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REVIEW ARTICLE

Interleukin-6 and TNF-alpha as biomarkers associated with ACE2 SNPs rs1978124 and rs2074192 in patients type II diabetes mellitus with COVID-19

Ghaleb Hussein Obaid Al-Saeedi[1] , Wisam Salih Abood[2]

1PhD Student College of medicine, Al-Qadisiyah Univercity Iraq

2Ph.D.Medical Virology, College of Medicine, Al-Qadisiyah University Iraq

*Corresponding author: Email: ghaleb1974hussein@gmail.com , Phone: +9647803042672

Abstract:

Background: Diabetics have a unique set of challenges as a result of the current SARS-CoV-2 Coronavirus illness 2019 (COVID-19) epidemic. It predisposes to a particularly severe course of the disease and doubles the risk of dying from COVID-19. Aim: was to study the analysis and comparison of the relationship between polymorphisms and immune cytokines in the pathogenesis of type 2 diabetes in SARS-CoV-2 patients. Methodes: From January 2022 to April 2022, blood samples were drawn from 120 patients who were divided into three groups for a cross-sectional and case-control study admitted to COVID-19 isolation wards. 40 of whom were infected with SARS-CoV-2 virus and were mainly diabetic, 40 of whom had diabetes only, and 40 control samples. They were randomly selected, with ages ranging from (18-75) years.

Results: The results of the analysis showed that most infections are between the ages of 40-49. Also, it was clearly shown that females were infected more than males, and the comparison between the molecular examination of ACE2 (rs 1978124 and rs2074192) and cytokines, including IL- 6, TNF--α, result in the relationship between the ACE2 (rs1978124) genotype and serum IL-6 levels. for COVID-19 patients with diabetes, SNP TT (162.14± 99.95)compared to the CC genotype and CT, but for Diabetic patients was CC (33.11± 50.88), the relationship between the ACE2 (rs1978124) genotype and serum TNF--αlevels. was genotype CT(204.52± 76.64) to COVID-19 patients with diabetes , while Diabetic patients genotype TT(82.08 ± 34.00) . While, the relationship between the ACE2 (rs2074192) genotype and serum IL-6 levels, in COVID-19 patients with diabetes CT (193.13± 86.75) compared to the CC and TT genotype,and Diabetic patients were CC(41.63± 54.04) pg/ml compared to TT genotype and CT. and ACE2 (rs2074192) genotype and serum TNF-α levels COVID-19 patients with diabetesCT (172.31± 84.77), Diabetic patients CC (79.92± 36.51). Conclusions: ACE2 is known to be a receptor for COVID-19. Which is expressed in most cells of the human body, an important factor and an exacerbation problem in genetically susceptible individuals. The morbidity and mortality rates of SARS-CoV-2 pneumonia are affected by innate immunity. It was observed through the results that the islets of Langerhans in the pancreas were infected, either with the virus or the cytokine storm, which leads to diabetes type 2 .

Keywords: SARS-CoV2, COVID-19 , diabetes mellitus type 2, Cytokine, ACE2

Introduction

When the SARS-CoV-2 virus infection was deemed a global pandemic by the World Health Organization [1]. The sickness, known as COVID-19, causes severe illness with lung damage, multi-organ failure, and death. The SARS-CoV-2 surface protein must bind to the ACE-2 receptors in order to connect with and enter the host cell [2]. The high expression of the ACE2 receptor in capillary-rich organs, notably the lungs, is responsible for the severity and predominance of COVID-19's respiratory symptoms [3].

Patients with coronavirus disease-19 who include a history of diabetes and chronic diseases are more likely to experience worsening outcomes such as acute respiratory distress syndrome (ARDS) and pneumonia [4]. A number of ACE2 variants, by way of type2 diabetes mellitus , according to COVID-19's global distribution, cases, and fatality rates have been greater in populations in Europe and the United States [5]

1.1. SARS-CoV-2 innate immune response :

The first steps in the innate immune response signaling cascade are the Toll-like receptors (TLR7 and TLR3), which rec-

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ognize pathogen-associated molecular patterns and function as pattern recognition receptors (PRRs) for RNA viruses in the lungs. [6]

This can lead to multiple organ failures, severe immune-mediated lung damage, and unchecked production of inflammatory cytokines. for individuals who have severe cases of COVID-19. In reaction to immune cell lysis or T cell activation, significant levels of TNF- and Interleukin-6 (IL-6) are produced [7].As a result, immune cells for instance dendritic cells , macrophages, and endothelial cells become active.A cytokine storm is created by an upregulated positive feedback loop of pro-inflammatory cytokines [8].

