Biosight 2026; 07(01): 25-38

Association of Vascular Endothelial Growth Factor 18-bp Insertion/Deletion Variant with Susceptibility to Diabetic Nephropathy.

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Abstract: Genetic polymorphisms in cytokine genes have been implicated as important contributors to susceptibility to diabetic nephropathy, a major microvascular complication of type 2 diabetes mellitus (T2DM). Vascular Endothelial Growth Factor (VEGF) is a multifunctional cytokine that plays a critical role in endothelial cell proliferation, angiogenesis, and regulation of vascular permeability. The VEGF gene is highly polymorphic, and several variants have been reported to influence the risk of T2DM and its associated microvascular complications, including diabetic nephropathy. The present study aimed to evaluate the association between the 18-bp insertion/deletion (I/D) rs35569394 polymorphism in the VEGF gene and susceptibility to diabetic nephropathy among individuals with T2DM. Genotyping was performed using conventional polymerase chain reaction. A total of 400 participants were enrolled (200 diabetic nephropathy patients and 200 healthy controls). Allelic frequencies and genotype distributions were compared between cases and controls using SPSS software. Significant differences in allelic frequency and genotype distribution were observed between the groups. Chi-square analysis revealed a significant association between the heterozygous I/D genotype and diabetic nephropathy ($\chi^2 = 14.93$, $p < 0.001$). The homozygous I/I genotype was significantly associated with high-density lipoprotein levels, glomerular filtration rate, and bilirubin levels ($p < 0.001$). Additionally, the heterozygous I/D genotype in the −2549 promoter region was more frequent in diabetic nephropathy cases in the Pakistani population. The deletion allele may influence VEGF expression; however, functional validation was not performed. Further studies are warranted to explore its potential as a biomarker or therapeutic target.

Keywords: Diabetic nephropathy, *VEGF*, T2DM, Pakistan, 18bp indel

Received: 30-10-2025
Accepted: 1-1-2026
DOI: 10.46568/bios.v7i1.243

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Introduction

Diabetes Mellitus (DM) is a major global health challenge. According to the International Diabetes Federation (IDF), the global prevalence of DM is estimated at 537 million people and is projected to rise to 643 million by 2030 and 784 million by 2045 [1]. Persistent hyperglycaemia is known to cause both microvascular and macrovascular complications. With the rising prevalence of diabetes, its complications are also increasing, posing a significant public health burden. Such complications typically arise in tissues directly exposed to glucose, where uptake occurs independently of insulin [2]. One such complication is diabetic nephropathy (DN), affecting approximately 30–40% of diabetic patients [3]. DN, a multifactorial condition primarily driven by

hyperglycaemia, is the leading cause of chronic kidney disease [4]. Clinically, DN is characterized by persistent macro- or microalbuminuria, a sustained decline in glomerular filtration rate, and hypertension [5].

The disease is driven by hyperglycaemia-induced metabolic dysregulation, accumulation of advanced glycation end products, inflammatory and profibrotic cytokine signaling, and thickening with mesangial expansion of the glomerular basement membrane, ultimately leading to fibrosis [4,5]. Risk factors for DN include poor glycaemic control, dyslipidaemia, family history, obesity, uncontrolled hypertension, and ethnic predisposition [6]. Previous studies have shown that poor glycaemic control, hypertension, duration of diabetes, elevated glomerular filtration rate (GFR), and the presence of microalbuminuria are major determinants of disease progression [7,8]. Increasing evidence also highlights the contribution of hereditary factors in predisposing individuals to DN. Cytokines and growth factors, in particular, exacerbate tissue injury induced by chronic hyperglycaemia, thereby amplifying the risk of complications [9].

