Original Article

Association between Single Nucleotide Polymorphisms of F3 Gene (rs12029080 T>G and rs11165176 C>T) with Thrombosis in SARS-CoV-2 Patients in Babylon Province

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Abstract

Background: Severe acute respiratory syndrome coronavirus 2 is a respiratory virus that has a strong association with pulmonary morbidity and thrombosis and has caused death in much of the sick population. The coagulation factor III gene (F3) produces tissue factor (TF) a cell-surface glycoprotein. It is the primary initiator of the extrinsic blood coagulation cascade. **Objectives:** The study aimed to identify the single nucleotide polymorphism (SNP) of F3 gene (rs12029080 T>G and rs11165176 C>T) and D-dimer linked to thrombosis in coronavirus disease (COVID-19) patients. **Materials and Methods:** Blood samples were collected from 30 patients infected by COVID-19 in Babylon Province. Detection of the polymorphism was done by using sequencing technique. **Results:** The results of the polymerase chain reaction of the amplification targeted region had shown two valid SNPs: rs11165176C>T and rs11165176. We discovered that heterozygous C/T had a higher genotypic frequency than homozygous C/C (odd ratio (OR) = 1.14, 95% confidence interval (CI) = 0.41–3.15, $P = 0.796$) and the homozygous variation T/T genotypic frequency is comparable to that of homozygous C/C (OR = 1.4, 95% CI = 0.43–4.86, $P = 0.543$). The C and T alleles frequencies showed statistical difference as reported in F3 (rs11165176) between COVID-19 patients and control ($P = 0.053$), 29 (48.3%) and 31 (51.6%), respectively, than in control groups, C allele 34 (56.7%) and T allele 26 (43.3%). Therefore, the T allele was most frequent in COVID-19 patients and control groups. **Conclusion:** According to the results of our statistical research, there was a significant link between F3 gene rs11165176 and the development of thrombosis in patients with COVID-19 to be a potential D-dimer associated with COVID-19 patients.

Keywords: COVID-19 patients, D-dimer, F3 gene, SNPs

Introduction

Coronavirus disease (COVID-19) is a large family that causes respiratory diseases from mild levels, such as a common cold, to more severe diseases, severe acute respiratory syndrome coronavirus (SARS-CoV-2), a virus belonging to the Coronovirdiae family, as it was discovered in the Chinese city of Wuhan in the wake of the acute respiratory syndrome epidemic of unknown origin and the cause of acute respiratory syndrome, and additional analyzes have shown that it has a great genetic similarity with many types of bat coronavirus, and this may indicate that bats are a reasonable reservoir for this animal virus. Although SARS-CoV-2 has a nearly identical sequence of 79% with SARS-CoV, the transmission rate of the first is much greater than that of the second.[1] Higher levels of D-dimer, cytokines, lower levels of lymphocyte subsets, and disease severity are all associated with COVID patients.[2] Tareq *et al.*[3] explained the individuals with COVID-19, lymphopenia may serve as a laboratory and clinical sign of disease severity.

Goldstein *et al.*[4] showed that children under the age of 10 have a significantly lower susceptibility to SARS-CoV-2 infection, whereas adults over the age of 60 have a higher susceptibility to infection and therefore require additional

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precautions to prevent infection. Children are particularly vulnerable to COVID-19 infection and have the capacity to spread the underlying virus to others. Although there is a great need to strictly follow infection prevention and control measures to reduce the incidence of infection or complications among children, the precise risk that children will acquire or transmit the infection is quite variable due to a number of factors, including the levels of community transmission.[5]

A new study indicates that thrombosis caused by degradation of clotting factors is probably the main cause.[6-11] D-dimer A protein created after fibrinolysis (FDP) to break down clots and break them up a decrease in D-dimer levels indicates that the drug is effective.[12] Elevated D-dimer values are the first sign of coagulopathy in critically ill patients.[13] Some studies have linked D-dimer levels to an increased risk of coronary heart disease, peripheral and peripheral artery disease, venous thrombosis (VT), and atherosclerosis.[14-16] Tissue factor (TF) is an integrative cytoplasmic membrane TF protein encoded by the F3 gene as the primary initiator of thrombin generation *in vivo* and is the key regulator responsible for the initiation of the extrinsic coagulation cascade.[17,18] Fitzgerald *et al.*[19] demonstrated that other respiratory viruses, such as influenza A, which did not significantly affect the expression levels of genes involved in the coagulation pathway, may not share the coagulation response with SARS-CoV-2. According to a recent study, SARS-CoV-2 is significantly associated with coagulation-related genes (such as F3, PROS1, ITGB3, and TFPI2) but not with other human coronaviruses (such as Middle East respiratory syndromeand SARS-CoV). These findings show that SARS-CoV-2 causes distinct reactions in alveolar epithelial cells and macrophages, resulting in a coagulative milieu in the lungs that might cause site thrombosis or pulmonary embolism in people who are vulnerable. Smith *et al.*[20] revealed the F3 rs12029080 variation, which was connected to higher plasma D-dimer levels, would also be linked to a higher risk of VT incidentally.

