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Evaluation of *IL-6* and *IL-17A* gene polymorphisms in Iraqi patients infected with COVID-19 and type 2 diabetes mellitus

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Abstract.
BACKGROUND: In patients with COVID-19, diabetes mellitus type 2 (T2DM) increases the risk of hospitalization and death. Patients who have *IL-6* and *IL-17A* single nucleotide polymorphisms (SNPs) are more likely to have severe COVID-19. This study aims to determine whether SNPs of the *IL-6* gene at rs1800795 (G > C) and the *IL-17A* gene at rs2275913 (G > A) are associated with COVID-19 and T2DM in the Iraqi population.
PATIENTS AND METHODS: Twenty-four people were divided into 4 groups as follows: six patients with severe COVID-19 and T2DM were placed in Group 1 as "G1", six patients with COVID-19 but no T2DM were placed in Group 2 as "G2", and six patients with T2DM were placed in Group 3 as "G3". There were also six healthy controls included in each group. Polymerase chain reaction (PCR) was used to amplify the target genes after genomic DNA from the blood samples was extracted. Sanger sequencing was used to find the SNPs in both the forward and reverse directions for each sample.
RESULTS: In the case of IL-6 SNP at rs1800795, the GG genotype was more common in "G3", the CC genotype was less common in all patient groups than in controls, and the GC allele was more common in "G2" than in the control group. In comparison to the controls, the three patient groups showed lower frequencies of the C allele and higher frequencies of the G allele. Regarding IL-17A gene polymorphism, the AA and GA genotypes were more prevalent in "G2" and "G3", respectively. The GG genotype and G allele frequency dropped in all patient groups compared to the control group, whereas the A allele frequency increased in all patient groups.
CONCLUSIONS: The *IL-6* gene at rs1800795 (G/C) and the *IL-17A* gene at rs2275913 (G/A) loci were associated with COVID-19 and T2DM in Iraqi population.

Keywords: COVID-19, T2DM, Polymorphism, *IL-6* gene, *IL-17A* gene

1. Introduction

Coronavirus disease 2019 (COVID-19) is a highly contagious viral illness with pneumonia-like symptoms that is brought on by the severe respiratory syndrome coronavirus 2 (SARS-CoV-2). In a short period of time after patients started exhibiting symptoms resembling those of the SARS outbreak, the cause was identified as a new strain of the coronavirus family known as 2019nCoV. On February 11th, 2020, the International Committee on Taxonomy of Viruses (ICTV) changed the designation to "SARS-CoV2" [1].

Globally, type 2 diabetes mellitus (T2DM) is a problem for public health. Since insufficient insulin secretion from the pancreas does not react to target cell receptors for glucose molecules, the condition is frequently accompanied with elevated blood glucose levels [2].

Patients who have COVID-19 are at risk for poor clinical outcomes if they have diabetes mellitus (DM) [3]. The epidemic of COVID-19 has had a significant impact on diabetic patients' ability to control their blood glucose levels. Patients who have COVID-19 experience severe metabolic alterations and blood glucose increases. Enhanced cytokine release and other inflammatory mediators are linked to it, leading to hyperglycemia and enhanced insulin resistance [4]. Ad-

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ditionally, COVID-19 may be contributing to the rise in acute DM by targeting the Angiotensin-Converting Enzyme 2 (ACE2) receptors in the pancreatic islets, which result in pancreatic dysfunction [5].

DM was the second most common chronic comorbidity in COVID-19 after hypertension. Patients with diabetes are more likely to die and experience severe COVID-19. Interleukin-6 (IL-6), C-reactive protein (CRP), ferritin, and D-dimer levels are much higher in diabetes patients than in non-diabetic individuals, indicating that an evident inflammatory cytokine storm is associated with a more dire prognosis [6].

A variety of pathological circumstances, such as infection and inflammation, trigger the secretion of IL-6 from mast cells, dendritic cells, and macrophages. In addition, in response to particular stimuli, fibroblasts, endothelial cells, and epithelial cells all express IL-6. The main mediator of an uncontrolled pro-inflammatory response that has been linked to lung injury in those with severe COVID is IL-6 [7]. Numerous investigations [8–10] have shown a clear correlation between elevated IL-6 levels and the extreme severity of COVID-19.

Interleukin-17A (IL-17A) appears to play a significant role in extreme COVID-19 pathogenesis [11]. This interleukin is a proinflammatory cytokine, produced by T helper 17(Th17) cells. Lung damage is found in serious COVID-19 patients due to an inflammation characterized by strong cell responses to Th17 [12].

Patients are more likely to develop severe type of COVID-19 due to cytokines and SNPs such as the *IL-6* and *IL-17A* gene polymorphism. These changes mostly affect gene expression and cause immune cells to react severely by taking part in a cytokine storm [13].

This study sought to explore the association of two SNPs of the *IL-6* gene at rs1800795 G > C, and *IL-17A* gene at rs2275913 G > A with COVID-19 and T2DM in Iraqi patients.

2. Materials and methods

2.1. Patient groups and sample collection

Four groups were used to determine the case-control research. Six severely ill patients with COVID-19 and T2DM made up the first group "G1", six patients with COVID-19 only made up the second group "G2", six T2DM patients made up the third group "G3", and six healthy volunteers made up the fourth group, which served as the healthy control group. In the recommended transitory hospital for COVID-19, Ibn-Al Khateeb Hospital in Baghdad, the patients were admitted. The specialist made a diagnosis of T2DM, and infections with COVID-19 were identified by PCR, CT scan; furthermore, the infection was confirmed by IgG and IgM levels investigation by fluorescence immuno-assay (FIA).