Recent evidence indicates that inflammation plays a critical role in DN progression. Although the underlying mechanisms remain incompletely understood, evidence shows that inflammatory cytokines such as MCP-1 and TNF-α are elevated in these patients [10]. VEGF is a key regulator of angiogenesis and vascular permeability; however, it is also described as a pro-inflammatory mediator in pathological contexts [11]. VEGF is associated with other diabetic complications, such as diabetic retinopathy, where studies confirm a correlation between ischemic retinopathy and intraocular VEGF concentration [12], and diabetic cardiomyopathy, where elevated VEGF levels are observed in patients with myocardial infarction [13]. VEGF plays a crucial role in angiogenesis through the regulation of inflammation in chronic diseases. Studies indicate that VEGF-A mediates the recruitment of inflammatory cells such as macrophages and neutrophils to damaged tissue via VEGF receptor signalling and activation of the nuclear factor-kappa B (NF-κB) pathway. In coronary heart disease, VEGF exhibits a dual role in disease progression [14]. Consistent with its pro-inflammatory role, VEGF also contributes to the progression of rheumatoid arthritis (RA). VEGF knockout animal models show reduced pathology and synovial angiogenesis in RA-induced rat models, suggesting that inhibition of VEGF-mediated angiogenesis may suppress inflammation in RA [15].

The pathogenesis of DN is multifactorial. Recent studies suggest that vascular endothelial growth factor (VEGF), also known as vascular permeability factor, is closely associated with DN [16]. Genetic polymorphisms within VEGF have been linked to diabetic microvascular complications [17]. Several single nucleotide polymorphisms (SNPs), particularly in the promoter region, are known to influence its expression [18]. Variants such as +936 C/T, −2578 C/A, −634 G/C, and −1154 G/A have been associated with chronic diseases, including diabetic complications [19]. In the kidney, VEGF is essential for maintaining glomerular function; however, therapeutic VEGF blockade has been reported to induce adverse renal effects, highlighting its importance in DN pathophysiology [20,21]. Among reported variants, VEGF rs2010963 and rs3025039 have been associated with increased DN risk in Asian populations, while the −2549 D/D genotype in the VEGF promoter has also been implicated [22,23]. VEGF polymorphisms contribute to diabetes-related complications due to their critical role in angiogenesis [24,25]. The insertion/deletion variant in the VEGF promoter is associated with DN, where the 18-bp deletion increases transcriptional activity by approximately 1.95-fold compared with the insertion allele [26,27].

The earlier onset of diabetes in Asian populations compared with Europeans, combined with longer life expectancy, likely contributes to the higher burden of diabetes-related complications due to prolonged hyperglycaemic exposure [28]. Pakistan ranks among the countries with the highest diabetes prevalence, underscoring the need for studies on diabetes and its complications.

Although VEGF variants have been extensively investigated in various populations, their clinical and genetic relevance is population-specific due to variations in allele frequencies and linkage disequilibrium patterns. Therefore, the present study aims to investigate the role of VEGF variants in susceptibility to DN in the Pakistani population. Specifically, this study evaluates the association between the 18-bp indel (rs35569394) variant in the VEGF promoter and diabetic nephropathy in individuals with T2DM. The findings may provide valuable insights for disease management and the development of targeted therapeutic strategies.

Methods

Ethical Approval and Subject Selection

This case–control study comprised 400 participants, divided into two groups: patients with diabetic nephropathy (n = 200) and healthy controls (n = 200). The sample size was determined through power analysis. Ethical approval was obtained from the Institutional Review Board (IRB) of the Karachi Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi (Reference No. KIBGE/ICE/188/10/10/23). Written informed consent was obtained from all participants prior to enrolment.

Participant eligibility was assessed according to predefined criteria. Cases included individuals aged 45–60 years with a duration of diabetes exceeding 10 years, meeting established diagnostic criteria for diabetic nephropathy, and having glycated haemoglobin (HbA1c) levels >6.5%. Individuals with a history of non-diabetic kidney disease, acute inflammatory conditions, hematological disorders, or other diabetic complications, including retinopathy, were excluded. The control group comprised individuals with no history of diabetes or renal disease, with normoglycaemic status and HbA1c levels <5.7%.

Sample collection and biochemical investigations

A total of 3 mL of venous blood was collected into EDTA tubes for genomic DNA extraction and glycated haemoglobin (HbA1c) estimation. Genomic DNA was isolated using the salting-out method (Miller et al., 1998). DNA concentration was measured using a NanoDrop spectrophotometer (IMPLEN NanoPhotometer®, Germany), and integrity was assessed by horizontal agarose gel electrophoresis. The extracted DNA was stored at −20 °C until further analysis. For biochemical analyses, 2 mL of blood was collected in gel vacutainers and centrifuged at 13,000 rpm for 14 minutes. The separated serum was aliquoted and used for the determination of urea, albumin, creatinine, heme oxygenase-1 (HO-1), iron, bilirubin, total cholesterol (CHOL), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels. An additional 2 mL of blood was collected in fluoride vacutainers to utilize their antiglycolytic properties, thereby preventing glycolysis in red blood cells. These samples were used to measure the levels of fasting blood sugar (FBS) and random blood sugar (RBS) in the subjects. For validation, sample measurements were replicated.