1.2. Adaptive Immunity(Humoral and cellular immunity):

Strong cellular immune responses against SARS-CoV-2 in COVID-19 patients' viral infection are significantly controlled by B-cell-associated humoral immunity, cellular immunity mediated by TH1 cells, and cytotoxic CD8+ T lymphocytes. According to data from other trials, individuals with moderate to severe sickness may produce a lot of SARS-CoV-2-specific humoral immunity but are unable to produce enough virus-specific cellular immunity. Lymphocytes play a significant role in the immune response to viral infections. [9]

1.3. Cytokine release syndrome

It is believed that the virus's cytopathic effects and capacity to elude the host defense system contribute to the severity of COVID-19 sickness [10].

1.3.1. The role of interleukin-6 IL-6 signaling in inflammatory status :

Several molecular secretions leukocytes produce have been called interleukins (IL). IL-6 is a short polypeptide with four alpha helices. It consists of 184 residues of amino acids and has a molecular weight between 19 - 28 kD. That practically all immune cells produce, along with stromal cells and other cells including endothelial, fibroblastic, keratinocyte, and tumor cells [11]. The role of IL-6 in the maturation of B-cells and the making of antibodies is long recognized. IL-6 also promotes the activity of cytotoxic T lymphocytes, regulates the production of T-helper 17, and keeps the right ratio of regulatory T cells in check.

Prior to binding to the IL-6/s, IL-6 first attaches to its receptor (IL-6R). To start intracellular signaling, the IL-6R complex binds to the membrane protein gp130 that transmits signals.[12]

1.3.2. Role of Tumor necrosis factor-α :

Endothelial cells, monocytes, and macrophages generate TNF- were the involved producers of TNF-α [13]. Additionally, fibroblasts, T-cells, and B-cells . TNF- α binds and activates the TNF receptor (TNFR) 1 and TNFR 2 to transfer messages. A significant proinflammatory cytokine that affects RAS is tumor necrosis factor. According to reports, it affects insulin signaling pathways and contributes significantly to the emergence of T2DM [14].

1.4.Angiotensin converting enzyme 2 (ACE2) :

Angiotensin II (Ang) is controlled in a homeostatic manner by the antagonistic effects of ACE2 and ACE activities. Angiotensinogen is turned by renin into Ang I, which is then transformed by ACE into Ang II, which interacts with the angiotensin receptors 1R and 2R (AT1R, AT2R) to cause vasoconstriction and hypertension [15].

The ACE2 receptor is internalized and then detached from its useful position when SARS-CoV-2 invades the lung epithelial cells, which upsets the equilibrium between ACE and ACE2 action. The protective benefits of the ACE2-Angiotensin (1-7)-MAS receptor axis are thereby negated. Vasoconstriction, enhanced inflammation, fibrosis, and pulmonary damage are eventually implicated in the severity of the symptoms of SARS-CoV-2 infection [16].

1.4.1.ACE2 stricture :

A crucial regulator of blood volume and systemic vascular resistance is the renin-angiotensin-aldosterone system (RAAS), which is made up of the three primary substances angiotensin II, renin, and aldosterone [17]. The 805-amino acid ACE2 protein is encoded by 18 exons in human ACE2, which is located on chromosomal Xp22 and frequently exhibits sequence variants. Due to their modulation of the RAAS pathway, ACE2 has also been recognized as contributing factors to DM illness. Severe COVID-19 outcomes in DM patients depend on ACE2 gene (ACE2) polymorphisms [18].

