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Association of the -2549 insertion/deletion polymorphism in the promoter region of vascular endothelial growth factor towards chronic obstructive pulmonary disease susceptibility and clinical factors in the North Indian population

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Informed consent: informed written consent was obtained from all individual participants before their enrolment in the study. Participant anonymity and data confidentiality were strictly maintained throughout the study.

Patient consent for publication: the patients were informed during the enrolment that data generated from the study will be used for publication.

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Abstract

Chronic obstructive pulmonary disease (COPD) is a progressive respiratory disorder characterized by persistent inflammation, airway remodeling, and irreversible airflow limitation. Vascular endothelial growth factor (VEGF) plays a critical role in angiogenesis and exerts antiapoptotic effects on endothelial cells. The -2549 insertion/deletion (I/D) polymorphism in the promoter region of the VEGF gene has been reported to modulate gene expression and may influence disease susceptibility. This case-control study aimed to investigate the association between the VEGF -2549 I/D polymorphism and susceptibility to COPD, including its relationship with clinical parameters. A total of 100 COPD patients and 100 age- and sex-matched healthy controls were genotyped for the VEGF -2549 I/D variant using polymerase chain reaction following DNA extraction from peripheral blood samples. Chi-square tests were employed to assess allelic and genotypic frequencies, while logistic regression was used to analyze associations with demographic and clinical factors. The DD genotype was more prevalent among COPD patients, and differences were observed in lung function (FEV₁), COPD Assessment Test scores, and Global Initiative for Chronic Obstructive Lung Disease classification; however, these associations were not statistically significant after adjustment for confounders. Subgroup analysis showed variations in genotype associations among male subjects and smokers, although these findings were not statistically significant. Overall, these findings do not demonstrate a significant association between the VEGF -2549 I/D polymorphism and COPD susceptibility. The observed subgroup and clinical patterns may warrant further investigation in larger studies, particularly in genetically diverse populations.

Key words: COPD, VEGF, North Indian population, polymorphism.

Abbreviations

COPD - Chronic Obstructive Pulmonary Disease

VEGF - Vascular Endothelial Growth Factor

ORs - odds ratios

CI - confidence intervals

INDEL - Insertion or deletion

GOLD - Global Initiative for Chronic Obstructive Lung Disease

CAT - COPD Assessment Test

mMRC - Modified Medical Research Council

Introduction

COPD is a severe respiratory disease that leads to persistent airflow obstruction and is not reversible. Globally, it is the third primary cause of death and a major contributor to morbidity and mortality [1]. COPD is an umbrella term, and it includes numerous lung conditions, such as emphysema and chronic bronchitis. Clinical symptoms for the illness include wheezing, breathing difficulties, sputum production, coughing, and a reduced capacity for physical work. Smoking is the major environmental risk factor for COPD; however, several individuals with similar levels of smoking exposure do not get the condition, even as some people with little to no smoking history do. This disproportion highlights how a person's vulnerability to COPD is influenced by host genetic makeup [2,3]. Other factors that increase the risk of developing COPD include respiratory infections, occupational exposure to dust and chemicals, long-term exposure to indoor air pollutants (such as biomass smoke), and socioeconomic status [4]. To understand the complex cause of COPD, it is important to understand the interaction between genes and environmental exposures. The complex pathophysiology of COPD encompasses oxidative stress, an imbalance between proteases and antiproteases, chronic inflammation, airway remodelling, and damage to the alveolar walls. Endothelial dysfunction and vascular remodelling play important roles in the development of COPD [5,6], and these vascular changes lead to hypoxia, pulmonary hypertension, loss of alveolar units, reduced gas exchange, and irreversible lung injury. For maintaining the integrity of the pulmonary arteries and the alveolar structure, VEGF plays a crucial role. It is a potent pro-angiogenic factor that regulates endothelial cell migration, survival, and proliferation [7]. VEGF acts on endothelial cells through its primary receptor, VEGFR-2 (KDR/Flk-1), and supports the proper growth and maintenance of the alveolar capillary network in the lungs. It also helps in the healing and regeneration of injured tissue [8,9]. Remarkably, different COPD phenotypes have different levels of VEGF expression. While emphysematous lungs have lower VEGF levels, chronic bronchitis is often associated with higher VEGF levels, which promote vascular permeability, inflammation, and airway wall thickening [10]. Because of its dual behaviour, VEGF expression must be tightly regulated, and dysregulation in either direction may result in distinct symptoms of COPD.