For all the study groups, results from the FBS and HbA1C tests were found by biochemistry automated device (Beckman Coulter AU480, USA). Six participants from each set of 24 participants had their veins pricked for five milliliters of blood, which was drawn with disposable syringes in an aseptic environment. 3 ml blood was drawn in a gel tube (Sail, China) and put in a centrifuge (Kokusan, Japan) to yield serum for chemical and immunological investigations. About 2 ml of venous blood was drawn in an ethylenediaminetetraacetic acid (EDTA) tube and stored at -20°C for DNA extraction and amplification by PCR technique, followed by investigations of the IL-6 gene polymorphism at rs1800795 loci and the IL-17A gene polymorphism at rs2275913 loci by Sanger sequencing as forward and reverse sequences for each sample.

2.2. Anti-SARS-CoV-2 IgG/IgM determination

2.2.1. Principle of the method

Sandwich immune-detection method is used in this test; fluorescence-labeled conjugates bind to antibodies in a human sample, forming antibody-antigen (Ab-Ag) complexes that migrate onto the intracellular matrix and captured by other immobilized anti-human IgG and anti-human IgM on a test strip. More antibodies in the serum sample form more antigen-antibody complexes which cause stronger fluorescence signal by (detector) antigen. This signal is processed by i chroma II device (Boditech, Korea) to display the anti-SARS-CoV-2 IgG and IgM concentration of the test sample [14,15].

2.2.2. Kit components

1. Test cartridges: The cartridge part contains a membrane called a test strip, which has anti-human IgM, anti-human IgG, and chicken IgY.
2. Detectors: The detector contains a granule of viral antigen-fluorescence conjugate, anti-chicken IgY-fluorescence conjugate, sodium azide as a preservative, and bovine serum albumin (BSA) as a stabilizer.
3. Detector diluent: contains salt, detergent, and sodium azide in Tris buffer.
4. Identification (ID) chip.

2.2.3. Test procedure

1) To prepare the detection buffer, 150 μl of detector diluents were transferred to the detector tube containing a granule that dissolved completely in the tube.
2) Ten microliters of human serum were added into detector tube immediately with shaking 10 times or more.
3) Sample mixture of 75 μl were pipetted and loaded into the sample well.
4) The cartridge was left at room temperature for 10 minutes and inserted into the holder.
5) After tapping the start button, the device calculated the test results automatically and displayed positive ($\geqslant$ 1.1, negative ($<$ 0.9, and indeterminate (0.9 $\leqslant$ Titer $<$ 1.1) on the display screen of the instrument.

2.2.4. Genomic DNA extraction

The DNA from the blood samples was extracted using a DNA extraction kit (Geneaid, Taiwan). The extraction steps are conferred by the company's instruction.

2.2.5. Assessment of DNA concentration and purity

Using a Nano-spectrophotometer (Biodrop, UK), the concentration and purity of the DNA were evaluated. The DNA concentration was stated as ng/μL. Each sample was pipetted into the nanodrop system in two microliter portions, and the optical density was calculated. In general, pure DNA is indicated by an A260/A280 ratio of 1.7–1.9 [16].

2.2.6. Agarose gel electrophoresis

1. After extraction, agarose powder (Carl Roth, Germany) was dissolved in 100 ml of 1X TBE buffer (Intron, Korea) in a flask (1% agarose for genomic DNA was used; while for PCR product, 1.5% agarose was used). Agarose powder was dissolved by boiling by water bath (Julabo, Germany) for 2 minutes until the solution became clear.
2. Agarose solution was allowed to cool to 50–55°C.
3. Three microliter of red stain (Intron, Korea) was added to the warm gel.
4. Combs was fixed in the tray of gel-casting.
5. Agarose solution was poured into the gel-casting tray slowly to prevent the formation of air bubbles.
6. Agarose solution was left to solidify at room temperature for 30 minutes. Carefully, the combs was pulled out and the tape was removed. The gel was transferred into an electrophoresis chamber and then covered with the 1X TBE buffer.

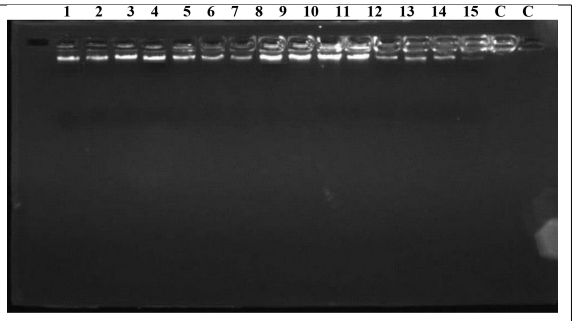

Fig. 1. Bands of genomic DNA extracted from human blood samples; Lanes 1–15 resemble the number of DNA samples; C: Control.

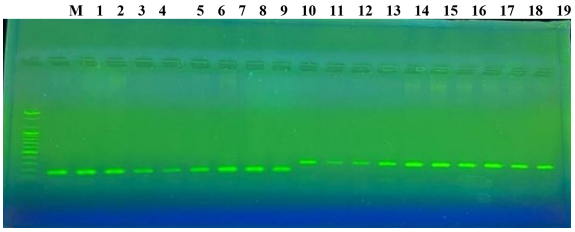

Fig. 2. Results of amplification of *IL-6* and *IL-17A* gene of blood samples were fractionated on 1.5% agarose gel stained with Red Safe. M = 100bp ladder marker. Lanes 1–10 resemble 197 bp PCR products of *IL-17* gene; 11–19 resemble 308 bp for *IL-6* gene.

7. DNA loading dye (3 μl) was mixed with each DNA sample (5 μl) and the mixture was loaded into each gel well.
8. Electrophoresis (Clarivate, Japan) of agarose gel was carried out at 70 V and 65 Amp for 60 minutes. The DNA bands were observed under a UV transilluminator (Scope-21, Japan). Figure 1 showed the optimization for the first 15 DNA samples [17]. The correct sizes of the DNA fragments were compared versus a 100bp ladder marker (Promega, USA) as shown in Fig. 2.

2.2.7. Primers used in the current study

Table 1 lists the primers (Alpha DNA, USA) utilized in the current study along with their molecular weight and sequence. The primers listed below were created using a web-based primer design service for conventional PCR and were then verified using the Primer-BLAST-NCBI database.