1.4.2. The virus entry mechanism into cells :

Cells are invaded by the severe acute respiratory syndrome coronavirus (SARS-CoV-2). The attachment of the spike protein to cell surface ACE2 signifies the beginning of the SARS-CoV-2 virus's cellular entrance (fig.(1)). Cellular proteases like TMPRSS2 and furin are responsible for the cleavage at the S1/S2 domains during the priming of the S protein. The virus can then cling to the cell surface because of this. Once within the cell, SARS-CoV-2 multiplies by using the cellular machinery already there. The virus is transported into endosomes. Angiotensin (Ang) I is changed into the octapeptide AngII by ACE, whereas AngII is changed into Ang1-7 by ACE2 [19].

Through the activation of Ang II type 1a receptors, AngII causes vasoconstriction and cell proliferation, whereas Ang1-7 encourages vasodilatation and inhibits cell growth. When Ang II levels are high, pro-inflammatory cytokines and soluble IL-6 receptors are generated. This triggers a positive feedback loop that results in an abundance of pro-inflammatory chemokines and cytokines Fig. (1).

1.4.3.ACE2 mutations and how they occur :

By changing transcriptional activity or modifying splicing efficiency, intron variations can impact how genes with introns are expressed. Splice site variations can result in the production of an aberrant transcription or non-functional proteins from the associated gene by causing the development of a cryptic splice site, improper recognition of the splice site, or disruption in the actions of regulatory components involved in splicing. Because gene expression regulatory components like enhancers can be found downstream of the target gene, downstream gene variations can also affect how a gene expresses itself [20].

1.4.4. Diabetes mellitus II (T2DM)and susceptibility to SARS-CoV-2 :

Infection The case series revealed that COVID-19 itself as well as more severe clinical course and death were both influenced by diabetes mellitus and other connected disorders [21]. Patients with diabetes make up a sizable share of COVID-19 hospitalized patients. The incidence of diabetes among COVID-19 patients was 7.4%, with some cases reaching 20% [22]. It suggests that people with diabetes have a higher risk of contracting SARS-CoV-2 infection. Diabetes mellitus and the COVID-19 clinical course vary depending on the severity of the disease. in comparison to individuals without diabetes [23]. As a result, acute respiratory distress syndrome (ARDS) development and diabetes were substantially related [1].

2. Materials and methods

2.1. Sample collection

Patient group: 120 people with SARS-CoV-2 who received a diagnosis from a specialist doctor were divided into three groups to conduct a cross-sectional study and a case-control study: each group consisted of only 40 people (the first group was 40 who were infected with the SARS-CoV-2 virus they mainly suffered from diabetes, the second was from the diabetes unit in the diabetes unit at Al-Diwaniyah Teaching Hospital and had no history of patients infected with SARS-CoV-2. The third control group also consisted of 40 healthy people who were randomly), selected individuals aged between (18-75) year.

Patients were collected as part of a sample and research population for serious situations. From the Shifa Center between the dates of January 1 and April 30, 2022, respectively,SARS-CoV-2 infections in the cases were verified by RT-PCR.

2.2. Collection of samples :

Five milliliters (5 ccs) of blood were drawn from each of the three groups, including the control, by puncturing a vein with a disposable syringe while the subject was seated or lying down and after the withdrawal site had been cleaned with 70% alcohol. As seen below

Two portions of the blood sample were taken. There was a tube of blood (3 ml). Following the completion of the separation procedure, three duplicates of the blood sample are transferred to 1.5 ml centrifuge tubes (supplement Eppendorf containers), which are then maintained at -20 °C. One of these tubes is then used to conduct an ELISA test to measure the levels of IL-6 and TNF (2 ml) of peripheral blood was collected in a K3-EDTA anticoagulant tube.

2.3. Exclusion criteria:

Patients under the age of 18 and those suffering from other chronic illnesses including high blood pressure and respiratory conditions were not included in the research. In addition, people vaccinated with one of the COVID-19 vaccines, Immunological diseases, and patients with cancer and kidney disease were also excluded; lack of data for patients at hospital discharge and a lack of information about.

2.4.Immunological diagnosis :

Execution of reagent preparation and assay procedures was carried out in accordance with accurate manufacturer descriptions.

2.4.1.Enzyme-linked immunosorbent assay human interleukin 6 Kit, Human Tumor necrosis factor -α:

According to the manufacturer's instructions, human ELISA kits (IL-6, TNF-) were employed in the current investigation for quantification from patient blood samples for both study and healthy control group groups (BT-LAB, Zhejiang, China). manufacturing a solid-phase antibody (Biotech, USA) as detailed below [24]. adopting the purified antibody for microtitration plate packing.

