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Original Article

Association of Polymorphisms in IL-10, TGF-β1, IFN-γ, and TNF-α Genes with the Susceptibility to Chronic Obstructive Pulmonary Disease in Kerman, Iran

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Abstract

Background: One of the principal cigarette smokes (CS) mediated diseases is chronic obstructive pulmonary disease (COPD).

Methods: In the current case-control study, the relationship between the polymorphisms of interleukin-10 (*IL-10*), transforming growth factor-β_1 (*TGF-β_1*) codon 10, *TGF-β_1* codon 25, interferon-γ (*IFN-γ*), and tumor necrosis factor-α (*TNF-α*) in 213 individuals with COPD and susceptibility to the disease, with 100 healthy age and gender-matched people as a control group, was investigated using PCR-ARMS (polymerase chain reaction-amplification refractory mutation system). Moreover, the combination of the polymorphisms of *TGF-β_1* codon 10.25 regarding this susceptibility was studied in the same condition.

Results: There was a significant difference between polymorphism of *TGF-β_1* codon 10 (+869 T/C), codon 25 (G+915C), and susceptibility to the disease (OR=0.50; (95 %CI=0.24-1.07, p=0.05), OR_{CC}=5.31; (95% CI: 1.22-23.2); p=0.02), thus polymorphism of IL-10 and *TGF-β_1* increased the risk of susceptibility to COPD but the polymorphisms of *TNF-α* (G-308A) and *IFN-γ* (+847 T/A) did not show any association.

Conclusion: All in all, it is recommended that the patients carrying the above-said genotypes should be paid proper attention, especially those who are exposed to chemicals at their workplaces, pollution, and cigarette smoke.

Keywords: Chronic obstructive pulmonary disease, Susceptibility, Gene polymorphism

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Introduction

Chronic obstructive pulmonary disease (COPD) is one of the most common chronic and complex diseases and is estimated, by WHO, to reach the sixth to the third cause of death by 2020 in the world (1). This disease causes mortality among patients, which, in turn, imposes high costs on health systems in countries (2). Evidence suggests that COPD is an inflammatory disease, and the oxidative stress created by oxidant compounds such as cigarette smoke in inhaled air plays an important role in creating this inflammation (3,4). Various studies have shown that the production and activity of inflammatory factors are different in people, which results from polymorphic genes involved in their production (5-7).

Interleukin-10 (*IL-10*), as a polymorphic gene, is an anti-inflammatory factor. It increases the survival of B-Cell by inhibiting apoptosis, located on chromosome 1 (1q31–q32), and is composed of five exons and four introns (8). The most important single nucleotide polymorphism (SNP) of *IL-10* is (G-1082A at rs1800896), this gene has a protective role, and the GG genotype is linked to higher *IL-10* production (9). Tumor necrosis factor-α (*TNF-α*) and transforming growth factor-β_1 (*TGF-β_1*) are involved in the chain of events leading to lung fibrosis (10). *TNF-α* is also a fundamental potent pro-inflammatory factor mapped on the short arm of the human sixth chromosome (6p21.31), including macrophages in the lung (11,12). Among the SNPs of *TNF-α*, (-308G/A at rs1800629) is the one that has been paid a lot of attention in inflammatory diseases (13). This polymorphism in the gene promoter

