## Original Article

# The severity of COVID-19 infection correlation with IL-17 polymorphism

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## ABSTRACT

Single nucleotide polymorphisms (SNPs) in many studied genes have been related to the severity of COVID-19. This study was carried out to investigate whether the polymorphisms of two SNPs (rs763780 and rs2275913) of the gene polymorphisms for Interleukin 17 (IL-17) are connected to the COVID-19 severity. The effect of these polymorphisms on the levels of IL-17 and the relationship between the level of IL-17 and the severity of COVID-19 were also investigated. The real-time polymerase chain reaction (RT-PCR) was performed to detect SARS-COV-2. Blood samples were obtained for the analyses of IL-17 levels by enzyme-linked immunosorbent assay, and Genomic DNA was extracted for genotyping. It should be noted that genotyping was performed using RT-PCR. The results indicated that the IL-17 level significantly increased in the case group, compared to the control group (healthy people), and there was a relationship between the severity of COVID-19 and IL-17 levels ($P$<0.01) in the severity groups. Mean±SE values of IL-17 were 67.99±2.05, 147.60±3.34, 218.15±6.27, and 283.97±5.59 ng/L for the control, mild, moderate, severe, and the critical group, respectively. Furthermore, the type of severity had a non-significant relationship with SNP80 and IL-17 levels (ng/L). Moreover, the type of severity also had a non-significant relationship with SNP13 and IL-17 levels (ng/L) ($P$≥0.05). In addition, the severity of COVID-19 and the prevalence of the AA genotype were shown to be significantly correlated. Besides, rs2275913 A-allele carriers were shown to be at risk ($P$=0.021) in the case group, compared to the control group. Furthermore, rs2275913 A-allele carriers were at a higher risk in the severe group ($P$=0.005, $P$ <0.05) and critical group ($P$=0.023, $P$<0.05), compared to the mild group. There was no relationship ($P$>0.05) between the prevalence of the GA genotype and the severity of COVID-19. Among C-allele carriers of rs 763780 at IL-17, a strong association was discovered between the frequency of the TC/CC genotype and the severity of COVID-19 risk ($P$=0.0001, $P$<0.05). Both of them increased the risk in all groups, including the mild or moderate group ($P$=0.0001, $P$<0.05), the severe group ($P$=0.0001, $P$<0.05 for TC genotype and $P$=0.04, $P$<0.05 for CC genotype), and critical group ($P$=0.0001, $P$<0.05). According to the results, COVID-19 prognosis and severity were substantially correlated with IL-17 level and two IL-17 SNPs, rs2275913 and rs763780. It demonstrated that the two SNPs might be potential markers for the prediction of COVID-19 risk and development. The different levels of severity also had a non-significant relationship with SNP13 and SNP80 as well as IL-17 levels (ng/L).