2.2.8. Protocol of gene amplification

The *IL-6* and *IL-17A* genes were amplified using a traditional polymerase chain reaction (PCR) thermal cycler Tc-3000X (Techne, USA). The PCR mixture contains 5 μl AccuPower PCR Premix (Bioneer, Korea) that is ready to use, 1 μl each of forward and reverse

Table 1
Primers and their information used in the current study

Primer name	The primer (5….. 3)	Size (bp)	Reference
IL-17 rs2275913	Forward: TGACCCATAGCATAGCAGCTC Reverse: GGTCACTTACGTGGCGTGTC	197	Designed in this study
IL-6 rs1800795	Forward : ATGCCAAAGTGCTGAGTCACTA Reverse : TCGAGGGCAGAATGAGCCTC	308	Designed in this study

primers, 13 μl of DNAse-free water, and 5 μl of extracted DNA for a total volume of 25 μl. Depending on the insertion program, the mixture was moved to a thermal cycler to initiate the reaction.

Thermocycling was designed to operate under the following conditions: an initial denaturation at 95°C for 5 min, 30 cycles of denaturation at 95°C for 30 sec., annealing at 60°C for 30 sec., and elongation at 72°C for 30 sec., and final single extension step at 72°C for 1 min for one cycle. On a 2% agarose gel, the PCR products were electrophoresed. DNA migrating bands were detected using a gel documentation system.

2.2.9. DNA sequencing

Sanger sequencing was performed using the ABI3730XL automated DNA sequencing by Macrogen Corporation in Korea on twenty-four amplified PCR product samples (forward and reverse separately) for the *IL-6* gene at rs1800795 loci and twenty-four sample (forward and reverse) for the *IL-17A* gene at rs2275913 loci.

2.3. Statistical analysis

Categorical variables were analyzed using NCBI-BLAST [18] and MEGA software version 6.0 [19] by comparing the outcome with NCBI control strains. The same program was used to examine query, pairwise alignment, and identity [20]. Odd ratio (OR) calculation was done using the MedCalc application to compare groups. Statistical significance was defined as a probability value of $p < 0.05$.

3. Results

Immunoglobulins IgG and IgM concentrations against SARS-CoV-2 were estimated for all the study groups by using fluorescence Immunoassay (FIA) with high sensitivity. The means and SE of IgM in G1, G2, G3 and control group were 2.97 $\pm$ 0.31, 8.32 $\pm$ 1.2, 0.605 $\pm$ 0.06 and 0.61 $\pm$ 0.05 respectively, whereas the means and SE of IgG in G1, G2, G3 and control group were 16.9 $\pm$ 1.3, 32.68 $\pm$ 3.8, 0.46 $\pm$ 0.05 and 0.44 $\pm$ 0.06 respectively. The mean of IgM and IgG in G2 was significantly increased (8.32 $\pm$ 1.2 and 32.68 $\pm$ 3.8 respectively) when compared to G1 (2.97 $\pm$ 0.31; 16.9 $\pm$ 1.3), G3 (0.605 $\pm$ 0.06; 0.46 $\pm$ 0.05), and the control group (0.61 $\pm$ 0.05; 0.44 $\pm$ 0.06), as shown in Table 2 and Figs 3 and 4.

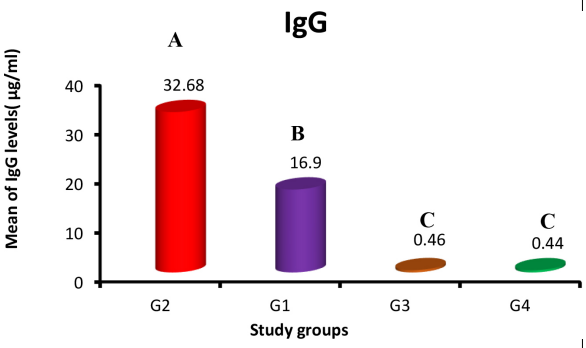

Fig. 3. Means of anti-SARS-CoV-2 IgG (μg/ml) among the study groups.

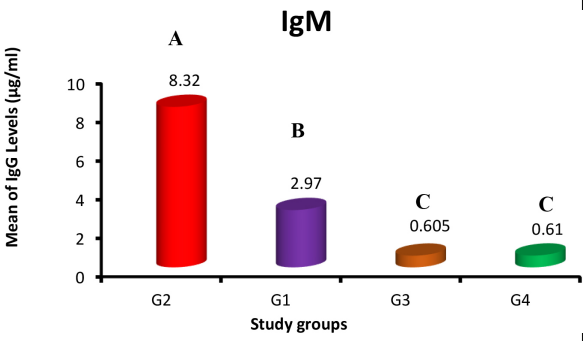

Fig. 4. Means of anti-SARS-CoV-2 IgM (μg/ml) among the study groups.

3.1. HbAIC

The mean and standard errors of HbA1C levels in G1 and G3 were 9.84 $\pm$ 0.49 and 9.0 $\pm$ 0.35 respectively, whereas in G2 and the control group were 5.27 $\pm$ 0.06 and 5.09 $\pm$ 0.09 respectively, as illustrated in Table 3 above.

3.2. DNA sequencing

Sanger sequencing was used to examine 24 DNA samples in order to find *IL-6* and *IL-17A* SNPs within these sequences. The human *IL-6* and *IL-17A* genes

Table 2
Means and SE of Anti-SARS-CoV-2 IgG and IgM in the study groups

Variable (s)	G1	G2	G3	Control	*p*-value
IgM (Mean ± SE)	2.97 ± 0.31	8.32 ± 1.2	0.605 ± 0.06	0.61 ± 0.05	0.001**
	B	A	C	C	
IgG (Mean ± SE)	16.9 ± 1.3	32.68 ± 3.8	0.46 ± 0.05	0.44 ± 0.06	0.001**
	B	A	C	C	

SE: Standard Error; p: probability; S: significant; G: Group; the letters (A, B, and C) for rows represented the levels of significant (LSD test), highly significant start from the letter (A) and decreasing with the last one. Similar letters mean there are no significant differences between tested mean.