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increases the transcription of the gene and production of *TNF-α* six to seven times. Individuals homozygous for the G allele are considered low manufacturers of *TNF-α* (*TNF-α* Lo), and those with genotype A as high manufacturers of *TNF-α* (*TNF-α* Hi) (14). *TGF-β_1* is a factor with different effects on the propagation and differentiation of inflammatory cells. The human *TGF-β1* gene is mapped in chromosome 19q13.1–3 (15). Although *TGF-β_1* is an anti-inflammatory factor, it can cause fibrosis (16). In the lungs, the secretion of *TGF-β_1* by bronchial epithelial cells stimulates fibroblast propagation (17). Moreover, *TGF-β_1* production is under genetic control, and several polymorphisms in the *TGF-β_1* gene have been identified (18), of which two of the most important SNPs were examined in the present study: the + 869 T/C (Leu/ Pro) at codon 10 (rs1982073) and + 915 G/C (Arg/ Pro) at codon 25 (rs1800471). For TGF-β1 at codon 10 individuals homozygous for the C allele are considered low manufacturers and those with genotype T as high manufacturers of TGF-β1 at codon 10 and for TGF-β1 at codon 25 individuals homozygous for the C allele are considered low manufacturers and those with genotype G as high manufacturers of TGF-β1 at codon 25. Interferon gamma (*IFN-γ*) has been demonstrated to play a key role in pathogen clearance and tumor surveillance (19). *IFN-γ*, a pro-inflammatory factor produced by activated CD4 + T cells and NK cells, defines the development of Th1 response and promotes cell-mediated immunity. The gene encoding IFN-γ is located on chromosome 12q24 and has four exons spanning around 5.4 kb (20-23). It has been reported that a novel SNP, T to A, at the 5' end of the CA repeat region in the first intron of the human *IFN-γ* gene (+ 874T/A at rs62559044) is considered the most important gene. This SNP in the first intron of the *IFN-γ* gene + 874T/A can putatively influence the secretion of *IFN-γ*. The analysis of the biological role of this SNP suggested that + 874A carriers were low *IFN-γ* producers (24). Therefore, the T to A polymorphism could directly influence the level of *IFN-γ* production (25).

According to the nature of the gene coding, these cytokines, such as *IL-10, TGF-β_1, IFN-γ, TNF-α,* and the results obtained from different populations cannot be applied to other populations. The current study aimed to investigate the relationship between these variants and COPD susceptibility in Iranian patients.

Material and Methods

Study population

In this case-control study, 100 healthy subjects (control group) and 213 patients with COPD (study group) were selected from Afzalipoor hospital and Besat clinic in Kerman. Participants were selected by convenience sampling. The number of samples was determined according to a previous similar study, in which the eligibility criteria for the cases were as follows (26): male, COPD diagnosis by pulmonologist based on GOLD (global initiative for obstructive lung disease) guideline and confirmed by performing two steps spirometry (Table 1). The inclusion criteria for healthy subjects were: male, smoking (the same number of cigarettes for more than 10 years), no symptoms of pulmonary involvement, and filling out the informed consent form. Then, the research objectives, study phases, and follow-up process were explained to all patients (27). The demographic data, general health conditions, lifestyle, and smoking habits were registered through a questionnaire. The written informed consent form was obtained from the subjects after describing the aim of the study.

Sampling

For genotyping, 5 mL of blood was drawn into an EDTA tube, and after centrifugation, stored at -70°C until DNA extraction was carried out. DNA was extracted using a standard salt precipitation technique (28) and quantified by measuring the optical density (OD) at λ = 260 nm. The 260/280 ratio was used to assess the quality of DNA, being close to 1.8. The polymerase chain reaction-amplification refractory mutation system (PCR-ARMS) method was used for genotyping. Then demographic data and disease susceptibility were recorded according to GOLD criteria and based on the result of spirometry in the previous year.

Detection and genotyping

IL-10 -1082 (G/A), *TGF-β_1* codon 10 + 869 (T/C), *TGF-β1* codon 25 + 915 (G/C), *IFN-γ* + 874 (T/A), and *TNF-α* -308 (G /A) SNPs were genotyped by ARMS-PCR technique using specific primers as described (9-31). ARMS method is an application of PCR in which DNA is amplified by allele-specific primers. This is due to the absence of 3' to 5' exonuclease checking activity of Taq polymerase. High-reliability DNA polymerases, that have this activity, cannot be used in ARMS. It is an extremely valuable method for the identification of point mutations or polymorphisms. Technical factors for genotyping these SNPs, which could affect sensitivity and specificity, included replications and sample retesting. As a result, all relevant measurements were made to ensure the study's

Table 1. Global Initiative for chronic obstructive lung disease (GOLD) criteria for the classification of COPD