Materials and Methods

Study population

Blood samples were collected from those infected with the SARS-CoV-2 virus who attended the hospitals of Babylon Governorate and those lying in Marjan and Al Hashemiyah Hospital in the epidemiological division. The number of samples was 30 patients infected with the SARS-CoV-2 virus diagnosed by polymerase chain reaction (PCR) and/or chest computer topography (CT) scan. 10 samples were taken from men and 10 blood samples from women and 10 healthy conditions as a control group), subsequently separated into three groups based on the severity of the illness (mild, moderate, and severe) for the period from December 2021 to February 2022.

Genotyping and D-dimer measuring

DNA was extracted using a DNA extraction kit (Promega, Madison, Wisconsin, United States) to assess the presence of the F3 gene in SARS-CoV-2 patients by using conventional PCR. Fifteen samples for PCR product were taken for sequencing polymorphism detection. F3 gene genotyping was performed with PCR using specifically designed primers:

Forward: GCTATCCCTCACTTATCTTTGG

Reverse: AAGCAAAAGTAGTCATGTGGT

The DNA amplification for the F3 gene includes a PCR condition: 93°C/3 min (one cycle), 93°C/30 s, 56°C/30 s, 72°C/35 s (30 cycles), 72°C/3 min (one cycle), 4°C (one cycle). Results identified one form of the highly polymorphic rs11165176C>T, namely the normal homozygous (CC), and the mutant heterozygous (TT). AFIAS-6 was used to measure D-dimer (Automited Immunoassay Analyzer with the all-in-one cartridge system, Korea).

Statistical analysis

Statistical calculations were performed using statistical package for the social sciences software (version 23.0; SPSS Inc., Chicago, Illinois), and *P*-value was considered at 0.05 as statistically significant.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number dated December 1, 2021 to get this approval.

Results and Discussion

Table 1 shows the D-dimer level for both the COVID-19 patient group and control group. The results show significant

Table 1: The mean differences of D-Dimer between study groups

Parameters	Control	COVID-19			Sig.
		Mild	Moderate	Severe	
D-dimer		704.00 ± 86.2a	1523.36 ± 252.27b	3903.13 ± 41.9c	0.022*
	140.37 ± 19.4a	1731.41 ± 114.5b			0.008*

*Significant at *P*-value ≤ 0.05, a, b and c refer to significance among groups according to Duncan's statistical test

differences between the three groups 0.022 at *P*-value 0.05 as 704.00±86.2, 1523.36±252.27 and 3903.13±41.9 for mild, moderate, and severe, respectively, mean of all three groups is 1731.41±114.5 whereas the control group was 140.37±19.4 with 0.008 significant differences between control and patients' groups. Our study aimed to detect on a genetic basis by examining the association at the genetic level of the high level of D-dimer and causing the formation of clots in the human body infected with the coronavirus.

High D-dimer readings may indicate increased blood coagulation in COVID-19 patients due to a systemic inflammatory response syndrome or as a direct result of the SARS-CoV-2 itself.[21,22]

A study presented by Grillet *et al.*[23] found abnormal coagulation particularly marked elevation of D-dimer and FDP are common in deaths with pneumonia caused by a novel coronavirus (NCP).

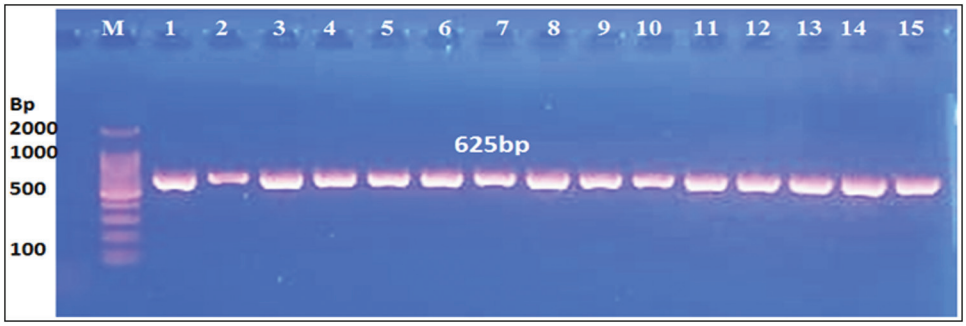

Figure 1: Amplification product with the band size of 625bp. The product was electrophoresed on a 3% agarose gel at 100 volt/cm² with 1× Tris-Borate-EDTA (TBE) buffer for 45 min. L: DNA ladder (100)