Table 3
Mean of HbA1C percentages among the study groups

Variable (s)	G1	G2	G3	Control	*p*-value
HbAIC% (Mean ± SE)	9.84 ± 0.49	5.27 ± 0.06	9.0 ± 0.35	5.09 ± 0.09	0.05
	A	B	A	B	

SE: Standard Error; p: probability; G: Group; the letters A and B for rows represented the levels of significant (LSD test), highly significant start from the letter (A) and decreasing with the last one. Similar letters mean there are no significant differences between tested mean.

are located on chromosomes 6 and 7, respectively, and have gene sizes of 308 and 197 base pairs, respectively. The outcome for the IL-6 gene's rs1800795 SNP is shown in Fig. 5 below. Single "C" and "G" peaks denote homozygous alleles CC and GG, while double peaks denote heterozygous alleles CG.

Table 4 displays the results of the IL-6 SNP (rs1800795) molecular analysis. In comparison to the control group (0%), the homozygous GG genotype was significantly more common in G1 (66.6%) (OR = 23.4; p-value = 0.05) and G3 (100%) (OR = 169; p = 0.0134).

Because the homozygous GG genotype was significantly more common in G1 (66.6%) and G3 (100%) than in the control group (0%) in a significant way (OR = 23.4, p = 0.05 and OR = 73.9, p = 0.01, respectively), and because the homozygous CC genotype was significantly less common in all patient groups (0%) than in the control group(100%) in a significant way (OR = 0.005, p = 0.0134), the GG genotype might increase the susceptibility to T2DM infection and the CC genotype might be associated with decreased susceptibility to COVID-19 and T2DM. At OR = 169 and p = 0.0134, the heterozygous GC genotype was considerably more prevalent in G2 (100%) than in the control group (0%), suggesting that the GC may be linked to a higher vulnerability to COVID-19. According to allele analysis, the G allele was highly prevalent in G1(83.3%), G2(50%), and G3(100%) compared to the control (0%), and the C allele was significantly less common in G1(16.6%), G2(50%), and G3(0%) compared to the control (100%). As a result, the C allele was associated with a decreased risk of COVID-19 and T2DM, and the G allele may increase the risk of infection with COVID-19 and T2DM.

Table 4
Genetic association of *IL-6* gene SNP in the study groups and control

IL-6 (rs1800795)	N (%)		OR	*p*-value
Genotype/allele	$N = 6$	$N = 6$		
Group 1				
GG	4 (66.6%)	0	23.4	0.05
GC	2 (33.3%)	0	7.2	0.2
CC	0	6	0.005	0.0134
C	2 (16.6)	12 (100%)	0.0095	0.0037
G	10	0	105	0.0037
Group 2				
GG	0	0	1	1
GC	6 (100%)	0	169	0.0134
CC	0	6 (100%)	0.005	0.0134
C	6 (50%)	12 (100%)	0.04	0.0372
G	6 (50%)	0	25	0.037
Group 3				
GG	0	0	1	1
GC	6 (100%)	0	169	0.0134
CC	0	6 (100%)	0.005	0.0134
C	6 (50%)	12 (100%)	0.04	0.0372
G	6 (50%)	12 (100%)	25	0.037

N: number; OR: odd ratio; p: probability.

The results for the SNP of the *IL-17A* gene at the rs2275913 locus are displayed in Fig. 6 below. The homozygous alleles AA and GG are indicated by single "A" and "G" peaks, respectively, while the heterozygous allele GA is indicated by double "A" and "G" peaks.

Regarding the *IL-17A* gene polymorphism, frequencies of the GG genotype were found to be significantly lower in the three patient groups (0%) than in the control group (100%) (OR = 0.005, p = 0.0134), and the AA genotype was found to be more frequently in G2

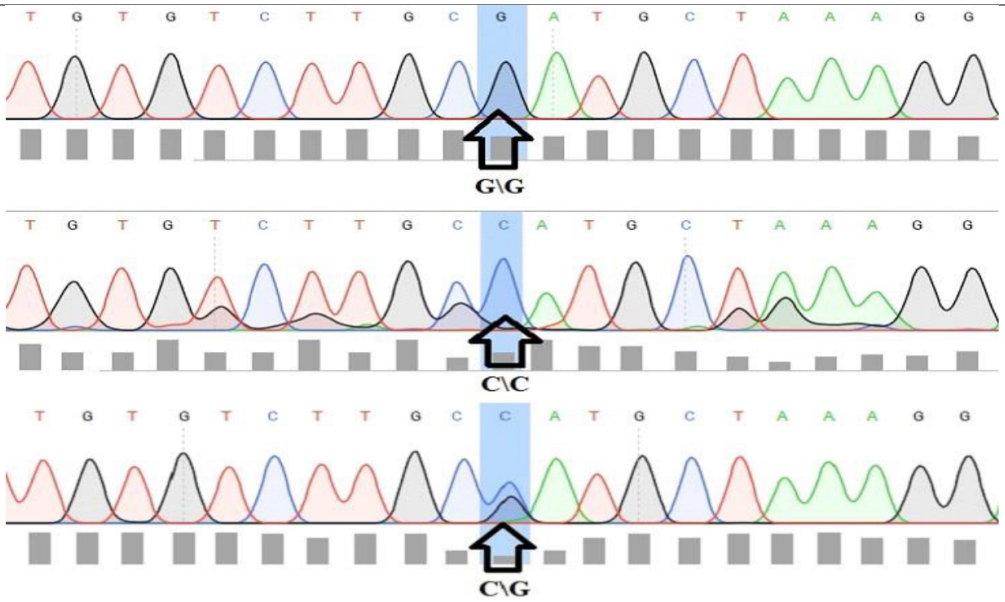

Fig. 5. Analysis of the *IL-6* gene's rs1800795 SNP. The homozygous alleles CC and GG are indicated by single C and G peaks, respectively, while the heterozygous allele, CG, is indicated by double peaks.