Genotyping of VEGF 18 bp Indel rs35569394 polymorphism

The present study investigated the 18 base pair insertion/deletion polymorphism at position −2549 in the promoter region of the VEGF gene in both case and control groups. Genotyping was carried out using conventional polymerase chain reaction (PCR). The primer sequences used for amplification of the VEGF 18 bp indel (rs35569394) variant were as follows: forward primer, 5′-GCTGAGAGTGGGGCTGACTAGGTA-3′, and reverse primer, 5′-GTTTCTGACCTGGCTATTTCCAGG-3′. The PCR reaction mixture comprised 5 µL of 10× buffer, 3 µL of DNA template (50 ng/µL), 0.4 µL of dNTPs (25 mmol), 1 µL each of forward and reverse primers (10 µmol), 3.8 µL of $MgCl_2$ (20 mmol), 0.5 µL of Taq DNA polymerase (5 U/µL), and DNase/RNase-free water to a final volume of 20 µL.

Amplification was performed under the following thermal cycling conditions: initial denaturation at 94 °C for 30 seconds, annealing at 61 °C for 45 seconds, and extension at 72 °C for 45 seconds, for a total of 35 cycles, using a thermal cycler (T100 Bio-Rad®, California, USA).
The amplified products were resolved by agarose gel electrophoresis on a 3% agarose gel. PCR amplification of the VEGF 18 bp indel yielded fragments of 219 bp for the insertion (I) allele and 211 bp for the deletion (D) allele, as confirmed by gel electrophoresis (Figure 1).

Data analysis

Statistical analyses were performed using SPSS version 16.0. Clinical characteristics of patients with diabetic nephropathy (DN) and controls were compared using independent *t*-tests. The distribution of genotypes i.e. homozygous mutant (D/D), heterozygous (I/D), and homozygous wild-type (I/I) for the VEGF 18 bp indel (rs35569394) variant was evaluated for compliance with Hardy–Weinberg equilibrium (HWE). The association between alleles (I and D) and disease risk was assessed using chi-square (χ^2) analysis and odds ratios (ORs) with corresponding 95% confidence intervals (CIs). Genetic models for the VEGF (rs35569394) variant were analyzed using SNPStats® software. Logistic regression analysis was performed to examine the association between VEGF genotypes and clinical parameters. Differences in clinical variables across genotypes were illustrated using bar graphs. A p-value < 0.05 was considered statistically significant. Genotype frequencies in the control group were consistent with Hardy–Weinberg equilibrium ($p > 0.05$), indicating no significant deviation.

Results

Anthropometric and biochemical characteristics of the participants

A total of 400 participants (n = 400) were included in this case–control study. Table 1 presents the anthropometric and baseline clinical characteristics of patients with diabetic nephropathy (DN) and healthy controls. The analysis revealed a significant increase in systolic blood pressure in the DN group compared with controls, indicating a strong association with disease status. In contrast, no significant differences were observed in diastolic blood pressure (DBP), body mass index (BMI), or age between the two groups.
Clinical parameters were further compared between the DN and control groups. Fasting blood glucose (FBG), random blood glucose (RBG), glycated haemoglobin (HbA1c), triglycerides (TG), glomerular filtration rate (GFR), and creatinine (Cr) levels were significantly elevated in patients with DN ($p < 0.001$). Additionally, low-density lipoprotein (LDL), albumin, heme oxygenase-1 (HO-1), and iron levels differed significantly between the groups ($p < 0.01$). In contrast, bilirubin levels were significantly reduced in the DN group compared with controls ($p < 0.05$).