2.4.2.Molecular diagnosis of ACE-2 :

Amplification refractory mutation system (ARMS), a method that may identify known mutations involving single base alterations or minor deletions, was employed for the molecular diagnostic; for this reason, sequence-specific PCR primers were used [25].

2.4.3.Primers :

ARMS-PCR primers for ACE2 (rs1978124) (rs2074192) gene polymorphism. These primers were provided by (Scientific Researcher. Co. Ltd. Iraq)

2.4.4.Statistical analysis

Using SPSS version 23 and Microsoft Office Excel 2010, the necessary data were gathered, condensed, examined, and presented. As long as the variable had a normal distribution, an independent sample t-test was used to compare the means of any two groups. The one-way ANOVA test was used to compare the mean differences between more than two groups, assuming that the variable was normally distributed. As long as they were non-parametric, the Kruskal-Wallis test was employed to analyze differences in mean rank between any number of groups [26].

3.Results:

The COVID-19 patients with diabetes had a mean age of (51.10 12.23) years, diabetic patients had a mean age of (47.35 13.69) years, and control subjects had a mean age of (45.25 13.21) years. The difference in mean age between the two groups was not statistically significant (P = 0.101). The frequency distribution of COVID-19 patients, diabetic patients (T2DM), and control individuals by age was also shown in table (3-1). Once more, there were no appreciable differences in the occurrence distribution of COVID-19 patients, diabetic patients, and control individuals by age groups (P = 0.057). Patients aged 40 to 49 were more likely to have diabetes and COVID-19 infection compared to other age groups .

The average age of COVID-19 diabetic patients was 51.10 (12.23) years old, the average age of diabetic patients was 47.35 (13.69) years old, and the average age of the control group was 45.25 13.21 years old. The mean age of the two groups did not differ statistically significantly from one another (P = 0.101). In Table 3-1, the frequency distribution of COVID-19 patients, diabetic patients, and control individuals was also shown by age. Again, there was no discernible difference in the frequency distributions of COVID-19 patients, diabetic patients, and control participants by age groups (P = 0.057). those 40 to 49 years of age were more likely to have diabetes and COVID-19 infection compared to those in other age groups.

In the COVID-19 with diabetes group, there was no statistically important difference in the incidence distribution of patients and controls by gender (table 3-2). Diabetic patients included 18 (45.0%) cases of males and 22 (55.0%) cases of females, whereas the control group included 15 (37.5%) cases of males and 25 (62.5%) cases of females. 16 (40.0%) cases were male and 24 (60.0%) cases were female. Given the necessity of such a case-control research, the aforementioned findings have ensured statistical parity in terms of age and gender between the patient groups and the control group.

3.1. Association between ACE2 (rs1978124) genotype and serum IL-6 levels in patients groups:

Table (3-3) below summarizes the relationship between the ACE2 (rs1978124) genotype and blood IL-6 levels. The current findings in Covid-19 patients with diabetes demonstrated a non-significant rise in blood IL-6 levels in the TT genotype (162.14 99.95 pg/ml) compared to the CC genotype and CT genotype 133.24 (75.99)pg/ml and 158.62 94.79 pg/ml, respectively) (P=0.610). The current findings also indicate a non-significant rise in serum IL-6 levels in diabetes individuals with the CC genotype, 33.11–50.88 pg/ml, compared to the TT genotype and CT genotype, 27.99–30.50 pg/ml and 20.95–10.30 pg/ml, respectively (P=0.752).

3.2. Association between ACE2 (rs1978124) genotype and serum TNF-α levels in patients groups.

The table (3-4) below summarizes the relationship between the ACE2 (rs1978124) genotype and serum TNF- levels. The current findings in Covid-19 patients with diabetes demonstrate non-significantly higher blood TNF- levels in the CT genotype (204.52 ± 76.64 pg/ml) compared to CC genotype and TT genotype, which were 158.82 ± 69.77 pg/ml and 136.12± 27.02 pg/ml, respectively (P=0.097). The current findings also indicate a non-significant rise in serum TNF- levels in diabetic individuals, with the TT genotype having 82.08 ± 34.00 pg/ml as opposed to the CC genotype and CT genotypes, which had 67.04± 35.55 pg/ml and 77.48 ± 33.23 pg/ml as corresponding values (P=0.490).