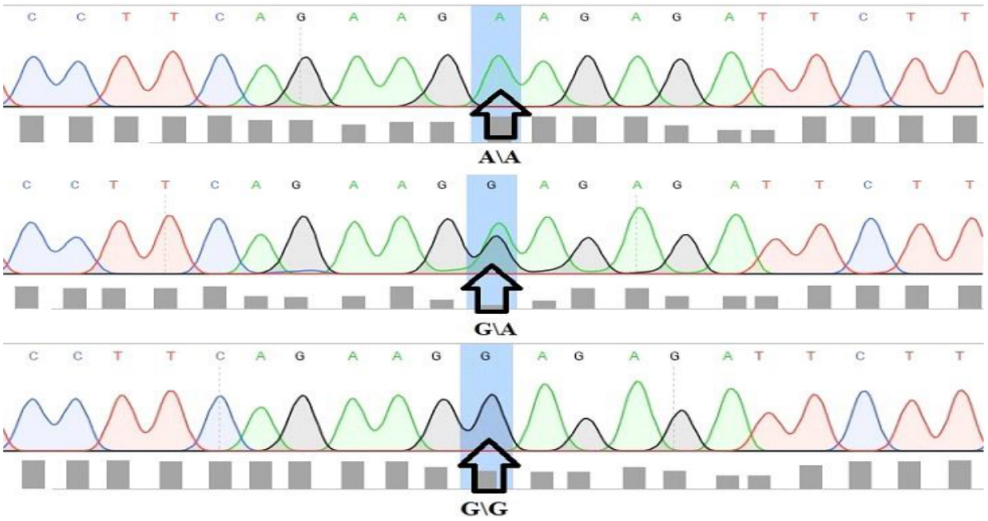

Fig. 6. Analysis of rs2275913 SNP of *IL-17* gene. Single A and G peaks are indicative of a AA and GG homozygous allele respectively, while double peaks are indicative GA heterozygous allele.

(100%) compared to the control group (0%) (OR: 169, $p = 0.01$); as a result, the AA was associated with increased susceptibility to COVID-19 infection; while the GA frequency was considerably higher in G3 (100%) compared to the control group (0%) (OR = 169, $p = 0.0134$), this indicates that the GA genotype was linked to an increased risk of T2DM illness.

In terms of allele frequency, the A allele was more prevalent in G1 (83.3%)at OR = 105 and $p = 0.01$, G2 (100%) at OR = 625 and $p = 0.01$, and G3 (50%) at OR = 25.0 and $p = 0.0372$ compared to the control group (0%); as a result, the A allele was associated with infected with COVID-19 and T2DM. The G allele was considerably lower in G1 (16.6%), G2 (0%) and G3 (50%) compared to the control group (100%), with OR values of 0.0095, 0.0016, and 0.04 and p-values of 0.001, 0.001, and 0.0372, respectively, as demonstrated in Table 5.

Table 5
Genetic association of *IL-17A* gene SNP in the study groups and control

IL-17A (rs2275913)	N (%)		OR	p-value
Genotype/allele	$N = 6$	$N = 6$		
Group 1				
GG	0	6 (100%)	0.005	0.0134
GA	2 (33.3%)	0	7.2	0.2
AA	4 (66.6%)	0	23.4	0.05
A	10 (83.3%)	0	105	0.01
G	2 (16.6%)	12 (100%)	0.0095	0.001
Group 2				
GG	0	6 (100%)	0.005	0.0134
GA	0	0	1	1
AA	6 (100%)	0	169	0.01
A	12 (100%)	0	625	0.01
G	0	12 (100%)	0.0016	0.01
Group 3				
GG	0	6 (100%)	0.005	0.0134
GA	6 (100%)	0	169	0.0134
AA	0	0	1	1
A	6 (50%)	0	25.0	0.0372
G	6 (50%)	12 (100%)	0.04	0.0372

N: number; OR: odd ratio; p: probability.

4. Discussion

T2DM is the most common comorbidities in COVID-19 patients, leads to poor outcomes. T2DM is among the variables that cause increased ACE2 expression in the lungs and other tissues; ACE2 is a cellular "receptor" and port of viral entry. The previous chronic inflammation, together with an amplified inflammatory response to the infection, results in a severe systemic immune response ("cytokine storm"), which is closely linked to higher COVID-19 severity [20].

In this study, serum levels of IgG and IgM antibodies against SARS-CoV-2 were investigated for all individual groups involved in this study. Samples were collected after the second week of infection from patients who have acute symptoms that related to infection with COVID-19.

Liu et al. observed that the timing of IgG and IgM Ab incidence in patients varies largely. This variation may be associated with age and comorbidity and they observed that the IgM antibody response against SARS-CoV-2 appeared earlier and peaked earlier than IgG and the response of IgM began to decline at week 3 of the disease, while the IgG response continued and was maintained in COVID-19 patients, and severe cases of COVID-19 patients tended to have a more robust response in both IgM and IgG antibodies to SARS-CoV-2 [21].

In response to infections, the immunoglobulins IgM and IgG are usually created by the adaptive immune system [22]. IgM and IgG can be specific and diagnostic markers for detecting of infections like COVID-19 [23]. This finding is the same to the results of another group that displayed a positive IgG titer in SARS-CoV-2 patients during the first 3 weeks after onset of symptom, though IgM showed a minor decrease in the third week [24]. Other studies have revealed that IgM concentrations elevated in patients during the 1st week of infection, with a high peak in the second week prior to a vast reduction near background levels in most COVID-19 infected patients. On the other hand, IgG antibody was observed after one week of infection and maintained at a high level for a long time after the occurrence of infection [21,25].