Table 1. Comparison of Biochemical and Anthropometric measures between diabetic nephropathy (DN) patients and controls

Variables	Groups	Mean ± SD	p-value
AGE (years)	DN	54.75 ± 10.3	0.400
	Control	51.42 ± 12.5	
BMI (kg m^{-2})	DN	27.05 ± 4.13	0.467
	Control	26.76 ± 3.79	
SBP (mmHg)	DN	142.4 ± 18.7	0.000***
	Control	126.8 ± 9.86	
DBP (mmHg)	DN	87.01 ± 12.1	0.340
		81.83 ± 6.20	
FBG (mg dL^{-1})	DN	172.7 ± 23.8	0.000***
	Control	82.64 ± 10.6	

RBG (mg dL⁻ ¹)	DN	327.9 ± 11.8	0.000***
	Control	115.4 ± 16.1	
UREA (mg dL⁻ ¹)	DN	54.26 ± 11.1	0.000***
	Control	30.65 ± 6.27	
LDL (mg dL⁻ ¹)	DN	119.1 ± 13.6	0.01**
	Control	99.46 ± 15.5	
HDL (mg dL⁻ ¹)	DN	42.70 ± 12.3	0.082
	Control	52.04 ± 6.50	
TG (mg dL⁻ ¹)	DN	250.4 ± 83.9	0.000***
	Control	86.49 ± 15.0	
CHOL (mg dL⁻ ¹)	DN	208.3 ± 55.4	0.067
	Control	188.6 ± 21.5	
GFR (mL min⁻ ¹)	DN	30.14 ± 7.39	0.000***
	Control	82.24 ± 13.9	
HbA1c (%)	DN	10.85 ± 3.63	0.000***
	Control	5.420 ± 0.84	
CREATININE (mg dL⁻ ¹)	DN	3.140 ± 3.42	0.000***
	Control	0.900 ± 0.63	
ALBUMIN (g dL⁻ ¹)	DN	14.00 ± 9.07	0.01**
	Control	8.950 ± 5.40	
HO-1 LEVEL (ng mL⁻ ¹)	DN	0.980 ± 0.37	0.01**
	Control	1.760 ± 0.91	
IRON (µg dL⁻ ¹)	DN	18.80 ± 1.65	0.01**
	Control	15.87 ± 2.20	
BILIRUBIN (mg dL⁻ ¹)	DN	1.000 ± 0.42	0.05*
	Control	1.540 ± 0.73	

DN = diabetic nephropathy; Data are presented as mean ± SD; Statistical significance: ***p < 0.001, **p < 0.01, *p < 0.05; Groups: DN = 200, Control = 200

Genotyping of *VEGF* polymorphism

PCR amplification followed by gel electrophoresis produced distinct bands at 219 bp and 211 bp, corresponding to the allele-specific fragments. The homozygous wild-type (I/I) genotype was observed in lane 1, showing a single band at 219 bp. The homozygous mutant (D/D) genotype was detected in lanes 4 and 5, exhibiting a single band at 211 bp. The heterozygous (I/D) genotype was identified in lanes 2, 3, and 6, where both 219 bp and 211 bp bands were present. Lane 7 contained the 100 bp DNA ladder used for size confirmation. These results confirm the successful genotyping of the VEGF polymorphism, as depicted in Figure 1.

Distribution of *VEGF* genotypes

Genotypes of the VEGF 18 bp indel (rs35569394) variant were analysed in 200 diabetic nephropathy (DN) patients and 200 control subjects, as presented in Table 2. The findings showed that a greater proportion of DN patients (60%) exhibited the I/D genotype compared with controls (51%). In contrast, the homozygous wild-type (I/I) and mutant (D/D) genotypes were more frequent in controls (20% and 29%, respectively) than in DN patients (26.5% and 13.5%, respectively). Furthermore, a significant association was found between the heterozygous genotype and an increased predisposition to DN, as determined by chi-square analysis ($\chi^2 = 14.93$, $p < 0.001$).

The allelic distribution of the VEGF 18 bp indel variant (rs35569394) was also assessed between the two groups. The I allele was significantly more prevalent in the DN group (56%) compared with controls (46%), whereas the D allele was less frequent in DN patients (44%) and more frequent in controls (54%). Odds ratio analysis indicated a significant protective effect of the mutant D allele against the risk of diabetic nephropathy (OR = 0.64, $p < 0.01$).