**Keywords:** COVID-19, HRM RT-PCR, Interleukin-17, Polymorphism, RT-PCR, SARS-CoV-2, SNP

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## 1. Introduction

The 2019 Coronavirus Disease (COVID-19) is an infectious disease caused by coronavirus 2 (SARS-CoV-2) that produces severe acute respiratory distress syndrome (ARDS). It originally surfaced in December 2019 in Wuhan, China, and since then, has expanded all over the globe (1). This 2019-nCoV is the third and most dangerous human pathogen following a zoonotic transmission epidemic of CoV, SARS-CoV (in 2003), and Middle East respiratory syndrome (MERS)-CoV (in 2012). Positive sense RNA was found, which affects birds and a variety of other creatures, including humans (2). Viral pneumonia and host inflammation characterize each of these diseases, which result in pulmonary edema and a state resembling ARDS (3). Coronaviruses are members of the Coronavirinae subfamily of the Coronaviridae family in the order Nidovirales. In humans and many other animals, it can induce respiratory, digestive, and neurological system problems. The Coronavirus particles are spherical, varying from 80 to 160 mm. The spike (S) protein is coated on the envelope's surface. The S proteins are membrane (M) and envelope (E). A helical nucleocapsid comprises genomic RNA and phosphorylated nucleocapsid (N) protein and can be found inside the envelope (4). The COVID-19 is a pleomorphic or spherical encased particle. The single-stranded (positive-sense) RNA is associated with a nucleoprotein within a matrix protein-based capsid. The envelope is adorned with club-shaped glycoprotein projections. Several coronaviruses have the hemagglutinin-esterase protein (5). Coronaviruses are the RNA viruses with the largest genomes (26.4-31.7 kb) with levels of G+C within the range of 32-43%. A variety of small open reading frames (ORFs, spike, membrane, envelope, nucleocapsid, and ORF1ab) reside downstream of the nucleocapsid gene, between many conserved genes, and in different coronavirus lineages. The unique N-terminal region of the spike protein is a defining feature of the viral genome. The S, E, M, and N are the genes that code for the key structural proteins in all coronaviruses and exist in the 5′–3′ order (6). The COVID-19 patients might present with a variety of symptoms. At least 26 of these symptoms have been found; which is only a small number of its symptoms. Given that general terminology, like neurological and dermatological symptoms, indicate a spectrum of individual reactions, the necessity of stressing the vast number of presentations cannot be emphasized. This might lead to a rise in the number of symptoms and signs. Dyspnea, fever, cough, and headache are non-specific common SARS-CoV-2 symptoms, from asymptomatic patients to those dying of severe pneumonia. The severity of infection may differ and shortness of breath, fever, and cough were the initial symptoms of the condition. The list was expanded to include chills, headache, muscle discomfort, sore throat, and loss of taste or smell (neurological manifestations) by the US Centers for Disease Control and Prevention (7). There are three severity degrees in the 2019 Coronavirus Disease levels. Flu, for example, may occur in the first stage due to viral pneumonia and viral infection which can cause patients to be admitted to the hospital or placed on a ventilator for a lengthy period. The second stage also distinguishes between pulmonary inflammation and coagulopathy, which can happen sequentially but overlap. Finally, the last stage of this disease is fibrosis. In individuals who require mechanical ventilation, two respiratory phenotypes can be distinguished by low and high elastance. The H-type has greater lung edema, resulting in higher lung weight and worse lung compliance (8). Due to the prominent involvement of the respiratory system in suspected COVID-19 cases, a chest computed tomography (CT) scan is strongly advised for initial screening and follow-up. The X-rays of the chest have a limited diagnostic value in the early stages, even though CT scans can be obtained before the beginning of symptoms. Furthermore, when an initial false-negative result utilizing real-time reverse-transcriptase polymerase chain reaction (RT-PCR) was obtained, CT results were diagnostic in a few cases (9). One of the key challenges for the facilitation of public health initiatives is the reliability of biomarker testing. Real-time PCR is commonly utilized to identify causal viruses in respiratory secretions in acute respiratory infections (10). Although coordinated cytokine stimulation is important for the host immune response, impairment of production regulation is linked to immunopathology, which can cause the onset of cytokine storms (11). Cytokines associated with COVID-19 include pro- and anti-inflammatory interleukins (ILs), chemokines, and interferons (12). The IL-17 is produced by T-helper 17 (Th17) cells and its signaling is associated with immune functions of barrier epithelial tissues and host defense against extracellular bacterial and fungal infections (13). The IL-17 includes unique structures, composed of IL-17A and IL-17F (17), and is an inducer of antimicrobial proteins, various chemokines, acute-phase response mediators, and inflammatory functions (14). Recent studies have found increased inflammatory responses in COVID-19 patients due to IL-17 overproduction (Amatya et al., 2017; Orlov et al., 2020). Genetic polymorphisms implicated in disease-origin research also contribute to the prevention of infection transmission and the development of potentially effective therapies. Single nucleotide polymorphisms (SNPs) are a common type of such polymorphisms that are known to play a crucial role in the pathogenicity of a microbial agent, disease immunity, susceptibility, and severity of diseases (AL-Suhail and Ali, 2021). A growing body of research suggests that the severe symptoms of COVID-19 might be caused by genetic differences in genes linked to immunological diseases, infections, and/or cytokine storms (15). Additionally, ARDS patients with genetic variants that have resulted in decreased IL-17 production had a higher 30-day survival rate, compared to others, according to the research performed on IL-17 gene polymorphisms in these patients. However, the lower survival rate was linked to a polymorphism that increased the production of IL-17 (16).

In order to explain the clinical course of COVID-19 infection and the survival from and/or death due to COVID-19 infection, it was postulated that SNPs in the genes encoding for IL-17A and IL-17F might be involved. On chromosome 6p12, the rs2275913 is located 2 KB upstream of the IL17A gene. The promoter region of the IL-17A gene contains the rs2275913 gene, and the A allele of this gene is linked to the promoter activity of the gene. Moreover, the rs763780 is located in exon 1 of the IL17f gene, and the C allele of this gene is linked to the increasing severity of the disease.

## 2. Materials and Methods

### 2.1. Sample collection

This study was performed in Al Yarmok Teaching Hospital, and Dar al-salam and Alshefaa hospitals in Iraq from November 2021 to January 2022. The Ethics Committee of the Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq approved this study (ref no. CSEC/0122/0020). This research included 100 Iraqi patients who tested positive for SARS-CoV based on nasopharyngeal swab samples through real-time reverse transcriptase PCR and were admitted to various isolation wards. Patients with vomiting, diarrhea, weakness, systemic diseases, and lower oxygen saturation were hospitalized. Blood specimens were collected on the second day of admission to the hospital by taking venous blood from each patient 5 ml divided into 2 ml of whole blood were placed into EDTA heparinized tube to test Genomic DNA isolation Kits used in the study included: EasyPure® Genomic DNA Kit TransGen, TransStart® Top Green qPCR Super Mix (biotech, China) and Eva Green® for high-resolution melt (HRM) (Wizbio, Korea) and 3 ml of whole blood in the non-heparinized tube (gel tube) to test human IL-17 level by ELISA KIT.