Quantitative detection of IgM and IgG could assist in evaluating the severity of COVID-19 infection and could establish a dynamic that help in predicting prognosis and the levels of IgM and IgG antibodies are raised in COVID-19 infected patients [26].

The results of this study showed increased significantly of HbA1C in G1 and G3 and not increased in G2 compared with the control group. Glycated hemoglobin (HbA1C) is an essential biomarker of long-term glycemic control since it can reflect the previous two to three months' cumulative glycemic history. HbA1c is not only a valid indicator of chronic hyperglycemia, but it also corresponds well with the risk of long-term diabetic complications [27]. Glycated hemoglobin (HbA1c) test is used to help investigate the prevalence of unrecognized diabetes, the cumulative prevalence of unrecognized and known diabetes, and the prevalence of poor glycemic control in both [28].

Single nucleotide polymorphism (SNP) is a change in single base of DNA sequence with a normal alternative of two possible nucleotides at a given site. This variation happens at a specific location in the genome, and has an allele frequency equal or greater than 1%. A single base pair polymorphism is the most common type of polymorphism [29]. Polymorphisms are important for determination of disease severity and susceptibility in the latest years. Polymorphisms are naturally occurring in DNA sequences [30]. SNPs are common markers in molecular genetic disease studies as well as pharmacogenomics researches. They consider as factors contributing to inflammatory diseases and cancer [31].

Genetic analysis regarding the SNP of the *IL-6* gene (rs1800795) suggested that the GG genotype might be linked to T2DM, the GC genotype might play a susceptibility role in COVID-19, the CC genotype might play a role in reducing susceptibility to infection with COVID-19 and T2DM, the C allele might play a protective role for infection with COVID-19, and the G allele

might be considered a risk factor for both COVID-19 and T2DM.

It has been demonstrated that the *IL-6* (-174G/C) polymorphism's promoter region has a direct impact on gene transcription. After IL-6 production, the interleukin can work on various immune cells to trigger and boost the inflammatory response and activate macrophages, which in turn causes organ damage (such diabetic nephropathy and neuropathy) [32]. In COVID-19 patients, the genetic makeup of a person is very likely to have an impact on the immune response induction and outcome. The SARS-COV-2 entrance, replication, and host immune response are all regulated by many host genes [33].

Verma et al. found that the patients with COVID-19 showed a considerably high frequency of the GC genotype in the North Indian population (adjusted OR 3.86, p 0.001), and the C allele of the rs1800795 SNP may represent a risk factor for the severity of COVID-19 [34]. The findings of Altamemi et al. and Fishchuk et al. revealed a significant association between the IL-6 polymorphism at rs1800795 and the risk of developing COVID-19, but in contrast with our findings, they found a significant increase in the frequency of the CC genotype and C allele in severe COVID-19 patients compared to controls [35,36].

According to the severity of the clinical symptoms, Rahimlou et al. discovered that the frequency of the rs1800795 SNP genotypes of IL-6 fluctuated in different groups of COVID-19 patients, and the G allele may likely have a protective function against COVID-19 [37]. Furthermore, Falahi et al. in the Iranian population observed no statistically significant differences in the genotype distribution of a particular SNP rs1800795 (174 G $>$ C) in the promoter region of the IL-6 gene in patients with severe and mild COVID-19 [38]. There is no correlation between the rs1800795 SNP and the likelihood of developing T2DM in the Isfahan community [39].

In the case of the rs2275913 SNP for the IL-17A gene, frequencies of homozygous GG genotype and G allele were lower in patient groups than in controls; consequently, the GG genotype and G allele might have a protective role against COVID-19 and T2DM; the frequency of AA genotype was significantly higher in COVID-19 patients compared to controls, and the GA genotype was significantly higher in T2DM patients compared to controls; therefore, the GA genotype may predispose patients to infection with T2DM, the AA genotype may enhance susceptibility to COVID-19, and the allele A may raise the likelihood of developing COVID-19 and T2DM.

The relationship between the IL-17A (rs2275913) polymorphism and vulnerability to COVID-19 and DM has been the subject of numerous investigations. The cytokine storm differed by the rs2275913 polymorphisms in the IL-17A gene, and the severity of disease might be related to the genetic host factors including immune profiling. In a previous study, it was discovered that the GG and AG genotypes of the rs2275913 SNP in the IL-17A gene were associated with COVID-19 susceptibility in the populations of Brazil and Spain [40].

Kadhum and Ahmed reported that this polymorphism is linked to an elevated risk of T2DM possibly by upregulating the expression of *IL-17A* cytokine [41]. They demonstrated that the heterozygous AG genotype of *IL-17A* G/A (rs2275913) polymorphism showed a risk among T2DM patients and the G allele was related to an increased risk of T2DM. However, there is no correlation between COVID-19 severity in Egyptian patients and *IL-17A* polymorphism [42]. Additionally, Abdulateef and Fouad discovered no correlation between COVID-19 and the frequency of the GA genotype in the rs2275913 locus. However, patients who had the A allele were shown to be at increased risk to infect with COVID-19 compared to the control group [43]. Differences in SNPs observed among populations could be influenced by a variety of factors, including genetic backgrounds, environmental conditions, and sample size; the small sample size may limit the study results, and a greater number of patients and controls may provide additional information about the role of *IL-6* and *IL-17A* gene variations in COVID-19 susceptibility.

5. Conclusion

The susceptibility to infect with COVID-19 and T2DM was associated with two possible SNPs of the *IL-6* gene at rs1800795 (G/C) and *IL-17A* gene at rs2275913 (G/A) loci in the Iraqi population. The relationship between genetic variants in *IL-6* and *IL-17A* and the infection with COVID-19 and T2DM has to be confirmed by additional research incorporating more SNPs and a larger sample size.

Ethical clearance

On an ethical level, this study was accepted by the research ethical committees of Iraq's ministries of environment and health, higher education and scientific research. in approval number BCSMU/1221/0004M in December, 1, 2021. . . .