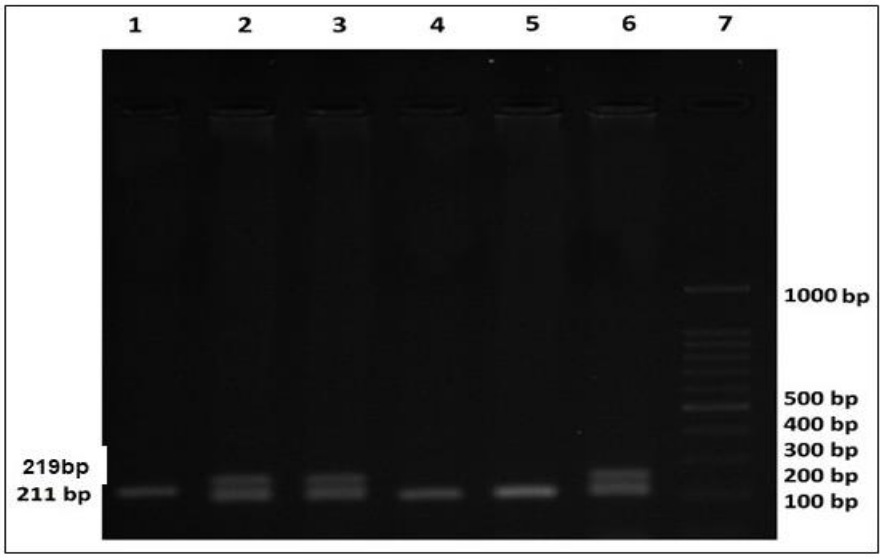

Figure 1. Agarose gel electrophoresis showing VEGF insertion/deletion (I/D) genotypes. Lane 1 represents the homozygous insertion (I/I) genotype with a 219 bp band. Lanes 4 and 5 show the homozygous deletion (D/D) genotype with a 211 bp band. Lanes 2, 3, and 6 display the heterozygous (I/D) genotype with both 219 bp and 211 bp bands. Lane 7 shows the 100 bp DNA ladder used as a molecular size marker.

Table 2. Distribution of VEGF 18bp indel genotypes and alleles in diabetic nephropathy (DN) patients and controls

Genotypes n=400	**DN n=200**	**Controls n=200**	**Chi-square**	**p-value**	**Odds Ratio 95⁒ CI**
I/I	53 (26.5%)	40 (20%)			
I/D	120 (60%)	102 (51%)	χ^2=14.93	0.001	
D/D	27 (13.5%)	58 (29%)			
Alleles n=800	**DN n=400**	**Controls n=400**		**p-value**	
I	226 (56%)	182 (46%)			OR=0.64
D	174 (44%)	218 (54%)		0.002	0.48-0.84

N = diabetic nephropathy; Controls = healthy subjects; χ^2 = Chi-square; 95% CI = 95% confidence interval; OR = odds ratio; I allele: wild-type; D allele: mutant; Total DN = 200, Controls = 200 for genotype

Genetic Models of *VEGF* Variant

Genetic models of the VEGF 18bp indel variant (rs35569394) were analyzed to assess the association of different genotypes with diabetic nephropathy, as summarized in Table 3. Four theoretical models were evaluated to compare DN patients with controls. The codominant model (OR = 1.13) and the recessive model (OR = 2.62) showed a significant association with an increased risk of diabetic nephropathy ($p < 0.001$). These results indicate that both the heterozygous and recessive genotypes of the VEGF 18bp indel variant are associated with susceptibility to diabetic nephropathy in diabetic patients.

Table 3. Association of VEGF 18bp indel genotypes with diabetic nephropathy under different genetic models

Model	Genotypes	DN	Controls	OR (at 95% CI)	p-value
Co-dominant	I/I	53 (26.5%)	40 (20%)	1.00	0.001***
	I/D	120 (60%)	102 (51%)	1.13 (0.69-1.83)	
	D/D	27 (13.5%)	58 (29%)	2.85 (1.54-5.26)	
Dominant	I/I	53 (26.5%)	40 (20%)	1.00	0.12
	I/D-D/D	147 (73.5%)	160 (80%)	1.44 (0.90-2.30)	
Recessive	I/I-I/D	173 (86.5%)	142 (71%)	1.00	0.001***
	D/D	27 (13.5%)	58 (29%)	2.62 (1.58-4.35)	
Over dominant	I/I-D/D	80 (40%)	98 (49%)	1.00	0.07
	I/D	120 (60%)	102 (51%)	0.69 (0.47-1.03)	

