Impact of Rs657152 Gene Polymorphisms on Inflammatory Markers in COVID-19 Patients With Type 2 Diabetes Mellitus

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Abstract- COVID-19 has significantly affected people with pre-existing conditions, particularly those suffering from type 2 diabetes mellitus (T2DM), as it increases the risk of complications and mortality. The dysregulated inflammatory response in T2DM patients is a critical factor contributing to severe disease progression in these individuals. Recent research suggests that genetic variations, such as Rs657152 polymorphisms, could influence inflammatory markers and immune responses in T2DM patients infected with COVID-19. Understanding this genetic relationship is crucial for improving treatment strategies and predicting outcomes in this high-risk group. The present was designed to evaluate the correlation of Rs657152 gene polymorphisms with inflammatory markers in COVID-19 patients with T2DM. This study enrolled 91 participants, including 31 healthy individuals, 30 COVID-19 patients with T2DM, and 30 non-diabetic COVID-19 patients. Inflammatory markers (CRP, IL-6, D-dimer, and ferritin) were measured, and Rs657152 polymorphisms were genotyped. Statistical analysis was conducted using SPSS version 23. COVID-19 patients with T2DM showed significantly higher BMI, greater severity of COVID-19, and increased levels of inflammatory markers compared to non-diabetic patients. A significant correlation was observed between the Rs657152 polymorphisms and elevated levels of IL-6, D-dimer, and ferritin in T2DM patients (P<0.05). The polymorphisms of the Rs657152 gene may exacerbate the inflammatory response in COVID-19 patients with T2DM, contributing to increased severity of the disease.
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Introduction

The COVID-19 pandemic, caused by the novel coronavirus SARS-CoV-2, has become one of the most significant global health crises in recent history (1). First identified in December 2019 in Wuhan, China, COVID-19 rapidly spread across the globe, leading to widespread illness, unprecedented strain on healthcare systems, and significant socio-economic disruption (2). The virus, characterized by its high transmissibility and potential for severe respiratory disease, has prompted extensive research and public health efforts to mitigate its impact (3).

The COVID-19 pandemic has posed unprecedented challenges to global health, significantly affecting various population groups, particularly those with underlying health conditions (4). Among them, patients with type 2 diabetes mellitus (T2DM) have emerged as a highly vulnerable cohort. The interplay between COVID-19 and T2DM often results in severe disease progression and increased mortality rates. Understanding the factors contributing to this heightened vulnerability is critical for developing effective therapeutic strategies and improving patient outcomes (5).

Patients with T2DM are predisposed to a chronic state of low-grade inflammation, which can exacerbate the inflammatory response triggered by COVID-19 (6). This heightened inflammatory state is characterized by the high levels of various markers, such as C-reactive protein (CRP), interleukin-6 (IL-6), D-dimer, and ferritin (7).

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These markers indicate systemic inflammation and are often correlated with disease severity in COVID-19 patients. The inflammatory response in T2DM patients can lead to severe complications, including acute respiratory distress syndrome (ARDS), multi-organ failure, and thromboembolic events, contributing to higher mortality rates (8).

While the link between T2DM and severe COVID-19 outcomes is well-documented, the role of genetic factors in this interplay remains an area of active research (9). Genetic polymorphisms and variations in DNA sequence among individuals can influence the immune response and the severity of infectious diseases. One such genetic factor is the Rs657152 polymorphism. Located in a genomic region associated with immune function, this polymorphism may modulate the inflammatory response in COVID-19 patients, particularly those with T2DM (10).

Previous studies have explored the relationship between various polymorphisms and disease severity. In a study conducted by Mahmood *et al*., in Iraq, it was reported that the ACE and ACE2 gene variants (rs4646994 and rs2285666, respectively) were not associated with an increased risk of developing COVID-19 (11). The study by Khalaf *et al*., identified a significant association between the IL-6 gene polymorphism at rs1800795 (G/C) and the IL-17A gene polymorphism at rs2275913 (G/A) with increased susceptibility to COVID-19 and Type 2 Diabetes Mellitus (T2DM) in the Iraqi population (12). The Rs657152 polymorphism has been implicated in various inflammatory and autoimmune conditions, suggesting it may modulate the body's response to infections (13). Patients with T2DM are highly susceptible to severe COVID-19 outcomes, including long-term disability caused by complications such as acute respiratory distress syndrome (ARDS) and thromboembolic events. By investigating the role of Rs657152 polymorphisms in modulating the inflammatory response in these patients, we aim to identify genetic markers that could predict disease severity. This knowledge is essential for developing personalized treatment strategies that target specific genetic predispositions, potentially improving patient outcomes, reducing the risk of severe complications, and addressing disabilities associated with prolonged inflammation. Moreover, by understanding the genetic factors contributing to inflammation, healthcare providers can better manage the therapeutic needs of this vulnerable population, ultimately improving survival and quality of life (13). Therefore, the present study was designed to investigate the correlation of Rs657152 gene polymorphisms with inflammatory markers (CRP, IL-6, D-dimer, and ferritin) in COVID-19 patients with T2DM.

