases for moderate symptoms at (28.9%), and 43 cases for severe symptoms at (47.8%).

| Age (year) | Mild Mean±SD | Moderate Mean±SD | Severe Mean±SD |
|-----------|--------------|------------------|----------------|
| 32.48±15.39| 57.04±16.91  | 66.51±14.18      |                |

4.2. Assessment of ABO blood group to severity.

Table 2 showed the distribution of COVID-19 patients according to the ABO blood group, where the highly percentage of patients was in A+ at 34.4% (31 out of 90 patients), while the lower percentage was in B- & O- at 2.2% (2 out of 90 patients).

| ABO     | Frequency | Percent |
|---------|-----------|---------|
|ope +   | 31        | 34.4    |
| A-      | 15        | 16.7    |
| B+      | 12        | 13.3    |
| B-      | 25        | 27.8    |
| A-      | 3         | 3.3     |
| O-      | 2         | 2.2     |
| Total   | 90        | 100.0   |

Table 3. The association between COVID-19 patients and ABO blood group according to the severity of disease.

| ABO     | Mild | Moderate | Severe | Total |
|---------|------|----------|--------|-------|
| A+      | 4    | 9        | 18     | 31    |
| B+      | 6    | 4        | 5      | 15    |
| AB+     | 3    | 3        | 6      | 12    |
| O+      | 4    | 10       | 11     | 25    |
| A-      | 1    | 0        | 2      | 3     |
| B-      | 2    | 0        | 0      | 2     |
| O-      | 1    | 0        | 1      | 2     |
| Total   | 21   | 26       | 43     | 90    |
Table 4. The association between COVID-19 patients and severity of disease according to the types of genotypes.

| Genotype | Mild | Moderate | Severe | Total |
|----------|------|----------|--------|-------|
| AT       | 9    | 4        | 11     | 24    |
| AA       | 12   | 18       | 30     | 60    |
| TT       | 0    | 4        | 2      | 6     |
| Total    | 21   | 26       | 43     | 90    |

Table 5. The association between COVID-19 patients with mild symptoms and COVID-19 patients with moderate and severe symptoms according to the Allele Frequency of rs179008.

| Allele | Mild | Moderate, Severe | OR (95% CI) | P-value |
|--------|------|------------------|-------------|---------|
| A      | 33   | 0.79             | 0.909 (0.341-2.421) | 0.08472 |
| T      | 9    | 0.21             | 1.100 (0.413-2.929) | -       |

Table 6. The association between COVID-19 patients with mild symptoms and COVID-19 patients with moderate symptoms according to the Allele Frequency of rs179008.

| Allele | Mild | Severe | OR (95% CI) | P-value |
|--------|------|--------|-------------|---------|
| A      | 33   | 0.79   | 1.291 (0.512-3.252) | 0.58741 |
| T      | 9    | 0.21   | 0.775 (0.308-1.951) | -       |

Table 7. The association between COVID-19 patients with mild symptoms and severe symptoms according to the Allele Frequency of rs179008.

| Model    | Genotype | Mild | Moderate, Severe | OR (95% CI) | P-value |
|----------|----------|------|------------------|-------------|---------|
| Codominant | A/A     | 12   | 48 (69.6%)       | 1.00        | -       |
|           | A/T     | 9    | 48 (69.6%)       | 0.42 (0.15-1.18) | 0.421 |
|           | T/T     | 0    | 6 (8.7%)         | 0.2 (0.016-5.66) | -       |
| Dominant  | A/A     | 12   | 48 (69.6%)       | 1.00        | -       |
|           | A/T-T/T | 9    | 48 (69.6%)       | 0.58 (0.21-1.59) | 0.3   |
|           | T/T     | 0    | 6 (8.7%)         | 0.27 (0.012-4.2) | 0.32  |
| Recessive | A/A-T/T | 12   | 48 (69.6%)       | 1.00        | -       |
|           | T/T     | 0    | 6 (8.7%)         | 0.27 (0.012-4.2) | 0.063 |

Table 8. The association between COVID-19 patients with mild symptoms and COVID-19 patients with moderate and severe symptoms according to the (A/T) SNP genotypes.

Table 9. The association between COVID-19 patients with mild symptoms and COVID-19 patients with moderate according to the (A/T) SNP genotypes.

Table 10. The association between COVID-19 patients with mild symptoms and COVID-19 patients with moderate according to the (A/T) SNP genotypes.
tients with mild symptoms and COVID-19 patients with severe symptoms according to the (A/T) SNP genotypes, where the co-dominant, dominant, recessive and over-dominant models don’t show any significant differences at p-value 0.05.

5. DISCUSSION
In this study when comparison between these group was found no significant association between ABO blood group and the severity of COVID-19 patients in statistic. But, according to Table 1 found the patients of (A) and (O) groups more frequency in severe patients. This study corresponds to French and Spanish studies was found not related with increased or decreased risk of COVID-19 infection (25). These data are opposite the previous studies. Which described they found group O in depressing risk of COVID-19 (26). Angiotensin-converting enzyme 2 (ACE2) has been described to be the SARS-CoV receptor, and the receptor binding domain is obtainable on the S proteins of the coronaviruses (27).

They wanted to see if ABO antibodies could end the interaction between the SARS-CoV receptor and ACE2 and, as a result, adhesion of S protein and ACE2 can be inhibited by anti-A and anti-B natural antibody, recent study give an explanation for the upper risk for blood group A, may absence of these antibodies “the genetic background of social populations can influence the vulnerability and result of infectious diseases. Reports suggested that the variations within the host’s genome play a role in COVID-19 disease progression (28). In this study TLR7 rs179008 polymorphism over dominant (AT) genotype was high significant between mild and moderate groups and play role as protective against COVID-19 disease progression and then was observed a no significant distribution of allele (A, T) for TLR7 gene rs179008 polymorphism between three groups.

TLR7 is a key factor in the production of interferon (IFN), which has a direct antiviral effect by inhibiting virus replication., As viral ssRNA binds to TLR7/8 upon entrance into the cell, increasing activation and antiviral immunity (21). TLR7 activation causes the adaptor molecule Myd88 to be recruited, resulting in the release of pro-inflammatory cytokines and chemokines (22), IFNs of type I (IFN-alpha and IFN-beta) and III (IFN-alpha and IFN-beta) (IFN-lambda) (23). It has been shown to aid in viral clearance and reduction of replication. The current study should be replicated in populations from other parts of Iraq with a larger sample size, so (AA) genotype for TLR7 rs179008 polymorphism was a risk factor and effect on severity of COVID-19 infection, so (AA) can consider as independent risk factor for development of COVID-19.

6. CONCLUSION
We conclude that the (AA) genotype for TLR7 rs179008 polymorphism was a risk factor and effect on severity of COVID-19 infection, so (AA) can consider as independent risk factor for development of COVID-19.

Acknowledgments: The authors would like to thank Dr Yasir Haider Al-Mawlah and Dr Ameer Mezher Hadi (DNA Research Center, University of Babylon. Pune for their kind support with all laboratory equipment and provide the suitable facilities, also for drafting the manuscript to make this work done.

Author’s contributions: Conception and design of the study: Zainab H. D. Al-Tamimi. Drafting the manuscript: Abdulsameh H. Alta’ee. Analysis and/or interpretation of data: Dr. Ahmed H. Jasim

Conflicts of interest: Author declare there are no conflicts of interest.

Financial support and sponsorship: Nil.

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