Stage of COPD	COPD Severity	Spirometry results based on post-bronchodilator FEV_1
I	Mild	$FEV_1/FVC < 70$ $FEV_1 \geq 80\%$ predicted
II	Moderate	$FEV_1/FVC < 70$ $50\% \leq FEV_1 < 80\%$ predicted
III	Severe	$FEV_1/FVC < 70$ $30\% \leq FEV_1 < 50\%$ predicted
IV	Very severe	$FEV_1/FVC < 70$ $FEV_1 < 30\%$

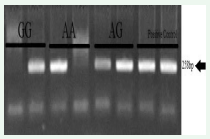
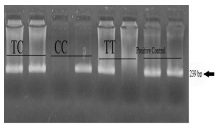
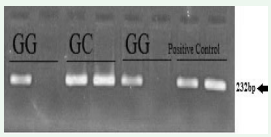
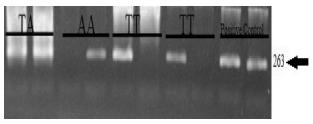
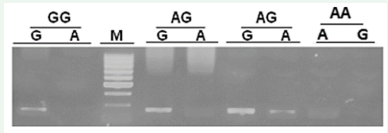
FEV_1: forced expiratory volume for 1 second; FVC: forced vital capacity

technical integrity. Aliquots of reagents were made, and each aliquot was only used once. For the PCR experiment, sterile microcentrifuge tubes and PCR tubes were employed. To prevent amplicon cross-contamination, reagent preparation, DNA extraction, DNA amplification, and detection were carried out in different spaces. The fragments and products of PCR were analysed on ethidium bromide-stained agarose gel, and visualized under ultraviolet light (Table 2). Finally, the resolute patterns of bands in the gel electrophoresis were obtained for the genotypes of each sample. The primer sequences of genes and demographic data for COPD and the healthy controls are shown in Tables 3 and 4.

Statistical analysis

Data analysis was done using IBM SPSS Statistics version 23. For the comparison of continuous variables, first, we checked the assumption that they were normally distributed. Logistic regression was used to determine the independent effect of each polymorphism on COPD risk. Additionally, the 95% confidence interval (CI) and odds ratio (OR) were calculated. Clinical and demographic differences between the two groups were tested using an independent student's *t* test or Fisher's exact test whenever

Table 2. Agarose gel electrophoresis of ARMS PCR products of the *IL-10, TGF-β1, IFN-γ*, and *TNF-α* genes

Gene	Genotyping pattern	Fragments size (bp)
IL10-1082G/A		258
TGF-β1 10+869T/C		239
TGF-β 25+915 G/C		232
IFN-γ+847T/A		263
*TNF-α*G-308A		184

Lane M: DNA molecular weight standard (DNA Marker 100 bp [Takara Biotechnology, Dalian, China])

Table 3. Primer sequences and annealing temperature of suggested genes

Gene	SNP	The sequence of forward (F) and reverse (R) primers	Annealing temperature (°C)
IL-10 (-1082G/A)	rs1800896	FG: 5′CTA CTA AGG CTT CTT TGG GAG-3′ FA: 5′CTA CTA AGG CTT CTT TGG GAA-3′ Rv.Com: 5′CAG TGC CAA CTG AGA ATT TGG-3′	62°C
TGF-β1 10 (+869T/C)	rs1982073	FT: 5′TCC GTG GGA TAC TGA GAC AC-3′ RT: 5′GCA GCG GTA GCA GCA GCA-3′ RC: 5′GCA GCG GTA GCA GCA GCG-3′	62°C
TGF-β 25 (+915 G/C)	rs1800471	FC: 5′GTG CTG ACG CCT GGC CC-3′ FG: 5′GTG CTG ACG CCT GGC CG-3′ Rv.Com: 5′GGC TCC GGT TCT GCA CTC-3′	62°C
IFN-γ (+847T/A)	rs62559044	FA: 5′TTC TTA CAA CAC AAA ATC AAA TCA-3′ FT: 5′TTCC TTA CAA CAC AAA ATC AAA TCT-3′ Rv.Com: 5′TCA ACA AAG CTG ATA CTC CA -3′	62°C
TNF-α (G-308A)	rs1800629	FG: 5′-ATAGGTTTTGAGGGGCATGG-3′ FA: 5′-ATAGGTTTTGAGGGGCATGA-3′ Rv.Com: 5′-TCTCGGTTTCTTCTCCATCG-3′	68°C

appropriate. The *P* value < *0.05* was considered significant.