VEGF expression is controlled by a variety of physiological and environmental variables, chief among them being hypoxia, which initiates VEGF transcription by activating HIF-1 α . However, genetic changes, especially in the promoter region, can significantly affect the transcriptional activity and mRNA stability of the VEGF gene [11,12]. The -2549 insertion/deletion (I/D)

polymorphism, present in the promoter region of the VEGF gene, is an example of a potentially functional variant. Due to an 18-base-pair insertion (I allele) or deletion (D allele), this polymorphism may alter transcription factor binding and affect VEGF expression levels [13]. Numerous diseases, including cancer, heart disease, complications from diabetes, and inflammatory ailments, have been linked to the D allele.

Additionally, it has been connected to altered VEGF production. Despite VEGF's well-established role in lung physiology and COPD pathophysiology, relatively little research has examined the genetic association between VEGF promoter polymorphisms and COPD susceptibility, particularly in Indian populations. Due to genetic variation and differing environmental exposures across communities, population-specific research is necessary to identify potential genetic risk factors for COPD.

Additionally, determining whether the -2549 I/D polymorphism is associated with clinical measures such as pulmonary function (FEV₁, FVC), symptom severity (CAT score, mMRC), or GOLD staging may help identify individuals at higher risk of disease progression and poor outcomes. The current investigation was carried out to ascertain the association between COPD susceptibility and the VEGF -2549 insertion/deletion polymorphism in a North Indian population. To explore this polymorphism's potential relationship with COPD phenotypic heterogeneity, disease risk, and symptom severity, the study also sought to determine its association with key clinical parameters.

Materials and Methods

Analysis of population and design

A case-control study was conducted to investigate the association between the VEGF -2549 I/D polymorphism and susceptibility to Chronic Obstructive Pulmonary Disease (COPD) in a North Indian population. From the Department of Pulmonary Medicine at the Government Medical College in Patiala, Punjab, India, 100 individuals diagnosed with COPD and 100 healthy controls, matched for age and gender, were selected. Each patient was diagnosed using spirometric parameters and clinical symptoms following the GOLD 2023 guidelines. The controls, who were selected from the general population and had no prior history of chronic respiratory disease or systemic inflammation, had normal lung function on spirometry. Individuals with asthma, interstitial lung disease, lung cancer, asthma-COPD overlap (ACO), occupational lung disease, or any acute respiratory infection were excluded. Participants had to be at least eighteen years old to be eligible. Before initiating the study, ethical permission

was secured from the Institutional Ethics Committee of the Government Medical College in Patiala (Approval No. Trg. 9(310)2022/38575). All subjects provided their informed consent before study participation, and the research adhered to the Declaration of Helsinki and its later amendments.

Sample collection and genomic DNA extraction

A 5 mL sample of peripheral blood was obtained and transferred into EDTA-coated vacutainers to prevent coagulation. After that, they were stored at -20°C until processing. To extract genomic DNA from peripheral blood leukocytes, a typical phenol–chloroform extraction procedure was modified to adhere to the protocol of Bartlett and White (2003). DNA quality was assessed by 2.0% agarose gel electrophoresis using ethidium bromide staining, while the concentration and purity of the extracted DNA were assessed using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, USA). Samples with absorbance ratios of 1.7 to 1.9 at 260/280 nm were judged appropriate for further examination.

Genotyping of VEGF -2549 indel polymorphism

Genotyping of the VEGF -2549 Indel polymorphism (rs35569394), situated in the promoter region of the VEGF gene, was performed using PCR. Each PCR amplification was carried out in a final reaction volume of 15 µL, containing 0.2 units of Taq DNA polymerase (Thermo Scientific, USA), 1.5× PCR buffer, 0.3 µM dNTPs, 1.5× bovine serum albumin (BSA), 0.75 µM of forward and reverse primers and 0.5 µL of genomic DNA (100 ng/µL). The PCR conditions included an initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 65°C for 45 seconds, and extension at 72°C for 30 seconds. A final extension was carried out at 72°C for 5 minutes.