### 2.2. Detection of SARS-COV-2 infections by real-time polymerase chain reaction

The SARS-COV-2 RNA was detected in the nasopharyngeal swabs using real-time PCR with the PCR Rotor-Gene, Zybio SARS-COV-2 Assay kit (Zybio company, China).

### 2.3. Real-time polymerase chain reaction detection protocol

This product qualitatively detected the RNA of SARS-CoV-2 in the specimen by measuring the change of fluorescence signal intensity during RT-PCR amplification with specific primers and probes against the conserved sequences of N, RdRP, and S genes.

### 2.4. RNA extraction

The RNA was extracted using the Viral Nucleic Acid Extraction Kit (Zybio company, China) and performed according to the instructions of the manufacturer.

### 2.5. Sample preparing

At this stage, 10 µl of the nucleic acid sample of SARS-CoV-2 Negative Control and 10 µl of SARS-CoV-2 Positive Control were added into each PCR reaction tube with filter tips and then covered. Afterward, they were transferred to the amplification detection zone after quick centrifugation to avoid the production of bubbles in tubes.

### 2.6. Polymerase chain reaction amplification

The PCR reaction tubes were put into a fluorescent PCR instrument (ABI 7500, USA) following the protocol tabulated in Table 1.

### 2.7. Human genomic DNA isolation

The protocol supplied by Transgenbiotech Company (*EasyPure*® Genomic DNA Kit) was followed to extract the genomic DNA. The purified DNA was stored at -20°C; subsequently, the extracted DNA was run in agarose gel electrophoresis to confirm its presence and integrity.

### 2.8. High-resolution melt real-time polymerase chain reaction

To detect genetic variation in the IL-17 gene, two SNPs (rs763780, rs2275913) were selected to investigate their association with the positive SARS-CoV-2 patient. The SNPs detection was achieved by using HRM real-time PCR.

### 2.9. Primers

Primers used in this study were designed according to their reference sequence in the National Centre for Biotechnology Information (NCBI) database. The Primer 3 plus, V4, and University Code of Student Conduct (UCSC) programs were used to design the primers which were synthesized by Alpha DNA, SENC (Montreal, Canada) and stored lyophilized. The sequences of primers used in the experiments in this study are summarized in Table 2.

### 2.10. Primer sequence matching

Detection of the primer-prepared gene (IL-17) was assessed for SNPs, which included rs 763780 and rs2275913 primers sequences that were designed according to their reference sequence (rs) in the database of NCBI. The Primer 3plus, V4, and UCSC programs were used to design the primers which were synthesized by Alpha DNA and SENC (Montreal, Canada), and finally, stored lyophilized. The sequences of primers used in the experiments in this study are summarized in Tables 3-8. Primer sequences were matched by the bioinformatics programs NCBI (Table Figure 3-2).

### 2.11. Interleukin-17 assay

The level of (IL-17) was assessed in the sera of patients and controls using ELISA (enzyme-linked immunosorbent assay) technique. The ELISA kit was produced by Sunlong Biotech Company, in China.

### 2.12. Statistical analysis

The Statistical Analysis System application (2012) was employed to determine how various factors affected the research parameters (17). The t-test, least significant difference test, and analysis of variance were used to compare the means significantly. In this study, a meaningful comparison between percentages (0.05 and 0.01 likelihood) was made using the chi-squared test (17). Moreover, Hardy-Weinberg equilibrium was calculated using a web tool (18).

**Table 1.** RT-PCR protocol used to detect SARS-CoV-2.

| Steps | Temperature | Time | cycle |
|---|---|---|---|
| **UNG reaction** | 37°C | 1 min | 1 |
| **Reverse transcription** | 50°C | 5 min | 1 |
| **Initial denaturation** | 95°C | 2 min | 1 |
| **Denaturation** | 95°C | 5 sec | 45 with amplification and fluorescence detection step |
| **Amplification and fluorescence detection** | 60°C | 30 sec | |

**Table 2.** Designed primers used in the study.

| Primer | Sequence (5′→3′ direction) |
|---|---|
| ***IL-17A (HRM)*** | |
| **Forward** | TCTTTAGGAACATGAATTTCTGC |
| **Reverse** | CTCCTTCTGTGGTCACTTACG |
| ***IL-17F (HRM)*** | |
| **Forward** | GCATTTCTACAGCTTCTTCAGC |
| **Reverse** | AAGGTGCTGGTGACTGTTGG |