Results

Evaluation of gene polymorphisms frequency in the study and control groups

Evaluation of IL-10 gene polymorphisms

The frequency of *IL-10* gene polymorphisms including A allele carriers and wild-type genotype (GG) in the two groups was listed in Table 5. According to the results, there was a significant relationship between *IL-10* gene polymorphisms and the disease ($OR_{A\ carrier}$ = 0.44; 95% CI: 0.24-0.80; *P* = 0.00).

The frequency of wild-type (GG), the heterozygous genotype (GA), and the homozygous genotype (AA) in the codominant *IL-10* gene were reported in Table 5, indicating a significant relationship between the GA genotype and the disease. A comparison of the G allele between the controls and COPD cases showed that the genotypes carrying the G allele are more common in the study group.

Table 4. Demographic and laboratory characteristics for COPD and the healthy controls

Characteristics	COPD No. (%)	Controls No. (%)
Age (y)[a]	60.38 ± 29.62	56.4 ± 28.6
Gender		
Male	163 (76.5)	100 (100)
Female	50 (23.5)	0 (0)
Smoking		
Former	42 (19.70)	40 (41.7)
Current	130 (61)	50 (48.5)
Never	41 (19.2)	10 (9.7)
Body mass index (kg/m²)	22.73 ± 7.47	27.33 ± 3.97
Education		
Educated	103 (48.6)	98 (95.1)
Uneducated	109 (51.4)	2 (4.9)
Job		
Office jobs	131 (61.5)	88 (85.4)
Workplaces with chemicals	38 (17.8)	2 (2.9)
Workplaces with suspended particles	44 (20.7)	10 (11.7)

[a] Data are shown as mean ± SD.

Evaluation of TGF-β_1 codon10 gene polymorphisms

The gene polymorphism frequency of the C allele carriers for *TGF-β_1* codon10 was shown in Table 6 for case and control groups. Based on the results, there was not a significant relationship between TGF-β1 codon10 gene polymorphisms and the disease ($OR_{C\ carrier}$ = 0.71; 95% CI: 0.41-1.24; *P* = 0.28).

According to the results in Table 6, Wild-type (TT) and heterozygous (GC) genotypes in the codominant *TGF-β_1* codon 10 gene did not show any association, but there was a significant relation between the homozygous genotype (CC) of the two groups (OR_{TC} = 0.78; 95% CI: 0.44-1.40; *P* = 0.40 and OR_{CC} = 0.50; 95% CI: 0.24-1.07; *P* = 0.05).

Evaluation of TGF-β_1 codon 25 gene polymorphisms

Table 7 shows the frequency of C allele carriers and Wild-type genotypes (GG) of *TGF-β_1* codon 25 gene polymorphisms in the case and control groups. The results indicated no significant relationship between *TGF-β_1* codon 25 gene polymorphisms and the disease ($OR_{C\ carrier}$ = 0.71; 95% CI: 0.41-1.24; *P* = 0.28).

The frequency of wild-type (TT) and heterozygous

Table 5. Comparison of the frequency of *IL-10* (-1082G/A) gene polymorphisms in case and control groups

IL10-1082G/A Genotype*	COPD cases n (%)	Controls n (%)	OR (95% CI)	[a]$OR_{adj.}$ (95% CI)
Dominant GG	66 (31)	14 (17)	1.00 (Ref.)	1.00 (Ref.)
GA + AA	147 (69)	86 (84)	0.44 (0.24-0.80)[b]	0.46 (0.22-0.97)
Codominant GG	66 (30)	14 (16)	1.00 (Ref.)	1.00 (Ref.)
GA	98 (46)	65 (63)	0.39 (0.20-0.72)[b]	0.43 (0.20-0.95)
AA	49 (23)	21 (29)	0.60 (0.29-1.25)	0.53 (0.21-1.34)

[a] Multivariate analysis of the adjusted odds ratio and 95 % confidence interval. The *P* value was estimated in a logistic regression model after considering sex, age, BMI, and cigarette smoking status.
[b] *P* < 0.001.