OR = odds ratio; 95% CI = 95% confidence interval; DN = diabetic nephropathy; Controls = healthy subjects; ***$p < 0.001$

Comparison of clinical parameters within *VEGF* genotypes

The levels of clinical parameters were compared across the three genotypes of the VEGF 18 bp indel variant (rs35569394) in both DN patients and controls, as presented in Figure 2. Bilirubin, heme oxygenase-1 (HO-1), albumin, creatinine, and glomerular filtration rate (GFR) showed significant differences among the I/I, I/D, and D/D genotypes between the DN and control groups ($p < 0.001$). In contrast, iron levels were significantly different only in individuals with the D/D genotype ($p < 0.01$), while no significant differences were observed for the I/I and I/D genotypes ($p > 0.05$).

Furthermore, lipid profile parameters, urea, and glucose levels were evaluated across VEGF indel genotypes, as illustrated in Figure 3. Glycated haemoglobin (HbA1c), urea, and triglyceride (TG) levels differed significantly among the three genotypes ($p < 0.001$). However, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and total cholesterol (CHOL) did not show significant variation across the I/I, I/D, and D/D genotypes in either DN patients or controls.

Clinical association by regression analysis

Regression analysis was performed to evaluate the association of clinical parameters with the genotypes of the VEGF 18bp indel variant (rs35569394) between DN patients and controls, as summarized in Table 4. The homozygous wild-type I/I genotype showed significant associations with high-density lipoproteins (HDL; OR = 1.20), glomerular filtration rate (GFR; OR = 1.22), and bilirubin (OR = 0.741) in relation to diabetic nephropathy ($p < 0.001$). The heterozygous I/D genotype was associated with GFR (OR = 1.22) and exhibited a weak protective trend for bilirubin (OR = 0.927, $p = 0.212$), along with protective associations for diastolic blood pressure (DBP; OR = 0.898), fasting blood glucose (FBG; OR = 0.797), random blood glucose (RBG; OR = 0.972), urea (OR = 0.740), LDL (OR = 0.879), triglycerides (TG; OR = 0.983), HbA1c (OR = 0.549), creatinine (OR = 0.681), albumin (OR = 0.982), and HO-1 (OR = 0.668) against the progression of diabetic nephropathy. The homozygous mutant D/D genotype did not show a significant association with DN risk (OR = 1.015 for bilirubin) but suggested minor protective effects for systolic blood pressure (SBP; OR = 0.997), FBG (OR = 0.995), total cholesterol (CHOL; OR = 0.999), and HbA1c (OR = 0.982).

Genotype distribution in the control group did not deviated from Hardy-Weinberg equilibrium ($p > 0.05$) as shown in table 5.

Table 4: Regression Evaluation of Clinical Factors in Relation to VEGF Polymorphisms among DN Patients

Clinical Variables	I/I Genotype		I/D Genotype		D/D Genotype	
	Exp (B)	Sig.	Exp (B)	Sig.	Exp(B)	Sig.
AGE (years)	0.980	0.350	0.930	0.021	1.000	0.951
BMI (kg/m^2	1.124	0.616	0.697	0.235	0.999	0.699
SBP (mmHg)	0.938	0.000	1.060	0.000	0.997	0.000***
DBP (mmHg)	1.010	0.013	0.898	0.000	1.005	0.000***
FBG ($mg\ dL^{-1}$)	0.811	0.000	0.797	0.000	0.995	0.000***
RBG ($mg\ dL^{-1}$)	0.959	0.000	0.972	0.000	1.000	0.001**
UREA ($mg\ dL^{-1}$)	0.814	0.000	0.740	0.000	0.998	0.105
LDL ($mg\ dL^{-1}$)	1.005	0.095	0.879	0.000	1.000	0.729
HDL ($mg\ dL^{-1}$)	1.204	0.000	1.086	0.000	0.999	0.513
TG ($mg\ dL^{-1}$)	0.994	0.000	0.983	0.000	1.000	0.002*
Cholesterol ($mg\ dL^{-1}$)	0.963	0.026	1.058	0.001	0.999	0.001**
GFR (mL/min)	1.218	0.000	1.220	0.000	1.002	0.000***
HbA1c (%)	0.549	0.000	0.549	0.000	0.982	0.000***
CREATININE ($mg\ dL^{-1}$)	0.770	0.001	0.681	0.000	0.993	0.103
ALBUMIN ($g\ dL^{-1}$)	0.964	0.000	0.982	0.000	1.000	0.318
HO-1 LEVEL ($ng\ mL^{-1}$)	0.167	0.000	0.668	0.000	1.008	0.451
IRON ($\mu g\ dL^{-1}$)	0.997	0.173	0.976	0.027	1.000	0.758
BILIRUBIN ($mg\ dL^{-1}$)	0.741	0.000	0.927	0.212	1.015	0.222