3.3.Association between ACE2 (rs2074192) genotype and serum IL-6 levels in patients groups.

The table (3-5) below summarizes the relationship between the ACE2 (rs2074192) genotype and blood IL-6 levels. The current findings in Covid-19 diabetic patients demonstrate a non-significant rise in blood IL-6 levels in CT genotype 193.13 ±86.75 pg/ml, compared to CC genotype and TT genotype, respectively, 132.51± 89.40 pg/ml and 126.19 ±72.81 In pg/In ml (P=0.108). The blood IL-6 levels in diabetes individuals with the CC genotype 41.63± 54.04, however, were found to be non-significantly elevated (P=0.221) than those in the TT genotype and CT genotypes, which were, respectively, 27.36 ±33.43 pg/ml and 11.07±4.49 pg/ml.

3.4.Association between ACE2 (rs2074192) genotype and serum TNF-α levels in patients groups.

The table (3-6) below summarizes the relationship between the ACE2 (rs2074192) genotype and serum TNF levels. The current findings in Covid-19 patients with diabetes demonstrate non-significantly elevated blood TNF levels in the CT genotype at 172.31± 84.77 pg/ml compared to CC genotype and TT genotype at 165.69 75.00 pg/ml and 155.71± 52.19 pg/ml, respectively (P=0.816). The current data, however, indicate a non-significant rise in serum TNF levels in diabetes individuals with the CC genotype, which were 79.92 ±36.51 pg/ml compared to TT genotype and CT genotype values of 74.84± 40.55 pg/ml and 67.13 ±30.78 pg/ml, respectively (P=0.586).

4.Discussion

The SARS-CoV-2 pandemic illness, a severe acute respiratory viral infection, has gained international attention when it was first discovered in December 2019 in Wuhan / China. As a representative sample of infected COVID-19 patients in Iraq, this investigation was conducted on patients infected with the SARS-CoV-2 virus in Al-Diwaniyah Governorate.In the second week following infection, people with severe and potentially fatal COVID-19 start to exhibit symptoms of cytokine interference, an excessive inflammatory response. As a result of the high levels of proinflammatory cytokines circulating in the blood, patients may experience acute respiratory distress syndrome (ARDS) and multiple organ failure.

The aforementioned research has the following drawbacks: First, because the COVID-19 wave only lasted a brief time, only a minimal number of research samples were chosen for each group. Second, the assessment and monitoring of cases in the hospital's isolation wards for people with type 2 diabetes or for those who contracted the SARS virus and started showing signs of diabetes. Third, the challenge of routinely monitoring the study subjects. Fourth, COVID-19 or other chronic illnesses complications caused fatalities in research samples taken from patients who were laying down during sample collection, resulting in their removal from the study. Fifth, COVID-19 participants who did not see any decline in blood sugar levels were disqualified.

Despite the fact that COVID-19 may affect people of any age, the study's findings at this time indicated that the age group with the highest infection rates was 40 to 49, followed by 50 to 59. People over the age of 40 are more likely than those under that age to develop COVID-19, which may be because they are older and more physically fragile or because they participate in more social activities.

which raises their risk of developing type 2 diabetes and severe pneumonia the majority of patients in the research were confined to being between the ages of 40 and 49 [27], which is similar to Jiawei et al., (2022)[28] and Jie et al., (2022)[29], Also, this study agrees with Fountain et al., (2022)[30] that females are more affected than males. While Acelajado et al. (2020)[31], who showed the highest prevalence of COVID-19 in individuals over 70 years of age, are at odds with these findings as well. Also, this study does not agree with Verdecchia et al.2021 [32] who stated that the prevalence rate of Covid-19 in the city of Diwaniyah is that those ages over 65 are the most infected with the virus.