## 3. Results and Discussion

In this study, COVID-19 infection was studied in 100 Iraqi patients, who were classified based on the severity of their infection (mild, moderate, severe, or critical). This study was performed on males and females between the ages of 20 and 92, from November 2021 to March 2022. The case group consisted of 30 patients who were diagnosed with SARS-CoV-2 based on a nasopharyngeal swab, using real-time PCR, while the control group consisted of 70 healthy individuals. There was a statistical difference ($P$<0.01) between the patients and the control group in terms of IL-17 levels. According to Table 3, the IL-17 levels in the control, mild or moderate, severe, and critical groups were 67.99±2.05 ng/L, 147.60±3.34 ng/L, 218.15±6.27 ng/L, 283.97±5.59 ng/L, respectively. Pulmonary inflammation due to COVID-19 is attributed to the release of specific pro-inflammatory cytokines, such as IL-17, leading to a cytokine storm (19, 20). In this regard, described an increase in the number of Th17 cells in the peripheral blood of patients presenting with SARS-CoV-2 (21). This finding strongly suggests an amplifier role for IL-17A in the inflammatory response, since it triggers the production of other pro-inflammatory cytokines, i.e., IL-1, IL-6, and TNF-α. Moreover, AL-Suhail and Ali (2021) observed a reduction in the population of lymphocytic subsets, together with an elevation in Th17 cell number and cytokines released by Th17 in these patients, consolidating the notion of a severe inflammation-derived immune response. Raised responses of Th17 or improved pathways of IL-17 were noticed in MERS-CoV and SARS-CoV patients as well (21). The death of lymphocytes might happen if inflammatory cytokines are released out of control. Primary data has approved that lymphocyte deficiency could be induced by specific pro-inflammatory cytokines, such as TNFα, IL-6, and others (22). Overall, the response of Th17 participates in the onset of the cytokine storm in pulmonary viral infection, including SARS-CoV-2, leading to tissue damage and probable promotion of pulmonary edema. Consequently, targeting the Th17 pathway may be beneficial to patients presented with a Th17-dominant immune pattern (23). A robust inflammatory response that results in a broad array of inflammatory mediators is a vital component of COVID-19 pathogenesis; one of them is interleukins. Excessive pro-inflammatory cytokine production during illness progression causes a cytokine storm, encouraging the severe development of acute organ damage (24). The fundamental mechanism is that SARS-CoV2 may quickly activate pathogenic T helper cell type 1 (Th1) cells to generate pro-inflammatory cytokines, such as interleukin-6 (IL-6), interleukin-10, and IL-17 (25). reported that the severity of COVID-19 infection is associated with increased concentrations of IL-10 and IL-6 alongside a decline in the cell number of $CD4^{+}$T and $CD8^{+}$T cells. Multiple studies have shown that patients with severe COVID-19 infections had more significant quantities of IL2, IL6, IL 17, and IL 10 than those with mild and moderate COVID-19 infections. Moreover, the COVID-19 patients hospitalized in the Intensive Care Unit developed increased levels of IL-17 in comparison to the control group (26). Therefore, it is necessary to grasp these essential inflammatory cytokines to comprehend the increased mortality in extreme instances caused by a cytokine storm (27). There was a non-significant relationship between SNP80 and IL-17 level (ng/L) with different types of severity. Moreover, there was a non-significant relationship between SNP13 and IL-17 levels (ng/L) with different types of severity ($P$≥0.05). The prevalence of the AA genotype at rs2275913 and the