The VEGF –2549 I/D polymorphism was genotyped using PCR with the primers listed below.

- Forward primer: 5'-GCTGAGGATGGGGCTGACTAGGTA-3'
- Reverse primer: 5'-GTTTCTGACCTGGCTATTTCCAGG-3'

After that, native PAGE was used to resolve the amplified PCR fragments with a 100 bp DNA ladder as a reference. The DD genotype (211 bp), ID genotype (229 and 211 bp), and II genotype (229 bp) were identified by migration pattern. For band detection, the gel was treated with ethidium bromide (0.5 µg/mL) and examined under ultraviolet light using a transilluminator, as shown in Figure 1. Complete concordance was observed after 20% of the samples were randomly re-genotyped to ensure correctness.

Clinical evaluation and lung function assessment

Spirometry was performed using a standardized spirometer (Model: RMS Helios 401) in compliance with the American Thoracic Society's recommendations. FEV₁, FVC, and FEV₁/FVC were measured following bronchodilator use. The severity of COPD was categorised according to the GOLD 2023 staging. Clinical symptoms were assessed using two validated measures: the CAT and the mMRC dyspnoea scale. BMI, smoking status, pack-years, duration of disease, and exacerbation history were recorded using structured interviews and medical records.

Statistical analysis

MedCalc software (version 20.0.27; MedCalc Software, Ostend, Belgium) was used to perform all statistical computations. The continuous variables, summarised as mean $\pm$ standard deviation, were analysed using the t-test. Gender and smoking status were among the categorical factors that were compared using the chi-square (χ^2) test. Allele and genotype frequencies were calculated for the patients and controls. A different Hardy–Weinberg equilibrium (HWE) test was administered to each group. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using logistic regression to assess the association between VEGF genotypes and COPD susceptibility, and the ORs reported in the overall analysis were adjusted for age, sex, and smoking exposure. The analysis incorporated codominant, dominant, and recessive genetic models. A p-value below 0.05 was used to indicate statistical significance.

Results

Overview of demographic and clinical features

The study population consisted of 100 clinically diagnosed COPD patients and 100 age and sex-matched healthy controls. The mean age of COPD patients was 58.34 ± 11.88 years, which was higher than that of the control group (57.19 ± 12.70 years; $p = 0.5029$). The gender distribution did not differ significantly, with 91% of the cases and 90% of the controls being male ($p = 0.8099$). Seventy-eight per cent of the two groups smoked, whereas twenty-two per cent did not. However, the mean smoking exposure, measured in pack-years, was substantially higher among COPD patients (27.93 ± 32.14) than controls (8.99 ± 13.05). As shown in Table 1, the difference in smoking exposure was statistically significant ($p < 0.0001$), indicating significantly higher smoking exposure among COPD patients compared with controls. COPD

patients had considerably worse lung function metrics, including FEV₁ (1.251 ± 0.513 L vs 3.81 ± 0.60 L), FVC (2.166 ± 0.626 L vs. 2.99 ± 0.89 L), and FEV₁/FVC ratio (0.558 ± 0.1080 vs. 0.85 ± 0.07). Individuals with COPD had an average BMI of 21.72 ± 5.50 . GOLD 2023 staging showed that most instances were in stage II (41%) or stage III (43%), with stage I (7%) and stage IV (9%). According to the GOLD ABCDE classification, 26% of participants were assigned to Group A, 54% to Group B, and 20% to Group E. Dyspnoea (98%) and reduced physical activity (80%) were the most prevalent symptoms, while coughing (23%) and mucus production (19%) were less frequent. Within less than two years, 42% of patients developed COPD; within two to five years, 32%; within five to ten years, 10%; and within more than ten years, 16%.