Table 6. Comparison of the frequency of TGF-β1 Codon 10 (+869T/C) polymorphisms in the case and control groups

TGF-β1 10 + 869T/C Genotype*	COPD cases n (%)	Controls n (%)	OR (95% CI)	[a]$OR_{adj.}$ (95% CI)
Dominant TT	61 (29)	20 (22)	1.00 (Ref.)	1.00 (Ref.)
TC + CC	152 (71)	80 (78)	0.71 (0.41-1.24)	0.68 (0.34-1.37)
Codominant TT	61 (29)	20 (22)	1.00 (Ref.)	1.00 (Ref.)
TC	125 (59)	60 (58)	0.78 (0.44-1.40)	0.73 (0.35-1.50)
CC	27 (13)	20 (20)	0.50 (0.24-1.07)	0.54 (0.21-1.40)

**P < 0.05*
[a] Multivariate analysis of the adjusted odds ratio and 95% confidence interval. The *P*-value was estimated in a logistic regression model after considering sex, age, BMI, and cigarette smoking status.

(GC) genotypes in codominant *TGF-β*$_1$ codon 25 genes (genotype) revealed no significant relationship between the genotype of the two groups, but there was a significant relationship between the homozygous genotype (CC) of the control and study groups (OR_{GC} = 0.76; 95% CI: 0.38-1.50; *P* = 0.43 and OR_{CC} = 5.31; 95% CI: 1.22-23.2; *P* = 0.02).

Evaluation of TGF-β1 codon 10.25 gene polymorphisms

The frequency of TT/GG and the rest of the genotypes in the *TGF- β1* C10.25 genes are shown in Table 8 for the two groups. Based on the results, there was no significant relationship between *TGF-β1* C10.25 gene polymorphisms and the disease ($OR_{C\ carrier}$ = 0.78; 95% CI: 0.43-1.40; *P* = 0.46).

Evaluation of IFN-γ gene polymorphisms

The frequency of the A allele carriers and wild-type (TT) genotype for *IFN-γ* gene polymorphisms revealed that there was no significant correlation between the study and control groups ($OR_{A\ carrier}$ = 0.69; 95% CI: 0.37-1.26; *P* = 0.30). The frequency of wild-type (TT), heterozygous (TA), and homozygous (AA) genotypes in the codominant *IFN-γ* gene, as shown in Table 9, indicated no significant relationship between the genotype of controls and the case group (OR_{TA} = 0.53; 95% CI: 0.28-1.02; *P* = 0.05 and OR_{AA} = 0.90; 95% CI: 0.46-1.80; *P* = 0.77).

Evaluation of TNF-α gene polymorphisms

Table 10 shows that there was no significant relationship between the genotype of the two groups.

Discussion

Since genetic agents are proposed as risk factors for COPD, many studies have examined the role of genetic polymorphisms and various diseases, including respiratory

Table 7. Comparison of frequency of *TGF-β1* codon 25 (+915 G/C) polymorphisms in the case and control groups

TGF-β 25+915 G/C Genotype*	COPD cases No. (%)	Controls No. (%)	OR (95% CI)	[a]$OR_{adj.}$ (95%CI)
Dominant GG	168 (79)	82 (83)	1.00 (Ref.)	1.00 (Ref.)
GC+CC	45 (21)	18 (18)	1.26 (0.69-2.32)	0.89 (0.4-1.99)
Codominant GG	168 (79)	82 (83)	1.00 (Ref.)	1.00 (Ref.)
GC	24 (11)	16 (16)	0.76 (0.38-1.50)	0.28 (0.10-0.77)
CC	21 (10)	2 (2)	5.31 (1.22-23.2)*	11.4 (1.81-71.85)

*$P < 0.05$.
[a] Multivariate analysis of the adjusted odds ratio and 95% confidence interval. The *P* value was estimated in a logistic regression model after considering sex, age, BMI, and cigarette smoking status.