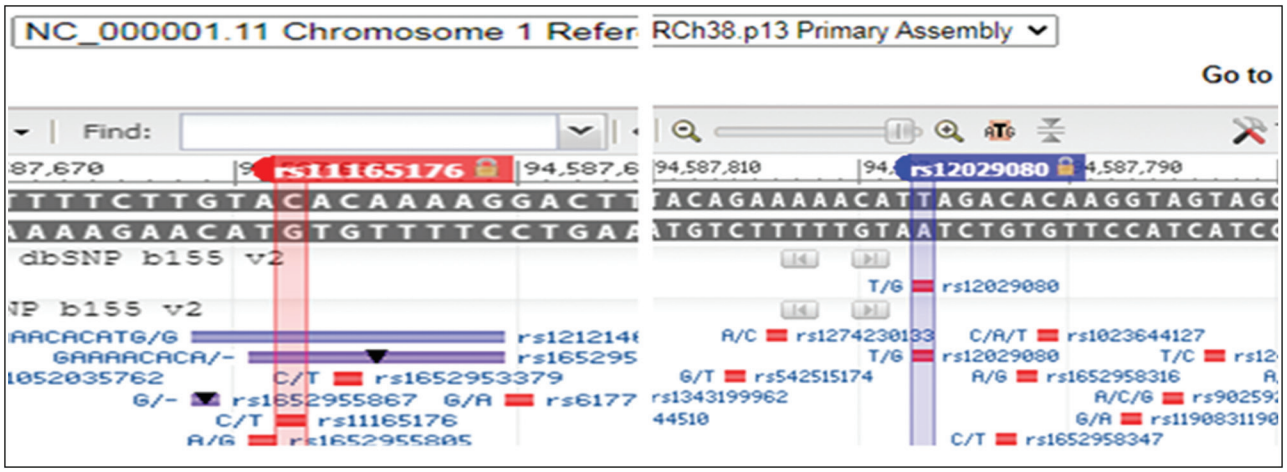

Figure 2: SNPs of rs11165176 and rs12029080 gene validity sites on chromosome 1 based on available data of national center for biotechnology information site

Table 2: Association between the COVID patients and the healthy control, the genotype distribution, and odd ratio of rs11165176 C>T polymorphisms

Genotypes rs11165176 C>T	Patients *N* = 30	Control *N* = 30	OR	95% CI	*P*-value
CC[a]	7 (23.3%)	10 (33.4%)			
CT	15 (50%)	14 (46%)	1.14	0.41–3.15	0.796
TT	8 (26.7%)	6 (20%)	1.4	0.43–4.86	0.543
Total number	30	30			
Allele	**Frequency**	**Frequency**			
C	29 (48.3%)	34 (56.7%)	0.47	0.22–1.0	0.053
T	31 (51.6%)	26 (43.3%)	2.13	0.99–4.6	0.053

CI, confidence interval; OR, odd ratio

P-value at 0.05; OR = (95% CI)

[a]Reference

In order to amplify the F3 gene target area, genomic DNA was isolated from blood samples. As shown in Figure 1, the results showed that the F3 gene target sequence appeared as a single band (625 bp) on an agarose gel.

The SNPs were screened for reference SNPs rs11165176 T>C and rs12029080 T>G fixed in the National center for biotechnology information database as shown in Figure 2.

For the SNPs with the number rs12029080 T>G, and based on the available data from previous research, this SNP is associated with COVID disease, whereas through our current study, it was found that the above SNPs registered in the GenBank were unrelated to COVID disease depending on the odd ratio (OR) value and its frequency was low. The reason for the low frequency may be due to the small sample size that was taken in the study as shown in Table 2.

Multiple alignments of chromatograms of the partial sequence of gene F3 in the patient group based on genius prime software. Figure 3 illustrates the collection chromatogram sequence of SNPs: rs11165176 and rs12029080, this software illustrated the homozygous wild type allele, heterozygous allele and homozygous mutant allele.

Figure 3: Multiple alignment chromatograms of partial sequences of F3 gene for the patient group. Two SNPs were observed: SNP rs12029080 T>G and rs11165176C>T

The result shows that the mutant T allele was high-risk in those infected with the coronavirus based on OR 1.4 (0.43–4.86) in the homozygous allele (TT), also the heterozygous allele showed a high OR value of 1.14 (0.41–3.15). These two values of homozygous allele (TT) and heterozygous allele have correlation with disease.

In Table 2, the results for F3 (rs11165176 C>T) polymorphism have shown that CT heterozygous genotype frequency was overrepresented among the COVID patients, and 15 (50%) which are more than the other two genotypes TT 8 (26.7%) and CC 7 (23.3%). Therefore, the CT heterozygous genotype was the most frequent in COVID patients. The F3 (rs11165176 C>T) CT heterozygous genotype was significantly associated with increased susceptibility to COVID-19. The individuals with CT heterozygous genotype were significantly overrepresented among COVID-19 patients, 15 (50%) were compared with other genotype, but no significance difference between COVID patients and control group (*P*-value = 0.796, OR = 1.14, 95% CI: 0.41–3.15 for CT genotype). The C and T alleles frequencies were statistical difference reported in F3 (rs11165176) between COVID-19 patients and control (*P* = 0.053), 29 (48.3%), and 31 (51.6%), respectively, than in control groups, C allele 34 (56.7%) and T allele 26 (43.3%). Therefore, the T allele was the most frequent in COVID-19 patients and control groups.

The valid SNPs rs11165176 C>T with these SNPs were correlated with SARS-CoV-2. The validity of these SNPs on chromosome 1 was verified and it was found by the values of the or that the SNPs are associated with corona and are a new record because they are associated with the disease.

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Conflicts of interest
There are no conflicts of interest.

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