Genotypic and allelic frequencies associated with the VEGF -2549 I/D variant

The distribution of VEGF-2549 I/D genotypes indicated that the ID genotype had the highest frequency in both patients (51%) and healthy individuals (58%). The DD and II genotypes were observed, with frequencies of 27% and 22% among cases and 24% and 18% among controls, respectively. The genotype distributions found in both groups were consistent with the Hardy-Weinberg equilibrium. However, there was no statistically significant association between VEGF -2549 genotypes and the incidence of COPD. As shown in Table 2 for the ID genotype compared to II, the adjusted OR was 0.6997, while for the DD genotype compared to II, the adjusted OR was 0.9058 (95% CI: 0.3807–2.1551; $p = 0.823$). When analysed under the dominant model (ID + DD vs II), the adjusted OR was 0.7696 ($p = 0.4661$), whereas under the recessive model (DD vs II + ID), it was 1.1795 ($p = 0.6139$). These results show no significant association between VEGF -2549 genotypes and the overall vulnerability to COPD in the North Indian population.

VEGF -2549 genotypes and their association with lung function

Pulmonary function test (PFT) results, including FEV₁ (% and L), FVC (L), and FEV₁/FVC ratio, were assessed in individuals with COPD to determine the impact of the VEGF -2549 I/D (rs35569394) polymorphism. Measurements were made both before and after the bronchodilator was given. The genotypes (II, ID, and DD) did not differ significantly ($p > 0.05$) in any spirometric parameter, according to Table 3. Although the DD genotype showed numerically higher post-bronchodilator values (FEV₁ = 1.3 L; FEV₁% = 49%; FEV₁/FVC = 0.614), these differences were not statistically significant ($p = 0.73, 0.90, \text{ and } 0.55$,

respectively). Figure 2 illustrates the distribution of lung function across genotypes, with orange dots representing individual participants and error bars indicating 95% CIs. The overlapping data points provide more evidence for the absence of a meaningful connection. The VEGF -2549 polymorphism did not show a significant association with lung function in this sample, and the numerically higher values observed among DD carriers should be interpreted cautiously.

Gender-stratified genotypic association

A stratified analysis was conducted to evaluate the influence of the VEGF -2549 I/D genetic variant across genders. In men, the dominant model showed an estimated OR of 0.5467 (95% CI: 0.2517–1.1875; $p = 0.12271$); however, this association was not statistically significant. The recessive model showed that DD homozygosity was not significantly associated with COPD in men, with an OR = 1.0019 ($p = 0.9957$). Because of the small sample size and the absence of the DD genotype in controls, statistical comparisons between females were restricted. A higher proportion of the DD genotype was observed among female cases than controls; however, this difference was not statistically significant.

Smoking and pack-year stratification

Using stratified analysis based on cumulative pack-years and smoking exposure, potential gene–environment interactions were examined. Among smokers, the DD genotype had an estimated OR of 0.5162 ($p = 0.109$); however, this association was not statistically significant. Among non-smokers, the odds ratio was greater for the DD genotype (OR = 2.9071; $p = 0.2177$), but this association was also not statistically significant. Using median pack-years to separate patients into low- and high-exposure groups, no significant association was observed with VEGF genotypes. Overall, these findings do not provide sufficient evidence that the VEGF -2549 I/D genotype significantly modifies COPD susceptibility in relation to smoking exposure.

Age-stratified analysis of VEGF -2549 I/D polymorphism

To assess the age effect on the relationship between the VEGF -2549 I/D polymorphism and COPD, participants were grouped by mean age (58.34 years). In individuals under this age, the DD genotype was less prevalent in cases than in controls; however, this association was not statistically significant (codominant model: adjusted OR = 0.25; 95% CI: 0.0655–0.9541; $p = 0.0525$). Similar patterns were seen in the dominant and recessive models; however, they

were not statistically significant. Among those aged 58.34 years or older, the DD genotype was more common in cases than in controls. The recessive model showed an estimated OR of 2.2871 (95% CI: 0.7246–7.2183; $p = 0.1583$); however, this association was not statistically significant. These results, given in Table 4, do not provide sufficient evidence to conclude that the VEGF -2549 I/D polymorphism is associated with COPD susceptibility in an age-dependent manner.