Materials and Methods

Study design

This cross-sectional observational study was conducted in Babylon from Marjan Medical Hospital (Al Ain, UAE) and Al-Turkish Hospital between July 2023 and November 2023.

Data collection

Patients diagnosed with COVID-19 based on RT-PCR testing, cases with a confirmed diagnosis of T2DM according to American Diabetes Association (ADA) criteria, patients aged between 30 and 70 years, and those willing to provide written informed consent were inclusion criteria. The exclusion criteria included patients with type 1 DM, cases with chronic inflammatory diseases other than T2DM, and patients on immunosuppressive therapy, as well as pregnant or lactating women. For the study, 91 samples were collected, including 31 healthy individuals, 30 COVID-19 patients with T2DM, and 30 non-diabetic COVID-19 patients.

Approximately five milliliters (ml) of venous blood were drawn from patients and control individuals. Blood samples were collected using EDTA tubes. Two ml of blood in EDTA tubes was utilized for measuring HbA1c levels and extracting DNA, with samples stored at -20˚ C. An additional 2 ml of blood was placed in serum-separating tubes, allowed to clot, and then centrifuged at 2000 rpm for 10 minutes to measure hematological parameters. One ml of serum was treated with sodium citrate to determine D-dimer levels. Demographic and clinical data, including body mass index (BMI) and COVID-19 severity, were finally collected.

Biochemical analysis

During a specified period, participants diagnosed with COVID-19 were identified through polymerase chain reaction (PCR) and/or chest computer tomography (CT). The study population was categorized into Control, COVID-19, and COVID-19 with T2DM. These groups were further classified based on the severity of infection into mild, moderate, and severe cases, with the latter receiving care in intensive care units (ICU). Four physiological parameters were measured: IL-6, CRP, D-dimer, and ferritin. Serological assessments were performed using assay kits from Crystal Chem. Co. (USA) with the AFIAS-6 automated immunoassay

analyzer and all-in-one cartridge system (Korea).

DNA extraction

Peripheral blood samples from study participants (patients and controls) were collected. DNA was extracted from all collected samples using a DNA extraction kit (Bioneer Company, South Korea) following the manufacturer's instructions.

Primer design and amplification conditions

Bioneer manufactured the primers employed in this study (14) under stringent conditions that adhere to ISO 9001:2000 standards, ensuring an environment free from DNase, RNase, and DNA contamination (Table 1). The company provided these primers in lyophilized form, with quantities measured in picomoles. For reconstitution, primers were dissolved in either sterile 1×TE buffer (1 mM of Tris and 0.1 mM of EDTA, pH 8.0) or sterile, nuclease-free water to create a master stock solution. These solutions were subsequently diluted to prepare working stock solutions. The PCR reaction was prepared in a total volume of 25 µL, comprising 12.5 µL of 2×PCR master mix, 1 µL of forward primer (10 µM), 1 µL of reverse primer (10 µM), 1 µL of genomic DNA (50-100 ng), and 9.5 µL of sterile nuclease-free water. Following the preparation of the PCR mixture, the thermocycler was programmed for 35 cycles (Table 2). The amplified PCR fragments were then electrophoresed on a 1.5% agarose gel and visualized using a DNA-safe stain.

Table 1. Primers used in the present study

Primer	Nucleotide sequence	Molecular weight (bp)
Forward	5'- GGGACCTTACTGGGGTGATT -3'	300
Reverse	5'- ACAAGGGCAAGCCTGAAAGG -3'	300

Table 2. Thermocycler program used for PCR

Time	Number of cycles	Temperature (c^{o})	Time
Primary denaturation	94	1	5 min
Denaturation	94		30 sec
Annealing	58	35	30 sec
Extension	72		45-sec
Final extension	72	1	10 min

Statistical analysis

Data analysis was conducted using SPSS software version 23 (SPSS Inc., Chicago, Illinois, USA). Various statistical tests were employed, including one-way ANOVA, exploratory analysis, Duncan's multiple range test, correlation analysis, and means comparison using the LSD test. The significance levels were established as $P \leq 0.05$.

Results

Demographic and clinical characteristics

The demographic and clinical characteristics of the participants are summarized in Table 3. There were no significant differences in age and sex distribution among the three study groups ($P>0.05$). However, the BMI was significantly higher in the T2DM group compared to the other groups, indicating elevated obesity rates among these patients ($P<0.001$). Additionally, the severity of COVID-19 was statistically higher in patients with T2DM than those without T2DM ($P=0.033$).