severity of COVID-19 were significantly correlated (*P*=0.021), while there was no significant correlation between the frequency of the GA genotype and severity of COVID-19 (*P*>0.05). The frequency of the TC/CC genotype at rs 763780 and the severity of COVID-19 were significantly correlated (*P*=0.0001, *P*<0.05). However, there was no significant correlation between the frequency of the TT genotype and the severity of COVID-19 (*P*>0.05) as seen in Tables 4 and 5. The prevalence of the AA genotype at rs2275913 and the severity of COVID-19 were significantly correlated (*P*=0.021, *P*<0.05, and odd ratio=3.9) more risk factors from other. However, no significant correlation was found between the frequency of GA genotype and severity of COVID-19 risk (*P*>0.05, *P*=0.3) among the A-allele carriers of rs2275913 at IL-17 (*P*=0.01, *P*<0.05) and odd ratio=1.8, control and case groups, as seen in Table 6. The mild group had no significant correlation with the genotypes GA and AA (*P*=0.5, *P*=0.8, respectively) (*P*>0.05) as seen in Table 7. There was a significant correlation between the frequency of the AA genotype and the severity of COVID-19, showing an increased risk in the severe group (*P*= 0.005, *P*<0.05, odd ratio=7.0) more risk factors from other. However, no significant correlation was found between the frequency of GA genotype and severity of COVID-19 (*P*=0.19, *P*>0.05) and control group, risk was found among A-allele carriers of rs2275913 at IL-17 (*P*=0.003, *P*<0.05, and odd ratio=2.5) as seen in Table 8. Table 8. Comparison of the genotype and Allele frequencies detected by Hardy-Weinberg equilibrium law of gene polymorphism rs13 between Patient Sever group and Healthy group. The frequency of the AA genotype and the severity of the COVID-19 were significantly correlated in the critical group, showing an increased risk in this group (*P*=0.023, *P*<0.0, and Odd ratio=4.8) more risk factor from other, risk was found among A-allele carriers of rs2275913 at IL-17, (*P*= 0.003, *P*< 0.05, and odd ratio=2.5) as seen in Table 9. The frequency of the TC/CC genotype at rs 763780 and the severity of COVID-19 had a significant correlation (*P*=0.0001, *P*<0.05) with odd ratio values of 11.4 and 17.1, respectively. A higher risk factor from other risks was found among C-allele carriers of rs763780 at IL-17, (*P*=0.0001, *P*< 0.05, and odd ratio=5.0) as seen in Table 10. The frequency of the TC/CC genotype at rs763780 was significantly correlated with the COVID-19 severity (*P*=0.0001, *P*< 0.05) with odd ratio values of 17.7 and 56.8, respectively. more risk factors from other both of them, risk was found among C-allele carriers of rs763780 at IL-17 in the mild group, (*P*=0.0001, *P*< 0.05, and odd ratio=8.2) as seen in Table 11. TC and CC genotypes were significantly correlated among C-allele carriers of the rs 763780 at IL-17, which show an increased risk in the severe group (*P*=0.0001, *P*< 0.05) TC genotype and (*P*=0.04, *P*<0.05) CC genotype, (*P*>0.05) with odd ratio values of 16.6 and 5.7, respectively more risk factor from other both of them, risk was found among C-allele in mild group, (P value = 0.0001,p< 0.05) and Odd ratio= 3.4 as seen in table 12. Among C-allele carriers of rs 763780 at IL-17, a strong link between the frequency of the TC and CC genotypes and the severity of COVID-19 risk was discovered. This increased risk was seen in both the TC and CC critical groups (P value = 0.0001, p< 0.05), Odd ratio = 9.2, and 15.3 more risk factors than others, as seen in Table 13. This study was the first to document the link of IL-17 (rs763780 and rs2275913) polymorphisms with ARDS susceptibility and COVID-19 prognosis. Based on the results, two functional polymorphisms of IL-17, rs2275913 and rs763780, had a relationship with the risk of ARDS and COVID-19 prognosis. These findings suggest that these two genetic variations can serve as promising indicators for COVID-19 risk and prognosis prediction. The clinical syndrome, known as ARDS, has several etiological agents and complex pathogenesis (28). Although the exact pathologic pathways are still unclear, it is well known that ARDS is an inflammatory disease and that immune dysregulation disorder may play a substantial role in the development of inflammation (29). The pro-inflammatory cytokine IL-17 has received much attention. The IL-17 family has several expression forms, including IL-17A-F, among which IL-17A can broadly trigger the inflammatory response. Moreover, it is important in a number of inflammatory disorders, indicating that IL-17 disruption may contribute to the early stages of ARDS development and the increase in COVID-19 severity (30). Additionally, increased levels of IL-17A in the blood and lungs have been linked to ARDS-related organ failure, an increased alveolar neutrophil proportion, and alveolar permeability (31). The IL-17A polymorphisms, such as rs2275913 and rs763780, have influenced the way many inflammatory disorders begin. The IL-17A gene, linked to ARDS risk and outcome, has the variant rs2275913. People with the wild G-allele of rs2275913 had ARDS-susceptibility that was at least as low as those with the mutant A-allele, suggesting that the former mutant A-allele is the pathogenic one (AA genotype) and the later wild G-allele is the protective one (GG, GA genotype). It should be mentioned that the IL-17F gene contains the variant rs763780. The risk and prognosis of ARDS were affected by the SNP of rs763780 (*P*>0.05) (32). Individuals with the wild T-allele had decreased ARDS susceptibility, demonstrating that the former mutant C-allele functioned as the pathogenic one while the latter wild T-allele played the protective role. The mutant C-allele carriers are at increased risk of having the ARDS (TC/CC) genotype (TT genotype). Additionally, the present research showed that IL-17 SNPs can modify IL-17 serum levels. Results showed no correlation between SNPs and blood levels of IL-17, indicating that rs2275913 and rs763780 were active polymorphisms with no ability to affect IL-17 production. Studies were also needed to explain the molecular processes that led to the connection between SNPs and IL-17 expression and the development of ARDS susceptibility and prognosis.

**Table 3.** Comparison between different Types of severity in IL-17.

| Type of severity | Mean ± SE of IL-17 (ng/L) |
|---|---|
| **Control** | 67.99 ±2.05 d |
| **Mild or Moderate** | 147.60 ±3.34 c |
| **Sever** | 218.15 ±6.27 b |
| **Critical** | 283.97 ±5.59 a |
| **LSD value** | 11.476 ** |
| **P-value** | 0.0001 |

This means that having the different letters in the same column differed significantly. ** (P≤0.01).