Clinical score correlation (CAT, mMRC, GOLD)

The relationship between VEGF genotypes and clinical severity was examined using the CAT score, mMRC grade, and GOLD categories. In the recessive model, the DD genotype was associated with nearly three times the risk of having more severe symptoms, defined as CAT ≥ 10 and mMRC ≥ 2 scores (OR = 2.856 for both; $p = 0.0587$). Although these relationships were not statistically significant, these findings do not establish an association between the DD genotype and greater symptom burden. GOLD spirometric stages ($p = 0.9661$), GOLD ABCDE classification ($p = 0.8000$), and disease duration ($p = 0.3192$) did not significantly correlate with VEGF genotype. Overall, no statistically significant association was observed between VEGF genotype and clinical severity measures or disease duration in this sample.

Symptom-based analysis

We evaluated the association between several clinical symptoms and VEGF -2549 genotypes. Patients with the DD genotype showed higher estimated odds of cough and expectoration; however, these associations were not statistically significant. According to the codominant model, the DD genotype was associated with a higher estimated odds of cough (OR = 3.82) and expectoration (OR = 7.83). Similarly, in the dominant model, the ORs for expectoration and cough were 5.0526 and 3.2416, respectively. None of these correlations were statistically significant ($p > 0.05$), and these findings should therefore be interpreted cautiously. Physical limitation, mucus production, and dyspnoea showed no significant relationships ($p = 0.998$), suggesting that these symptoms are common among people with COPD across all genotypes.

Discussion

This case-control study aimed to explore the relationship between the VEGF -2549 INDEL polymorphism and the risk of developing chronic obstructive pulmonary disease (COPD) in a North Indian population. While the analysis did not reveal a significant link between VEGF -

2549 I/D genotypes or alleles and COPD susceptibility, the DD genotype was somewhat more common in COPD cases than in controls. These findings are in line with previous studies that failed to establish a direct connection between this polymorphism and susceptibility to COPD in an Iranian community [14] and in a Korean population [15]. The ID genotype was the most prevalent in both patients and controls in our sample, where genotype frequencies were in Hardy-Weinberg equilibrium. Although no general correlation was found, additional analysis revealed patterns that may warrant further investigation into the potential relationship between the polymorphism and the clinical characteristics of COPD. It's interesting to note that, although not statistically significant, patients with the DD genotype had somewhat greater post-bronchodilator FEV₁ and FEV₁/FVC values than those with the II or ID genotypes. These findings may reflect the complex role of VEGF in pulmonary physiology, as it regulates angiogenesis, alveolar-capillary integrity, and vascular remodelling—processes that are altered in the pathophysiology of COPD [16-18]. Importantly, our study observed higher estimated odds for symptoms, including expectoration and cough, among DD genotype carriers; however, these associations were not statistically significant. For the DD genotype, higher CAT and mMRC scores were associated with higher estimated odds of greater symptom burden, although the association was not statistically significant ($p = 0.0587$). These findings do not establish a direct association between the D allele and increased VEGF expression or symptom severity, although the potential biological mechanisms warrant further investigation (Kanazawa et al., 2003; Kasahara et al., 2001). Breathlessness was extremely prevalent (98%) across all genotypes, regardless of genetic origin, suggesting that it is a common characteristic of COPD. Gender-stratified analysis showed different estimated associations between males and females; however, none were statistically significant. In contrast, the absence of the DD genotype among controls in females prevented meaningful interpretation. These gender-based disparities could be due to changes in environmental exposures, such as women's use of biomass fuel, or to hormonal regulation of VEGF expression [19]. The DD genotype showed a lower estimated OR in smokers and a higher estimated OR in non-smokers; however, neither association was statistically significant. These patterns are consistent with research showing that smoking-induced oxidative stress can alter VEGF expression, thereby affecting its subsequent effects, even if the effects were not statistically significant (Kim et al., 2008). Although the VEGF-2549 I/D genetic variant and the risk of COPD did not statistically correlate, this study provides valuable new information by highlighting the potential effects of this variant on phenotypic expression and clinical symptoms. It's interesting to note that a