Inflammatory markers

The levels of inflammatory markers, including CRP, IL-6, D-dimer, and ferritin, were measured, and compared among the three groups (Table 4). The results showed a significant elevation of these markers in COVID-19 patients with T2DM compared to those without T2DM, indicating an exacerbated inflammatory response in diabetic patients with COVID-19 infection.

Table 3. Demographic and clinical characteristics of study participants

Variables		COVID-19+ T2DM (30)	COVID-19 (30)	Healthy Individuals (31)	*P*
Gender	Male, N (%)	16 (53.3)	15 (50)	17 (56.6)	0.69
	Female N (%)	14 (46.7)	15 (50)	13 (43.4)	0.88
Age (years) (Mean±SD)		55.7 ± 8.4	58 ± 7.8	61.3 ± 9.5	0.094
BMI (kg/m²) (Mean±SD)		28.6 ± 3.7	26.3 ± 2.6	24.5 ± 2.3	<0.001*
Severity of infection	Mild, n (%)	6 (20)	11 (36.6)	-	0.074
	Moderate, n (%)	12 (40)	11 (36.6)	-	0.16
	Severe, n (%)	12 (40)	8 (26.8)	-	0.033*

Statistically significant at $P \leq 0.05$.

Table 4. Mean differences of the biomarkers according to study groups

Parameters	COVID-19+ T2DM (30)	COVID-19 (30)	Healthy Individuals (31)	*P*
IL-6	26.14±3.4	26.02±9.6	16.08±3.1	0.042*
CRP	73.63±10.7	39.11±7.4	36.00±9.7	0.036*
D-dimer	1555.75±22.7	710.54±50.1	433.00±24.1	0.032*
Ferritin	743.65±36.8	263.98±12.6	107.46±17.4	0.049*

According to Duncan's statistical test, * shows significance

Correlation with Rs657152 polymorphisms

To genotype the rs657152 SNP, genomic DNA was amplified using specific primers and processed in a thermal cycler under optimal conditions. Agarose gel electrophoresis results showed a single band of 300 bp, indicating successful amplification of the target sequence of the rs657152 gene (Figure 1).

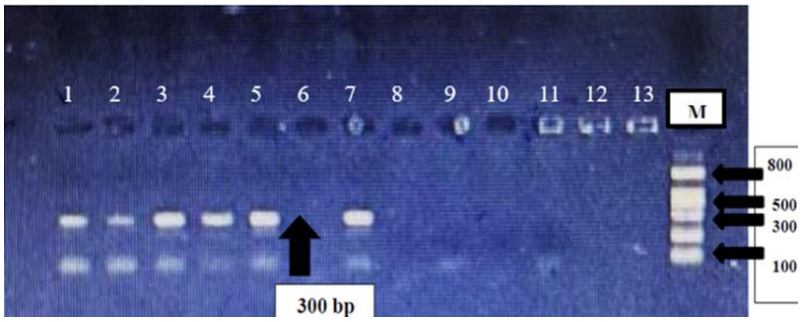

Figure 1. The electrophoresis pattern of the PCR product for rs657152 shows a single band at 300 bp. Lanes 1-3: PCR product of COVID-19 only, lanes 4-8: PCR product for COVID-19 + T2DM, and lanes 9-13: control groups. M: DNA ladder; 1% agarose, 75V, 20 mA for 1 hour (5 µ l in each well) annealing temperature 58 C°

The results demonstrated significant positive correlations between the presence of specific Rs657152 polymorphisms and higher levels of inflammatory markers. This observation indicates the association of specific gene polymorphisms with increased inflammatory responses in COVID-19 patients with T2DM (Table 5).

Table 5. Correlation of Rs657152 gene polymorphisms with inflammatory markers

Parameters	COVID-19 patients + T2DM with Rs657152 gene polymorphisms (18)	COVID-19 patients + T2DM without Rs657152 gene polymorphisms (15)	*P*
IL-6	28.06±2.17	21.14±1.9	0.042*
CRP	78.16±8.9	69.22±9.04	0.477
D-dimer	1887.68±25.09	1297.08±43.78	0.045*
Ferritin	874.06±32.7	645.1±22.8	0.033*

Statistically significant at $P \leq 0.05$.

Discussion

The present study aimed to determine whether Rs657152 polymorphisms contribute to an exacerbated inflammatory response and increased severity of COVID-19 in patients with T2DM. Our study provides insights into the potential genetic factors influencing disease outcomes in this high-risk group. It underscores the exacerbated inflammatory response observed in COVID-19 patients with T2DM, particularly those with the Rs657152 polymorphisms.