Table 5: Hardy-Weinberg Equilibrium (HWE) test for DN patients and control groups in *VEGF*

Groups	I/I	I/D	DD	X^2	Df	p (HW Test)
Control (n=200)	40	102	58	0.160	1	0.68
DN (n=200)	53	120	27	9.735	1	< 0.001***

(*** $p<0.001$)

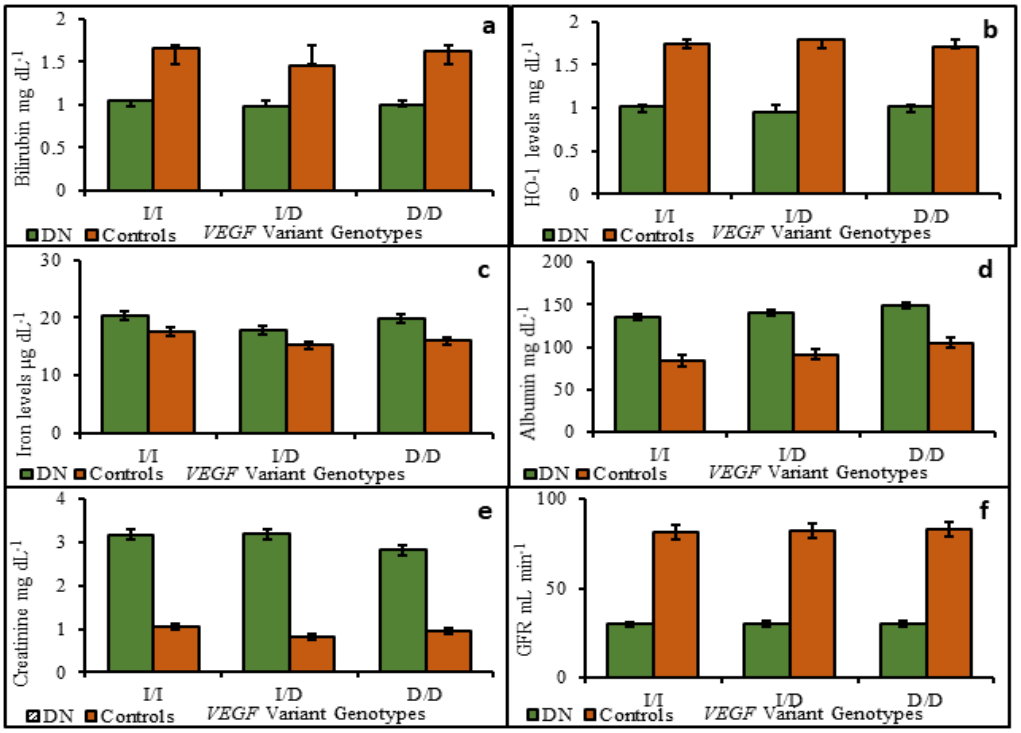

Figure 2: Comparison of biochemical and renal function markers among VEGF variant genotypes in diabetic nephropathy and control groups. Comparison of biochemical parameters among VEGF genotypes (I/I, I/D, D/D) in diabetic nephropathy (DN) patients and controls. Panels show (a) albumin (g/dL), (b) total protein (g/dL), (c) iron (µg/dL), (d) albumin (mg/L), (e) creatinine (mg/dL), and (f) GFR (mL/min). Green bars indicate DN patients and orange bars indicate controls; Data are presented as mean ± standard error (SE).