SARS-CoV-2 penetrates several organs, counting endocrine organs, according to sex during ACE2 receptors and produces a clinical picture in many spectrums that can fluctuate depending on the person's immune system, gender, age concurrent disorders, and from moderate to severe, and even result in death. As seen in figure (4-1), Covid-19 was discovered to be more common in girls than in males in this study.because there were fewer men among the afflicted patients than women.linked this circumstance to the potential that a larger viral load might affect the synthesis of hormones, similar to the study of Ghafouri et al.,(2020[33].

This is in line with prior Chinese research as a putative shared genetic locus for T2D, 8 Yinha SNPs (rs1978124, rs2074192) that are thought to be linked with T2D were discovered. Co-morbidity is linked to various ACE2 polymorphisms, according to genetic research studies [34]. By altering the interaction between ACE2 and S1 proteins, non-synonymous variants of ACE2 may play a role in regulating viral entrance into host cells [35].

Summarizing the relationship between ACE2 genotype (rs1978124) and serum IL-6 levels. In Covid-19 patients with diabetes, the results showed a non-significant increase in serum IL-6 levels in the TT genotype, compared to the CC genotype and the CT genotype, and COVID-19 patients who developed diabetes, while in diabetic patients, the results showed an increase non-significant in serum IL-6 levels in the CC genotype compared to the TT genotype and the CT genotype. This explains the presence of a strong TT genotype relationship in diabetics who carry the CC genotype and are not infected with the virus.

The association between serum TNF- levels and the ACE2 genotype (rs1978124). The findings indicated a non-significant rise in blood TNF- levels in the CT genotype, but only in COVID-19 individuals with diabetes of that nature. The CC genotype and the CT genotype are contrasted with the TT genotype. Given the rise in cytokine levels in patient's blood serum, this suggests a connection between TT and TNF in the occurrence of pancreatic damage.

In COVID-19 patients with diabetes and COVID-19 patients who developed diabetes, there were differences in the gen-

otypes of the angiotensin-converting enzyme-2 (ACE2), TMPRSS2, interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-), with heterozygous C/T and homozygous TT being risk factors but not statistically significant for these groups at the systemic level.In this investigation, the distribution of ACE2 genetic variants linked to COVID-19 comorbidities was examined for interethnic and interpopulation heterogeneity.

Conclusion:

Disruption of ACE2 function is one of the effects of severe SARS-CoV-2 infection, and this results in the pathogenesis of COVID-19. On the other hand, the innate immune system has an impact on the morbidity of SARS-CoV-2 pneumonia. Interaction of SARS-CoV-2 with ACE2 receptors in the pancreas leads to infection of the Islets of Langerhans with SARS-CoV-2, which is evidenced by the subsequent loss of the ability to secrete insulin, as well as the immune response to get rid of the virus, which leads to the release of (cytokines), killing pancreatic tissue and then impaired capacity On the sense of the amount of glucose in the blood and the secretion of insulin. The immune response to the virus also impairs the liver's ability to detect changes..

Ethics statement:

After receiving clearance from the National Security Agency, the Health Department, and the isolation wards at Al-Diwaniyah Teaching Hospital designated for receiving Covid patients, blood samples from patients will be taken for the study.The current study was accepted by the regional medical ethics board, and all participants were patients or healthy controls.Prior to beginning the process of gathering test blood samples for the study, all participants or their families gave their consent.

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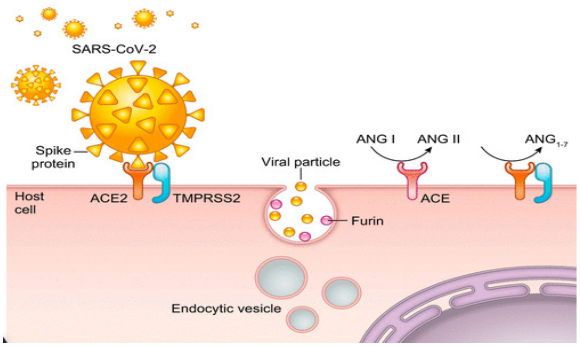

Fig. (1): Ischemic of SARS-CoV-2 entry into cells [42].

Table (3-1): Comparison of the age distribution of the patients and the controls.