**Table 4.** Relationship between SNP80 and IL-17 level (ng/L) with different types of severity.

| Genotype / SNP80 | Type of severity | | | |
|---|---|---|---|---|
| | Control | Mild or Moderate | Sever | Critical |
| TT | 63.51 ±2.84 | 132.54 ±9.86 | 237.02 ±18.35 | 303.82 ±20.41 |
| TC | 79.38 ±0.98 | 151.89 ±6.29 | 213.04 ±7.25 | 283.93 ±5.08 |
| CC | 68.16 ±0.00 | 146.40 ±3.32 | 237.24 ±5.80 | 274.13 ±12.41 |
| LSD value | 17.03 NS | 39.74 NS | 42.91 NS | 48.031 NS |
| P-value | 0.079 | 0.337 | 0.511 | 0.381 |

NS: Non-Significantly.

**Table 5.** Relationship between SNP13 and IL-17 level (ng/L) with different types of severity.

| Genotype / SNP13 | Type of severity | | | |
|---|---|---|---|---|
| | Control | Mild or Moderate | Sever | Critical |
| **GG** | 62.09 ±3.08 | 148.93 ±6.19 | 217.51 ±13.64 | 284.56 ±9.24 |
| **GA** | 77.46 ±1.78 | 145.08 ±2.98 | 222.73 ±8.85 | 293.64 ±5.57 |
| **AA** | 61.56 ±0.00 | 155.16 ±0.00 | 211.01 ±10.48 | 266.21 ±15.48 |
| **LSD value** | 18.57 NS | 42.196 NS | 61.02 NS | 49.82 NS |
| **P-value** | 0.081 | 0.659 | 0.703 | 0.423 |

NS: Non-Significantly.

**Table 6.** Comparison of the genotype and Allele frequencies detected by Hardy-Weinberg equilibrium law of gene polymorphism rs13 between Patient group and Healthy group.

| polymorphism rs13 | Frequencies (%) | | P value | Odd ratio (95% CI) |
|---|---|---|---|---|
| | Control group (n=70) | Patients Group (n=100) | | |
| Codominant | | | | |
| GG | 55.7 % (n=39) | 42 % (n=42.0) | --- | 1.00 (Reference) |
| GA | 38.5 % (n=27) | 41.0 % (n=41) | 0.3 | 1.4 (0.7-2.7) |
| AA | 5.8 % (n=4) | % (n=1717.0) | 0.021 | 3.9 (1.2-12.7) |
| Dominant | | | | |
| GG | 55.7 % (n=39) | 42 % (n=42.0) | --- | 1.00 (Reference) |
| GA+AA | 4.3 % (n=31)4 | 58.0 % ( n=58) | 0.079 | 1.7 (0.9-3.2) |
| Recessive | | | | |
| GG+GA | 94.2 % (n=66) | 83.0 % (n=83) | --- | 1.00 (Reference) |
| AA | 5.8 % (n=4) | % (n=1717.0) | 0.03 | 3.3 (1.0-10.5) |
| Allele | | | | |
| G | 75.0 % ( n=105) | 62.5 % ( n=125) | --- | 1.00 (Reference) |
| A | 25.0 % ( n=35) | 37.5 % ( n=75) | 0.01 | 1.8 (1.1-2.9) |

**Table 7.** Comparison of the genotype and Allele frequencies detected by Hardy-Weinberg equilibrium law of gene polymorphism rs13 between Patient Mild group and Healthy group.

| Polymorphism rs13 | Frequencies (%) | | P value | Odd ratio (95% CI) |
|---|---|---|---|---|
| | Control group (n=70) | Patients (Mild) Group (n=34) | | |
| Codominant | | | | |
| GG | 55.7 % (n=39) | 17 % (n=50.0) | --- | 1.00 (Reference) |
| GA | 38.5 % (n=27) | 44.1 % (n=15) | 0.5 | 1.2 (0.5-2.9) |
| AA | 5.8 % (n=4) | % (n=25.9 ) | 0.8 | 1.1 (0.1-6.8) |
| Dominant | | | | |
| GG | 55.7 % (n=39) | 17 % (n=50.0) | --- | 1.00 (Reference) |
| GA+AA | 4.3 % (n=31)4 | 50.0 % ( n=17) | 0.5 | 1.2 (0.5-2.8) |
| Recessive | | | | |
| GG+GA | 94.2 % (n=66) | 94.1 % (n=32) | --- | 1.00 (Reference) |
| AA | 5.8 % (n=4) | % (n=25.9 ) | 0.9 | 1.0 (0.1-5.9) |
| Allele | | | | |
| G | 75.0 % ( n=105) | 69.1 % ( n=47) | --- | 1.00 (Reference) |
| A | 25.0 % ( n=35) | 30.9 % ( n=19) | 0.5 | 1.2 (0.6-2.3) |

**Table 8.** Comparison of the genotype and Allele frequencies detected by Hardy-Weinberg equilibrium law of gene polymorphism rs13 between Patient Sever group and Healthy group.