different pattern of association was found by age-stratified analysis. Age-stratified analysis showed different estimated associations in participants younger than 58.34 years and those aged 58.34 years or older; however, these findings were not statistically significant. These findings do not provide sufficient evidence for an age-dependent association between the VEGF -2549 INDEL polymorphism and COPD susceptibility. The study's strengths include thorough clinical profiling using validated instruments such as CAT, mMRC, GOLD classification, and spirometry, as well as stratified analyses by smoking status and gender. It is important to recognise certain limitations of the study, such as the relatively small sample size, the lack of longitudinal follow-up, the focus on a single VEGF polymorphism, and the absence of data on VEGF protein expression. The relatively small sample size and multiple subgroup analyses may have limited the study's statistical power and increased the likelihood of chance findings. Furthermore, the lack of VEGF expression or functional data limits our ability to establish a biological link between the -2549 I/D polymorphism and the observed clinical characteristics. To gain a more comprehensive understanding of VEGF's role in COPD pathogenesis and its potential as a biomarker for disease progression and targeted therapy, future research should include larger, multicentre populations and functional studies assessing VEGF serum levels and haplotype analysis."

Conclusions

Our findings did not demonstrate a significant association between the VEGF -2549 I/D variant and the risk of developing COPD in the North Indian population studied. However, genotype-based analyses revealed some variations in clinical characteristics, although these findings were not statistically significant. Stratified analysis also suggested a potential age-related effect, with the DD genotype showing different estimated associations in younger and older participants, although these findings were not statistically significant. No statistically significant differences in lung function were observed among genotypes. The complexity of gene–environment interactions in COPD was further illustrated by stratified analyses by smoking and gender, even in the absence of a conclusive genotype effect. These findings do not establish that the -2549 I/D variant alters the clinical phenotype of COPD, although the observed patterns warrant further investigation. More comprehensive, multi-ethnic investigations, including functional assays and haplotype analyses, are required to elucidate the specific role of VEGF gene variants in COPD development and clinical progression.

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Table-1 Demographic and clinical parameters among cases and controls.

Parameters	Cases, (n%) N = 100	Controls, (n%) N = 100	p-value
Age (Years)			0.5029
MEAN $\pm$ SD	58.34 $\pm$ 11.88	57.19 $\pm$ 12.70	
Range	21-80	25-86	
Gender			0.8099
Male	91(91)	90(90)	
Females	9(9)	10(10)	
Smoking Status			1
Smokers	78(78)	78(78)	
Non-Smokers	22(22)	22(22)	
Pack Years			<0.001
Mean $\pm$ SD	27.93 $\pm$ 32.14	8.99 $\pm$ 13.05	
BMI	21.72 $\pm$ 5.50		
FEV1 (% predicted)	45 $\pm$ 0.164	88.79 $\pm$ 11.35	<0.001
FEV1 (L)	1.251 $\pm$ 0.513	3.81 $\pm$ 0.60	<0.001
FVC (L)	2.166 $\pm$ 0.626	2.99 $\pm$ 0.89	<0.001
FEV1/FVC, ratio	0.558 $\pm$ 0.1080	0.85 $\pm$ 0.07	<0.001
GOLD Severity			
I	7(7)		
II	41(41)		
III	43(43)		
IV	9(9)		
mMRC			
mMRC <2	29(29)		
mMRC $\geq$ 2	71(71)		
CAT Score			
CAT <10	29(29)		
CAT $\geq$ 10	71(71)		
GROUP ^{ABE}			
A	26(26)		
B	54(54)		
E	20(20)		
Duration of COPD			
<2 years	42(42)		
2–5 years	32(32)		
5–10 years	10(10)		
>10 years	16(16)		
Cough	23(23)		
Breathlessness	98(98)		
Mucus Production	19(19)		
Expectoration	19(19)		
Physical Movement	80(80)		

SD, standard deviation, N, total number of case patients or control subjects, BMI, body mass index, p-values were derived from Pearson Chi – square test except age; Student t-test was used for age. All p-values are two – sided. $p < 0.05$ was considered statistically significant.

Table 2. Genotype and genetic model-based distribution of VEGF -2549 I/D Polymorphism (rs35569394) among COPD cases and controls with adjusted odds ratios, confidence intervals, and p-values.