Table 8. Comparison of frequency of *TGF-β1* codon 10.25 polymorphisms in the case and control groups

TGF-β C10.25 Genotype*	COPD cases No. (%)	Controls No. (%)	OR (95% CI)	[a]$OR_{adj.}$ (95% CI)
TT+GG	48 (23)	16 (18)	1.00 (Ref.)	1.00 (Ref.)
The rest	165 (78)	84 (82)	0.78 (0.43-1.40)	0.78 (0.36-1.67)

[a] Multivariate analysis of the adjusted odds ratio and 95% confidence interval. The *P-value* was estimated in a logistic regression model after considering sex, age, BMI, and cigarette smoking status.
Genotype*: TGF-β1 C10.25: TT+GG=0 The Rest=1,

Table 9. Comparison of frequency of *IFN-γ* (+874T/A) polymorphisms in the case and control groups

IFN-γ+847T/A Genotype*	COPD cases No. (%)	Controls No. (%)	OR (95% CI)	[a]$OR_{adj.}$ (95 %CI)
Dominant TT	48 (23)	15 (17)	1.00 (Ref.)	1.00 (Ref.)
TA+AA	165 (78)	85 (83)	0.69 (0.37-1.26)	0.55 (0.25-1.24)
Codominant TT	48 (23)	15 (17)	1.00 (Ref.)	1.00 (Ref.)
TA	78 (37)	52 (51)	0.53 (0.28-1.02)	0.42 (0.18-1.01)
AA	87(40%)	34(33%)	0.90(0.46-1.80)	0.74(0.30-1.78)

[a] Multivariate analysis of the adjusted odds ratio and 95% confidence interval. The *P* value was estimated in a logistic regression model after considering sex, age, BMI, and cigarette smoking status.

Table 10. Comparison of frequency of *TNF-α* (-308A/G) polymorphisms in the two groups

TNF-α G-308A genotype	COPD cases No. (%)	Controls No. (%)	OR (95% CI)	*P* value
Dominant GG	176 (82.6)	85 (85.4)	1.00 (Ref.)	ns
GA+AA	37 (17.4)	15 (14.6)	0.81 (0.42-1.5)	ns

ns: Not statistically significant.
The *P*-value was estimated in a logistic regression model after considering sex, age, BMI, and cigarette smoking status. Genotype: TNF-α=TT: Wilde type, TA: Heterozygote, AA: Homozygote.

diseases. However, sometimes conflicting results have been achieved. On the other hand, investigating the effects of polymorphisms of *TNF-α, IL-10, TGF-β_1* codon 10, *TGF-β_1* codon 25, and *IFN-γ* on COPD has not been done in Iran. Accordingly, in the present study, the relationship and susceptibility between COPD and polymorphisms of *TNF-α, IL-10, TGF-β_1* codon 10, *TGF-β_1* codon 25, and *IFN-γ* were investigated.

IL-10 is an anti-inflammatory factor (32). The results of the current study showed that there was a significant relationship between *IL-10* gene polymorphisms and the disease. The existence of one or two A alleles increases the anti-inflammatory factor production in the IL-10 G-1082A polymorphism. Since the heterozygous genotype (GA) was a high producer, polymorphism in COPD was higher than in the control group (84% vs. 69%). As the frequency of the AA genotype in our population was very low, and no significant difference was found among GA, AA, and anti-inflammatory activity, these two groups were merged (Table 5). In the current study, A allele carriers were less susceptible to COPD, and this significance remained despite the presence of confounding factors, which means this allele has a protective role. In other words, the risk of COPD in individuals with the GA genotype was 0.44 times more than in normal people. These results are incompatible with the result achieved by Huang et al (33) reporting that the *IL-10* genotypes are associated with COPD, and a significant relationship between the IL-10 gene polymorphism and the disease was reported by Sangil et al (34). In 2015, Larocca et al reported that *IL-10* (-1082G/A) genotypes were associated with COPD (35).