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Authors' contributions

Each of the below authors made a significant contribution to the research and writing of this publication. Conceptualization, K.H. Rasool; Interpretation or analysis of data, E.N. Naji, Q.A. Khalaf; Soft-ware, Q.A. Khalaf; validation, K.H. Rasool, E.N. Naji; Formal analysis, Q.A. Khalaf and E.N. Naji, investigation, Q.A. Khalaf; Resources, Q.A. Khalaf; data curation, E.N. Naji; Preparation of the manuscript, Q.A. Khalaf; writing-review and editing, K.H. Rasool, E.N. Naji; Revision for important intellectual content, K.H. Rasool; Supervision, K.H. Rasool, E.N. Naji. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] M.A.U. Din and L.K.T. Boppana, An update on the 2019-nCoV outbreak, *American Journal of Infection Control* **48**(6) (2020), 713.

[2] R. Rafeh, A. Viveiros, G.Y. Oudit and A.F. El-Yazbi, Targeting perivascular and epicardial adipose tissue inflammation: therapeutic opportunities for cardiovascular disease, *Clinical Science* **134**(7) (2020), 827–851.

[3] S. Elamari, I. Motaib, S. Zbiri, K. Elaidaoui, A. Chadli and C. Elkettani, Characteristics and outcomes of diabetic patients infected by the SARS-CoV-2, *The Pan African Medical Journal* **37** (2020).

[4] A. Ceriello, Hyperglycemia, and COVID-19: What was known and what is really new? *Diabetes Research and Clinical Practice* **167** (2020), 108383.

[5] M. Nassar, N. Nso, C. Gonzalez, S. Lakhdar, M. Alshamam, M. Elshafey and V. Rizzo, COVID-19 vaccine-induced myocarditis: case report with literature review, *Diabetes & Metabolic Syndrome* **15**(5) (2021), 102205.

[6] M. Han, K. Ma, X. Wang, W. Yan, H. Wang, J. You and X. Luo, Immunological characteristics in type 2 diabetes mellitus among COVID-19 patients, *Frontiers in Endocrinology* **12** (2021) 596518.

[7] T. Kishimoto, IL-6: from its discovery to clinical applications, *International Immunology* **22**(5) (2021), 347–352.

[8] E.A. Coomes and H. Haghbayan, Interleukin-6 in COVID-19: a systematic review and meta-analysis, *Reviews in Medical Virology* **30**(6) (2020), 1–9.

[9] B.M. Henry, M.H.S. De Oliveira, S. Benoit, M. Plebani and G. Lippi, Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis, *Clinical Chemistry and Laboratory Medicine (CCLM)* **58**(7) (2020), 1021–1028.

[10] X. Liu, S. Shi, J. Xiao, H. Wang, L. Chen, J. Li and K. Han, Prediction of the severity of Corona Virus Disease 2019 and its adverse clinical outcomes, *Japanese Journal of Infectious Diseases*, JJID **73**(6) (2020), 404–410.

[11] V. Bulat, M. Situm, M.D. Azdajic and R. Likic, Potential role of IL-17 blocking agents in the treatment of severe COVID-19? *British Journal of Clinical Pharmacology* **87**(3) (2021), 1578.

[12] V. Mendoza, Interleukin-17: A potential therapeutic target in COVID-19, *The Journal of Infection* **81**(2) (2020), e136–e138.

[13] M.K. Vakil, Y. Mansoori, G.R.L. Al-Awsi, A. Hosseinipour, S. Ahsant, S. Ahmadi and A. Ghasemian, Individual genetic variability mainly of Proinflammatory cytokines, cytokine receptors, and toll-like receptors dictates pathophysiology of COVID-19 disease, *Journal of Medical Virology* **94**(9) (2022), 4088–4096.

[14] L.G. Giménez, J. Rojas, A. Rojas, J. Mendoza and A.G. Camacho, SARS-Associated Coronavirus Diagnostic kit: development of an ELISA-based antibody detection test with a cocktail of nucleocapsid and spike SARS-CoV proteins (2008), *Clinical and Vaccine Immunology*.

[15] L.R. Huang, C.M.S.H. Yeh, W.H. Huan, P.R. Hsueh, W.Z. Yang and P.J. Chen, Evaluation of antibody responses against SARS coronaviral nucleocapsid or spike proteins by immunoblotting or ELISA, *Journal of Medical Virology* **73**(3) (2004), 338–346.

[16] G. Lucena-Aguilar, A.M. Sánchez-López, C. Barberán-Aceituno, J.A. Carrillo-Avila, J.A. López-Guerrero and R. Aguilar-Quesada, DNA source selection for downstream applications based on DNA quality indicators analysis, *Biopreservation and Biobanking* **14**(4) (2016), 264–270.

[17] W. Ream, B. Gellar, J. Trempy and K. Field, Molecular microbiology laboratory (2nd ed.) Elsevier, Oxford, UK, (2013).

[18] T.A. Hall, BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In *Nucleic acids symposium series* **41** (1990), pp. 95–98. [London]: Information Retrieval Ltd., c1979–c2000.

[19] K. Tamura, G. Stecher, D. Peterson, A. Filipski and S. Kumar, Molecular evolutionary genetics analysis version 6.0, *MolBiolEvol* **30**(12) (2013), 2725–2729.

[20] A. Rajpal, L. Rahimi and F. Ismail-Beigi, Factors leading to high morbidity and mortality of COVID-19 in patients with type 2 diabetes, *Journal of Diabetes* **12**(12) (2020), 895–908.

[21] X. Liu, J. Wang, X. Xu, G. Liao, Y. Chen and C. Hu, Patterns of IgG and IgM antibody response in COVID-19 patients, *Emerg Microbes Infect* **9** (2020), 1269–1274.

[22] S. Panda and J.L. Ding, Natural antibodies bridge innate and adaptive immunity, *The Journal of Immunology* **194**(1) (2015), 13–20.