Genotype rs35569394	OVERALL	CASES (100), N (%)	CONTROLS (100), N (%)	adjusted OR	CI	p-value
Codominant	II	22 (22)	18 (18)	Reference		
	ID	51 (51)	58 (58)	0.6997	0.3340 to 1.4657	0.3438
	DD	27 (27)	24 (24)	0.9058	0.3807 to 2.1551	0.823
Dominant	II	22 (22)	18 (18)	Reference		
	ID+DD	78 (78)	8 (82)	0.7696	0.3806 to 1.5563	0.4661
Recessive	II+ID	73 (73)	76 (76)	Reference		
	DD	27 (27)	24 (24)	1.1795	0.6211 to 2.2401	0.6139

a Adjusted Odds ratios 95 % confidence intervals and their corresponding p-values were calculated by unconditional logistic analysis after adjusting for age, gender, smoking status, two-sided χ^2 test for either genotype distribution or allelic frequencies between the cases and controls, $P < 0.05$ indicates statistical significance.

Table 3. Association of allele frequencies with lung function.

Genotype rs35569394	Gene Types					Gene Types				
	Before	II	ID	DD	P	After	II	ID	DD	P
	FEV1 (%)	44	43	46	0.36	FEV1 (%)	48	47	49	0.9
	FEV1 (L)	1.125	1.050	1.330	0.23	FEV1 (L)	1.21	1.21	1.3	0.73
	FVC (L)	2.17	1.92	2.21	0.2	FVC (L)	2.26	2.24	2.31	0.9
	FEV1/FVC	0.54	0.57	0.57	0.47	FEV1/FVC	0.575	0.575	0.614	0.55

Table 4. Distribution of VEGF -2549 I/D genotypes and their association with COPD in age-stratified groups (<58.34 years and $\geq$ 58.34 years).

Genotype rs35569394	<58.34	Cases (43), N (%)	Controls (61), N (%)	adjusted OR	CI	p-value
Codominant	II	11 (26)	9 (15)	Reference		
	ID	24 (56)	34 (56)	0.4968	0.1722 to 1.4327	0.1954
	DD	8 (19)	18 (30)	0.25	0.0655 to 0.9541	0.0525
Dominant	II	11 (26)	9 (15)	Reference		
	ID+DD	32 (74)	52 (85)	0.4116	0.1484 to 1.1413	0.088
Recessive	II+ID	35 (81)	43 (70)	Reference		
	DD	8 (19)	18 (30)	0.4883	0.1762 to 1.3534	0.1682
	$\geq$ 58.34	Cases (57), N (%)	Controls (39), N (%)	adjusted OR	CI	p-value
Codominant	II	11 (19)	9 (23)	Reference		
	ID	27 (47)	24 (62)	0.7726	0.2311 to 2.5834	0.6753
	DD	19 (33)	6 (15)	1.6015	0.2614 to 9.8115	0.6106
Dominant	II	11 (19)	9 (23)	Reference		
	ID+DD	46 (81)	30 (77)	1.0079	0.3125 to 3.2508	0.9895
Recessive	II+ID	38 (67)	33 (85)	Reference		
	DD	19 (33)	6 (15)	2.2871	0.7246 to 7.2183	0.1583

Adjusted Odds ratios 95 % confidence intervals and their corresponding p-values were calculated by unconditional logistic analysis after adjusting for age two-sided χ^2 test for either genotype distribution or allelic frequencies between the cases and controls, $p < 0.05$ indicates statistical significance.