The *TGF-β_1* has various effects on cell proliferation, differentiation, and inflammation; it also has anti-inflammatory properties but can improve pulmonary fibrosis (36). The existence of one or two C alleles provokes the anti-inflammatory cytokine production in the *TGF-β_1* codon 10 (T + 869C) polymorphism. This polymorphism in the control group was higher than in the COPD group (78% vs. 71%, Table 6). In the current study, C allele carriers were less likely to develop COPD, and there was also a trend relationship in the presence of confounding factors, demonstrating the protective role of *TGF-β_1* codon 10 (T + 869 C). In other words, individuals with the CT genotype were likely to develop COPD 0.7 times more than normal people. In this study, there was a possible significant relationship found between *TGF-β_1* gene polymorphisms and COPD. Liao et al reported that the *TGF-β_1* polymorphisms were not associated with COPD risk (37). In addition, studies by Gong et al and Zhang et al could not find any association between *TGF-B_1* rs1800470 polymorphism and COPD (38,39).

The presence of one or two C alleles enhances the anti-inflammatory cytokine production in the *TGF-β_1* codon 25 (G + 915C) polymorphism. This polymorphism in the control group was lower than that in COPD patients (Table 7). In the present study, C allele carriers were more susceptible to COPD even in the presence of confounding factors. In other words, the risk of COPD in the individuals with the GC genotype was 1.26 times more compared to normal people, depicting codon 25 of the *TGF-β_1* genotype as a risk factor in susceptibility to COPD. In line with our results, Celedón et al (40) and Ogawa et al (41) reported the *TGF-β_1* genotypes were associated with COPD, which was also congruent with the results obtained by Ito et al (42).

The genotype frequency in *TGF-β_1* codon 10.25 in the patient group was 23%, while it was 18% in the control group. The frequency of the rest of the genotypes in the patient group was 78%, whereas it was 83% in the control group. According to the results, there was no significant relationship between *TGF-β_1* C10.25 gene polymorphisms and the disease ($OR_{C\ carrier}$ = 0.78; 95% CI: 0.43-1.40; P = 0.46). The only study in Iran on *TGF-β_1* C10.25 polymorphism has been carried out by Mandegary et al (43). The evidence shows that *TGF-β_1* may have a dual role in the lungs; on one side, it reduces the production of inflammatory cytokines, and on the other side, it induces pulmonary fibrosis (44,45).

One of the productions of Th1 lymphocytes and the crucial factor of the host immune responses to pathogens is *IFN-γ*. In the current study, the A allele carrier people were less susceptible to COPD even in the presence of confounding factors, which means this allele has a protective role. In other words, the risk of COPD in individuals with the TA genotype was 0.69 times more than in normal people. Since the frequencies of the AA genotype in the Iranian population were very low, and there was no significant difference between AT, AA, and inflammatory activities, these two groups were merged. In line with Di Stefano et al (46), we confirmed that the *IFN-γ* gene as an inflammatory factor was not a risk factor in susceptibility to COPD.

Several studies have demonstrated that *TNF-α* is relevant to the pathogenesis of COPD, including involvement in the neutrophil release from the bone marrow and neutrophil activation. Increased levels of *TNF-α* have been found in the sputum, bronchoalveolar lavage fluid, bronchial biopsies, and circulation of COPD patients. The outcomes of the current study showed that there was no significant relationship between *TNF-α* G-308A gene polymorphisms and susceptibility to COPD (OR = 0.81, CI = 0.42-1.5). Zhang et al concluded that there was a significant relationship between the above-said gene polymorphisms and COPD in Asian populations, but not in the Caucasian population (39).

Conclusion

Altogether, this is the first report demonstrating that the *TGF-β_1* (rs1982073) and (rs1800471) SNPs are related to the progression of COPD in the Kerman population. The

IL-10 (rs1800896) polymorphism may be less susceptible to COPD, with the genotypes carrying the G allele more common in COPD cases. There was no association between *TNF-α* (rs1800629) and *IFN-γ* (rs62559044) with COPD risk. Further studies with larger various populations are needed for definitive associations and results, future applications, and the pathways involved in the susceptibility of COPD. All in all, it is substantial to give special care to carriers of such genotypes, especially those who are exposed to chemicals at work, pollution, and cigarette smoke.

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Competing Interests
No conflict of interest exists in relation to the submitted manuscript.

Ethical Approval
The ethical approval was obtained from the Ethics Committee of Kerman University of Medical Sciences (Code: IR.KMU.REC.1392.598).

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