| Polymorphism rs13 | Frequencies (%) | | P value | Odd ratio (95% CI) |
|---|---|---|---|---|
| | Control group (n=70) | Patients (Sever ) Group (n=33) | | |
| Codominant | | | | |
| GG | 55.7 % (n=39) | 11 % (n=33.3) | --- | 1.00 (Reference) |
| GA | 38.5 % (n=27) | 42.4 % (n=14) | 0.19 | 1.8 (0.7-4.6) |
| AA | 5.8 % (n=4) | % (n=824.3 ) | 0.005 | 7.0 (0.1-2.8) |
| Dominant | | | | |
| GG | 55.7 % (n=39) | 11 % (n=33.3) | --- | 1.00 (Reference) |
| GA+AA | 4.3 % (n=31)4 | 67.7 % ( n=22) | 0.03 | 2.5 (1.0-5.9) |
| Recessive | | | | |
| GG+GA | 94.2 % (n=66) | 76.7 % (n=25) | --- | 1.00 (Reference) |
| AA | 5.8 % (n=4) | % (n=824.3 ) | 0.011 | 5.2 (1.4-19.0) |
| Allele | | | | |
| G | 75.0 % ( n=105) | 54.5 % ( n=36) | --- | 1.00 (Reference) |
| A | 25.0 % ( n=35) | 45.5 % ( n=30) | 0.003 | 2.5 (1.3-4.6) |

**Table 9.** Comparison of the genotype and Allele frequencies detected by Hardy-Weinberg equilibrium law of gene polymorphism rs13 between Critical Patient group and Healthy group.

| Polymorphism rs13 | Frequencies (%) | | P value | Odd ratio (95% CI) |
|---|---|---|---|---|
| | Control group (n=70) | Patients (Crit.) Group (n=33) | | |
| Codominant | | | | |
| GG | 55.7 % (n=39) | 14 % (n=42.4) | --- | 1.00 (Reference) |
| GA | 38.5 % (n=27) | 36.3 % (n=12) | 0.2 | 1.2 (0.4-3.0) |
| AA | 5.8 % (n=4) | % (n=721.3 ) | 0.023 | 4.8 (0.1-1.9) |
| Dominant | | | | |
| GG | 55.7 % (n=39) | 14) % (n=42.4 | --- | 1.00 (Reference) |
| GA+AA | 4.3 % (n=31)4 | 57.6 % ( n=19) | 0.2 | 1.7 (0.7-3.9) |
| Recessive | | | | |
| GG+GA | 94.2 % (n=66) | 78.7 % (n=26) | --- | 1.00 (Reference) |
| AA | 5.8 % (n=4) | % (n=721.3 ) | 0.02 | 4.4 (1.1-16.4) |
| Allele | | | | |
| G | 75.0 % ( n=105) | 54.5 % ( n=36) | --- | 1.00 (Reference) |
| A | 25.0 % ( n=35) | 45.5 % ( n=30) | 0.003 | 2.5 (1.3-4.6) |

**Table 10.** Comparison of the genotype and Allele frequencies detected by Hardy-Weinberg equilibrium law of gene polymorphism rs80 between Patient group and Healthy group

| Polymorphism rs80 | Frequencies (%) | | P value | Odd ratio (95% CI) |
|---|---|---|---|---|
| | Patients Group (n=) | Control group (n=) | | |
| Codominant | | | | |
| TT | 13.0 % (n=13) | 65.7 % (n=46) | --- | 1.00 (Reference) |
| TC | % (n=5858.0) | 25.7 % (n=18) | 0.0001 | 11.4 (0.5-25.6) |
| CC | .0 % (n=2929) | 8.6 % (n=6) | 0.0001 | 17.1 (0.5-5.0) |
| Dominant | | | | |
| TT | 13.0 % (n=13) | 65.7 % (n=46) | --- | 1.00 (Reference) |
| TC+CC | % (n=8787.0) | 34.3 % (n=24) | 0.0001 | 12.8 (0.5-2.7) |
| Recessive | | | | |
| TT+TC | 71.0 % (n=71) | 64 % (n=91.4) | --- | 1.00 (Reference) |
| CC | .0 % (n=2929) | 8.6 % (n=6) | 0.0001 | 4.3 (1.6-11.1) |
| Allele | | | | |
| T | 42.0 % ( n=84) | 91.7 % ( n=110) | --- | 1.00 (Reference) |
| C | 58.0 % ( n=116) | 8.3 % ( n=30) | 0.0001 | 5.0 (3.0-8.2) |

**Table 11.** Comparison of the genotype and Allele frequencies detected by Hardy-Weinberg equilibrium law of gene polymorphism rs80 between Patient Mild group and Healthy group.