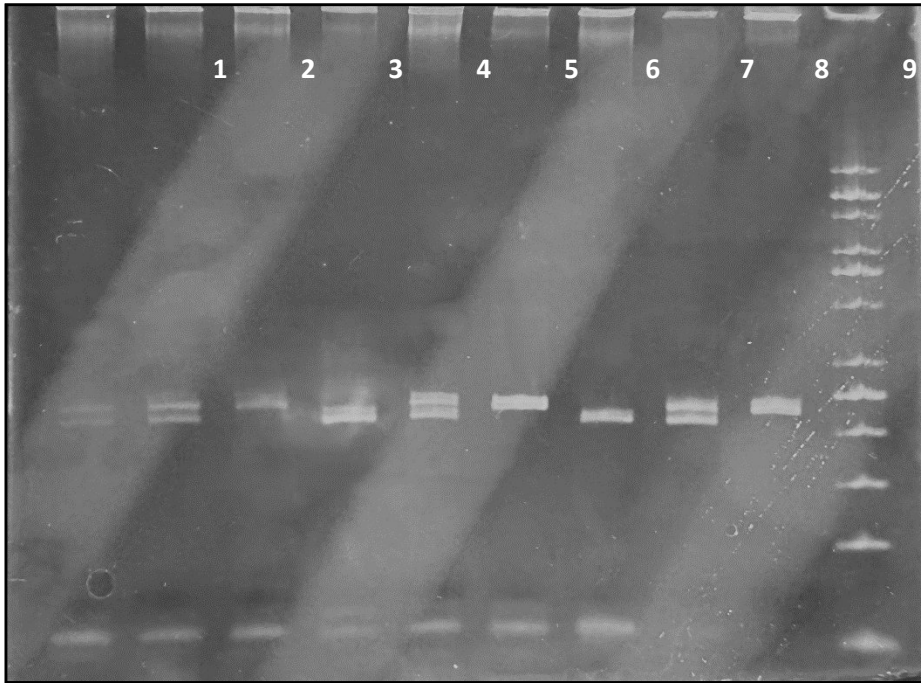
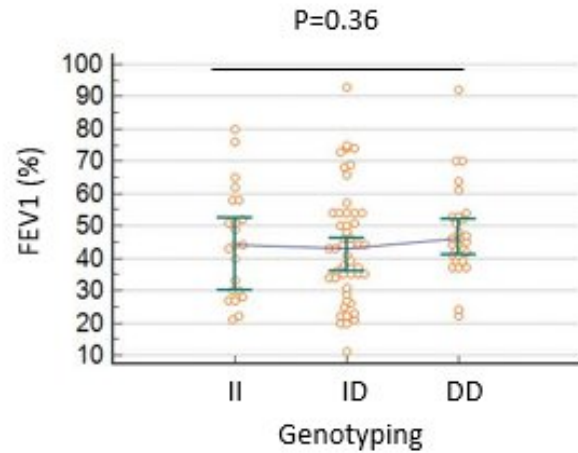
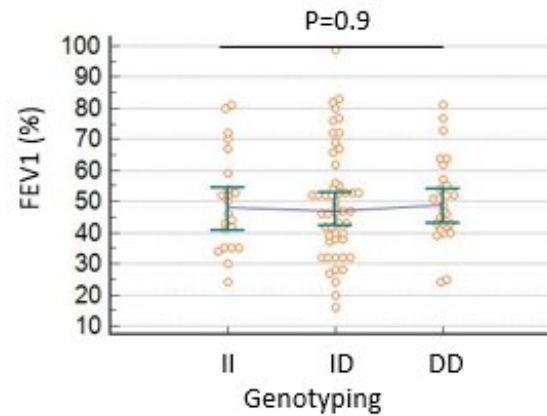


Figure 1. Representative Native Image Illustrating VEGF –2549 I/D Genotypes. Native PCR product image displaying VEGF –2549 I/D polymorphism genotypes. Lanes 1, 2, 5, and 8 show the presence of two bands corresponding to the heterozygous genotype (ID). Lanes 3, 6, and 9 show a single upper band representing the insertion homozygous genotype (II), while lanes 4 and 7 show a single lower band corresponding to the deletion homozygous genotype (DD). The final lane contains the 100 bp DNA ladder used for approximate product size determination.



(A)



(B)

Figure 2: Distribution of FEV1 (%) among VEGF -2549 I/D genotypes. A) Before treatment, patients with genotype DD showed slightly higher mean FEV1 (%) compared to II and ID genotypes; however, the difference was not statistically significant ($p=0.36$); B) after treatment, all genotypes (II, ID, DD) showed marginal improvement in FEV1 (%), with genotype DD still presenting slightly higher values. No significant association was observed between VEGF genotypes and FEV1 (%) post-treatment ($p=0.9$). These results suggest that the -2549 I/D polymorphism does not significantly influence FEV1 (%) either at baseline or in response to therapy in COPD patients.