Characteristic	Covid-19with Diabetes N=40	Diabetic patients N=40	Control subjects n=40	P
Mean ±SD	51.10± 12.23	47.35 ± 13.69	45.25 ± 13.21	0.101 †
Range	28–73 years	18 – 69 years	18- 65 years	NS
<29, n (%)	2 (5.0 %)	5 (12.5 %)	8 (20.0 %)	
30-39, n (%)	3 (7.5 %)	8 (20.0 %)	10 (25.0 %)	
40-49, n (%)	11 (27.5 %)	10 (25.0 %)	10 (25.0 %)	0.057¥
50-59, n (%)	11 (27.5 %)	8 (20.0 %)	7 (17.5 %)	NS
60-69, n (%)	9 (22.5 %)	9 (22.5 %)	5(12.5 %)	
≥ 70, n (%)	4 (10.0 %)	0 (0 %)	0 (0%)	

n: number of cases; SD: standard deviation; †: one way ANOVA; ¥: Chi-square test; NS: not significant at P> 0.05

Table (3.2): Patients and controls are distributed according on gender.

Gender	Covid-19with Diabetes N=40		Diabetic patients N=40		Control subjects n=40		χ^2	P
	N	%	n	%	N	%		
Male	16	40.0	18	45.0	15	37.5	3.838	0.279¥ NS
Female	24	60.0	22	55.0	25	62.5		

:¥ Chi-square test; n: number of cases; NS: not significant at P ≤ 0.05

Table (3-3): Association between ACE2 (rs1978124) genotype and serum IL-6 levels patients groups.

Serum IL-6	ACE2 genotype			
	CC genotype	CT genotype	TT genotype	P
COVID-19 patients with diabetes				
Mean± SD	133.24± 75.99	158.62± 94.79	162.14± 99.95	P=0.610 † NS
Range	52.78 –265.21	33.32-301.69	55.49-302.85	
Diabetic patients				
Mean± SD	33.11± 50.88	20.95± 10.30	27.99 ± 30.50	P=0.752 † NS
Range	1.23 –195.22	10.50 –37.96	5.48– 95.96	

NS: not significant at P ≤ 0.05; # †: Anova test.

Table (3-4): Association between ACE2 (rs1978124) genotype and serum TNF-α levels patients groups.

Serum TNF-α	ACE2 genotype			
	CC genotype	CT genotype	TT genotype	P
COVID-19 patients with diabetes				
Mean± SD	158.82 ± 69.77	204.52± 76.64	136.12± 27.02	P=0.097 † NS
Range	88.80 –350.48	124.83-307.23	90.65-171.22	
Diabetic patients				
Mean± SD	67.04± 35.55	77.48± 33.23	82.08 ± 34.00	P=0.490 † NS
Range	15.06 –148.86	23.38 –128.53	24.49– 138.69	

NS: not significant at P ≤ 0.05; # †: Anova test.

Table (3-5): Association between ACE2 (rs2074192) genotype and serum IL-6 levels patients groups.

Serum IL-6	ACE2 genotype			
	CC genotype	CT genotype	TT genotype	P
COVID-19 patients with diabetes				
Mean± SD	132.51± 89.40	193.13± 86.75	126.19± 72.81	P=0.108 † NS
Range	52.78 –302.85	58.20-301.69	33.32-253.48	
Diabetic patients				
Mean± SD	41.63± 54.04	11.07± 4.49	27.36 ± 33.43	P=0.221 † NS
Range	9.73 –195.22	2.95 –18.75	1.23– 120.71	

NS: not significant at P ≤ 0.05; # †: Anova test.

Table (3-6): Association between ACE2 (rs2074192) genotype and serum TNF-α levels patients groups.

Serum TNF-α	ACE2 genotype			
	CC genotype	CT genotype	TT genotype	P
COVID-19 patients with diabetes				
Mean± SD	165.69± 75.00	172.31± 84.77	155.71± 52.19	P=0.816 † NS
Range	95.27 –350.48	88.80-329.41	102.84-307.21	
Diabetic patients				
Mean± SD	79.92± 36.51	74.84± 40.55	67.13 ± 30.78	P=0.586 † NS
Range	32.06 –148.86	23.38 –128.53	15.06– 138.69	

NS: not significant at P ≤ 0.05; # †: Anova test.