[23] M.A. Chowdhur, N. Hossain, M.A. Kashem, M.A. Shahid and A. Alam, Immune response in COVID-19: a review, *J Infect Public Health* **13** (2020), 1619–1629.

[24] Q.X. Long, B.Z. Liu, H.J. Deng, G.C. Wu, K. Deng, Y.K. Chen and A.L. Huang, Antibody responses to SARS-CoV-2

in patients with COVID-19, *Nature Medicine* **26**(6) (2020), 845–848.

[25] H. Hou, T. Wang, B. Zhang, Y. Luo, L. Mao, F. Wang, S. Wu, Z. Sun, Detection of IgM and IgG antibodies in patients with coronavirus disease 2019, *Clin Transl Immunol* **9**(2020): e01136.

[26] W. Aljabr, A. Al-Amari, B. Abbas, A. Karkashan, S. Alamri, M. Alnamnakani and A. Al-Qahtani, Evaluation of the Levels of Peripheral CD3+, CD4+, and CD8+ T Cells and IgG and IgM Antibodies in COVID-19 Patients at Different Stages of Infection, *Microbiology Spectrum* **10**(1) (2022), e00845–21.

[27] S.I. Sherwani, H.A. Khan, A. Ekhzaimy, A. Masood and M.K. Sakharkar, Significance of HbA1c test in diagnosis and prognosis of diabetic patients, *Biomarker Insights* **11**, BMI-S38440, (2016).

[28] N. Nanayakkara, H. Nguyen, L. Churilov, A. Kong, N. Pang, G.K. Hart and E.I. Ekinci, Inpatient HbA1c testing: a prospective observational study, *BMJ Open Diabetes Research and Care* **3**(1) (2015), e000113.

[29] W. Sukhumsirichart, Polymorphisms. In *Genetic diversity and disease susceptibility*. IntechOpen, (2018).

[30] M. Jackson, L. Marks, G.H. May and J.B. Wilson, The genetic basis of disease, *Essays in Biochemistry* **62**(5) (2018), 643–723.

[31] N. Deng, Z. Heng, F. Hua and Y. Yuan, Single Nucleotide Polymorphisms and Cancer Susceptibility, *Oncology Target* **8**(66) (2017), 35–49.

[32] B. Ayelign, M. Negash, H. Andualem, T. Wondemagegn, E. Kassa, T. Shibabaw and M.D. Molla, Association of IL-10 (-1082 A/G) and IL-6 (-174 G/C) gene polymorphism with type 2 diabetes mellitus in Ethiopia population, *BMC Endocrine Disorders* **21**(1) (2021), 1–8.

[33] S. Das, C. Gupta, N. Akhter, C. Jain, S. Sonthalia, S. Ahmad and S.A. Dar, COVID-19: Variable Immune Response in Indian Population, Egyptian Academic Journal of Biological Sciences. C, *Physiology and Molecular Biology* **13**(1) (2021), 133–147.

[34] S. Verma, F.H. Khan, Z. Siddiqi, S.T. Raza, M. Abbas and F. Mahdi, Genetic polymorphisms of IL6 gene–174G $>$ C and –597G $>$ A are associated with the risk of COVID-19 severity, *International Journal of Immunogenetics* **50**(1) (2023), 5–11.

[35] I.A. Altamemi and A.H. Khlebos, IL-6 Predict Disease Severity and Potential Therapeutic Target in COVID-19 Patient, *Annals of the Romanian Society for Cell Biology* **25**(2) (2021), 2173–2180.

[36] L. Fishchuk, Z. Rossokha, V. Pokhylko, Y. Cherniavska, S. Tsvirenko, S. Kovtun and N. Gorovenko, Modifying effects of TNF-α, IL-6 and VDR genes on the development risk and the course of COVID-19. Pilot study, *Drug Metabolism and Personalized Therapy* **37**(2) (2021), 133–139.

[37] B. Rahimlou, S. Ghaffarpour, M.S. Zamani, M.M. Naghizadeh and T. Ghazanfari, Association between interleukin-6-174G/C single nucleotide polymorphism with the COVID-19 severity, *Daneshvar Medicine* **30**(3) (2022), 10–22.

[38] S. Falahi, M.H. Zamanian, P. Feizollahi, A. Rezaiemanesh, F. Salari, Z. Mahmoudi and A.G. Karaji, Evaluation of the relationship between IL-6 gene single nucleotide polymorphisms and the severity of COVID-19 in an Iranian population, *Cytokine* **154** (2022), 155889.

[39] R. Ghavimi, M. Sharifi, M.A. Mohaghegh, H. Mohammadian, S. Khadempar and H. Rezaei, Lack of association between rs1800795 (-174 G/C) polymorphism in the promoter region of interleukin-6 gene and susceptibility to type 2 diabetes in Isfahan population, *Advanced Biomedical Research* **5** (2016).

[40] L.K. Batur and N. Hekim, Correlations of IL-6, IL-6R, IL-10 and IL-17 gene polymorphisms with the prevalence of COVID-2019 infection and its mortality rate, *Research Square* (2020).

[41] M.S. Kadhum and I.H. Ahmed, The influence of IL-17A genetic polymorphism on the susceptibility of Type 2 diabetes mellitus in Iraqi patients, *Chinese Journal of Medical Genetics* **31**(3) (2022), 288–291.

[42] M. Rushdy, M.S. Elsayed, R. Ahmed, A.G. Gaber and R. El-Asady, (IL-17A rs2275913; G197A) gene polymorphism as predictor for disease severity and its correlation with IL-17 serum levels in COVID-19 patients, *The Egyptian Journal of Immunology* **29**(3) (2022), 90–98.

[43] Z. Abdulateef Abdullah and L. Fouad Ali, Severity of COVID-19 infection correlation with IL-17 polymorphism, *Archives of Razi Institute*, 2022.