Research consistently exhibits that a higher BMI is a significant risk factor for severe COVID-19 (15,16). Recent studies conducted by Petrilli *et al*., (17) and Lighter *et al*., (18) have demonstrated that obesity is linked to an increased risk of hospitalization and severe outcomes. The present study's findings regarding higher BMI in COVID-19 patients with T2DM and its correlation with disease severity align with the abovementioned studies. This outcome underscores the necessity for targeted interventions in obese and diabetic populations.

Studies exploring genetic predispositions in COVID-19 patients have identified various polymorphisms influencing disease severity. For instance, studies on ACE2 and TMPRSS2 gene polymorphisms have shown that these genetic variations can affect the susceptibility and severity of COVID-19 (19). The association of Rs657152 polymorphism with higher inflammatory markers in the current study mirrors the findings of other genetic research, emphasizing the significant impact of disease outcomes by genetic variations. This study uniquely highlights Rs657152, adding to the broader genetic landscape influencing COVID-19 severity.

Numerous studies have documented the heightened inflammatory response in COVID-19 patients with T2DM. An investigation by Bornstein *et al*., (20) and Zhang *et al*., (21) found similar increases in inflammatory markers, such as CRP, IL-6, and ferritin, in diabetic patients. These markers are often correlated with disease severity and poor outcomes. Our findings agree with these observations, reinforcing that T2DM exacerbates inflammation in COVID-19 patients. Specific genetic data regarding Rs657152 polymorphisms adds a new layer of understanding, suggesting that certain genetic profiles may further enhance this response.

Research on the impact of genetic polymorphisms, like rs657152, on the severity of COVID-19 has become increasingly significant, especially in high-risk groups such as individuals with T2DM. Depending on population groupings and treatment protocols, some studies have examined the relationship between these genetic markers and the course of the disease, yielding varying conclusions. It suggests that genetic factors, such as rs657152, may not influence the efficacy of certain antiviral medications. Abdullaev *et al*.; (22) in 2024. According to their findings, these polymorphisms had no discernible impact on the outcomes for patients in this particular cohort, including the length of hospital stay or the requirement for intensive care. This is significant because it highlights the complexity of COVID-19 etiology, and the possibility of variability based on individual genetic profiles by suggesting that genetic factors like rs657152 may not affect the efficacy of specific antiviral medications. Recently, Biswas (23) examined the association between severe COVID-19 and the prevalence of the rs657152 and rs11385942 polymorphisms across various global populations. The study revealed significant variations in allele frequencies, with South Asian ethnic groups exhibiting a higher prevalence of the risk allele linked to severe COVID-19 (46.1%). These findings align with our results and demonstrate the usefulness of genetic screening in identifying individuals more prone to suffer disastrous outcomes. Balanovsky *et al*., (24) investigated the distribution of the polymorphisms, as mentioned earlier, throughout Russia and the adjacent countries to advance this field of inquiry further. Their findings demonstrated a connection between the rs657152 gene and higher COVID-19 death rates in Russian populations. This link suggests that genetic changes in a particular area may impact geographical variations in COVID-19 results, even though it was not observed elsewhere. Moreover, it emphasizes the importance of researching specific groups to understand how genetic variations impact disease severity fully.

Our results indicated a complex relationship between genetic susceptibility and the severity of COVID-19. Specifically, the polymorphism rs657152 may influence outcomes in certain populations, but it may not be a reliable indicator for others. These findings suggest that personalized medicine approaches could be helpful in managing COVID-19, particularly for high-risk groups such as those with type 2 diabetes and specific ethnic populations. Implementing personalized treatment plans and genetic screening may significantly improve patient outcomes and reduce mortality among those genetically predisposed to severe illnesses.

Identifying Rs657152 polymorphisms as a factor in increased inflammatory response opens avenues for personalized medical approaches. Screening for this polymorphism could help identify high-risk individuals who may benefit from more aggressive treatment strategies or preventive measures.

Like any study, ours has some limitations. First, genetic backgrounds, environmental conditions, and sample size could influence differences in SNPs observed among populations. Second, the small sample size may limit the study results, and a more significant number of patients and controls may provide additional information about the role of rs657152 gene variations in COVID-19 susceptibility.

The Rs657152 gene polymorphisms are correlated with increased levels of inflammatory markers such as CRP, IL-6, D-dimer, and ferritin in COVID-19 patients with T2DM, indicating a potential genetic influence on the severity of the inflammatory response. The findings of our study highlight the significance of genetic screening and personalized medical approaches in managing COVID-19 among diabetic patients. Future research should broaden its scope to include additional genetic polymorphisms and their interactions with various inflammatory markers and clinical outcomes. This attempt could provide a more comprehensive understanding of the genetic factors that influence the severity of COVID-19.

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