| Polymorphism rs80 | Frequencies (%) | | P value | Odd ratio (95% CI) |
|---|---|---|---|---|
| | Control group (n=) | Patients Mild Group (n=) | | |
| Codominant | | | | |
| TT | 65.7 % (n=46) | 3 % (n=8.8) | --- | 1.00 (Reference) |
| TC | 25.7 % (n=18) | 44.1 % (n=15) | 0.0001 | 17.7 (0.4-6.8) |
| CC | 8.6 % (n=6) | % (n=1647.1) | 0.0001 | 56.8 (0.1-25.2) |
| Dominant | | | | |
| TT | 65.7 % (n=46) | 3 % (n=8.8) | --- | 1.00 (Reference) |
| TC+CC | 34.3 % (n=24) | 91.2 % ( n=31) | 0.0001 | 19.8 (0.5-7.1) |
| Recessive | | | | |
| TT+TC | 64) % (n=91.4 | 62.9 % (n=18) | --- | 1.00 (Reference) |
| CC | 8.6 % (n=6) | % (n=1647.1) | 0.0001 | 9.4 (0.3-2.7) |
| Allele | | | | |
| T | 42.0 % ( n=84) | 30.0 % ( n=21) | --- | 1.00 (Reference) |
| C | 58.0 % ( n=116) | 70.0 % ( n=47) | 0.0001 | 8.2 (0.4-1.5) |

**Table 12.** Comparison of the genotype and Allele frequencies detected by Hardy-Weinberg equilibrium law of gene polymorphism rs80 between Patient Sever group and Healthy group.

| Polymorphism rs80 | Frequencies (%) | | P value | Odd ratio (95% CI) |
|---|---|---|---|---|
| | Control group (n=) | Patients Sever Group (n=33) | | |
| Codominant | | | | |
| TT | 65.7 % (n=46) | 4 % (n=12.1) | --- | 1.00 (Reference) |
| TC | 25.7 % (n=18) | 26 % (n=78.8) | 0.0001 | 16.6 (0.5-5.4) |
| CC | 8.6 % (n=6) | % (n=39.1) | 0.04 | 5.7 (0.1-3.2) |
| Dominant | | | | |
| TT | 65.7 % (n=46) | 4 % (n=12.1) | --- | 1.00 (Reference) |
| TC+CC | 34.3 % (n=24) | 87.9 % ( n=29) | 0.0001 | 13.8 (0.4-4.4) |
| Recessive | | | | |
| TT+TC | 64 % (n=91.4) | 90.9 % (n=30) | --- | 1.00 (Reference) |
| CC | 8.6 % (n=6) | % (n=39.1) | 0.9 | 1.0 (0.2-4.5) |
| Allele | | | | |
| T | 42.0 % ( n=110) | 51.5 % ( n=34) | --- | 1.00 (Reference) |
| C | 58.0 % ( n=30) | 48.5 % ( n=32) | 0.0001 | 3.4 (1.8-6.4) |

**Table 13.** Comparison of the genotype and Allele frequencies detected by Hardy-Weinberg equilibrium law of gene polymorphism rs80 between Patient Crit. group and Healthy group.

| Polymorphism rs80 | Frequencies (%) | | P value | Odd ratio (95% CI) |
|---|---|---|---|---|
| | Control group (n=) | Patients Crit. Group (n=33) | | |
| Codominant | | | | |
| TT | 65.7 % (n=46) | 5 % (n=15.2) | --- | 1.00 (Reference) |
| TC | 25.7 % (n=18) | 18) % (n=54.5 | 0.0001 | 9.2 (0.2-2.8) |
| CC | 8.6 % (n=6) | % (n=1030.3) | 0.0001 | 15.3 (0.3-6.0) |
| Dominant | | | | |
| TT | 65.7 % (n=46) | 5 % (n=15.2) | --- | 1.00 (Reference) |
| TC+CC | 34.3 % (n=24) | 84.8 % ( n=28) | 0.0001 | 10.7 (0.3-3.1) |
| Recessive | | | | |
| TT+TC | 64) % (n=91.4 | 69.7 % (n=23) | --- | 1.00 (Reference) |
| CC | 8.6 % (n=6) | % (n=1030.3) | 0.007 | 4.6 (1.5-14.1) |
| Allele | | | | |
| T | 42.0 % ( n=110) | 42.5 % ( n=28) | --- | 1.00 (Reference) |
| C | 58.0 % ( n=30) | 57.5 % ( n=38) | 0.0001 | 4.9 (2.6-9.3) |

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**Authors' Contribution**
Study concept and design: Z. AA, L. FA.
Acquisition of data: Z. AA, L. FA.
Analysis and interpretation of data: Z. AA, L. FA.
Drafting of the manuscript: Z. AA, L. FA.
Critical revision of the manuscript for important intellectual content: Z. AA, L. FA.
Statistical analysis: Z. AA, L. FA.
Administrative, technical, and material support: Z. AA, L. FA.

**Ethics**
We hereby declare all ethical standards have been respected in preparation of the submitted article.

**Conflict of Interest**
The authors declare that they have no conflict of interest.

**Data Availability**
The data that support the findings of this study are available on request from the corresponding author.

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