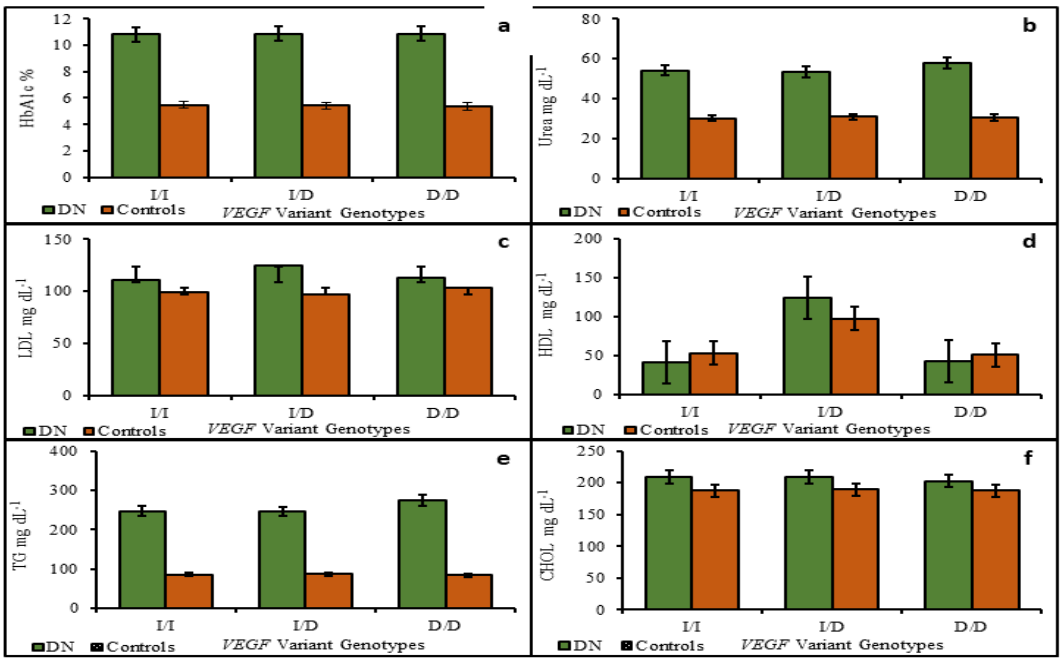

Figure 3: Clinical comparison of glycemic, renal, and lipid profile parameters among VEGF variant genotypes in diabetic nephropathy patients and controls

Clinical comparison of HbA1c (a), urea (b), LDL (c), HDL (d), triglycerides (TG) (e), and total cholesterol (CHOL) (f) among VEGF genotypes (I/I, I/D, D/D) in diabetic nephropathy (DN)

patients and controls. Green bars represent DN patients and orange bars represent controls; values are expressed as mean ± SE

Discussion

The association between diabetic nephropathy (DN) and vascular endothelial growth factor (VEGF) has received considerable attention due to VEGF's critical role in enhancing vascular endothelial permeability and modulating the glomerular filtration barrier [29, 30]. VEGF is essential for cell migration and proliferation and serves as a key regulator of vasculogenesis, angiogenesis, and vascular permeability. Evidence indicates a strong link between diabetic microvascular complications and VEGF polymorphisms [31], with single nucleotide polymorphisms in the VEGF gene associated with susceptibility to diabetes-related complications, including DN. Investigating functional genetic variants may, therefore, provide a more comprehensive understanding of DN pathogenesis. To our knowledge, no previous study has evaluated the association of the VEGF 18bp insertion/deletion (indel) variant with DN in the Pakistani population. Our study demonstrated that patients with DN had significantly higher fasting blood glucose (FBG), random blood glucose (RBG), glycated hemoglobin (HbA1c), triglycerides (TG), low-density lipoprotein (LDL), heme oxygenase-1 (HO-1), serum albumin, and glomerular filtration rate (GFR) compared to controls. These findings suggest that dysregulation of glycemic control is associated with DN risk, as FBG, RBG, and HbA1c differed significantly across groups. In contrast, age, body mass index (BMI), diastolic blood pressure (DBP), high-density lipoprotein (HDL), and total cholesterol were not significantly different, indicating these factors may not be major contributors to DN risk in this cohort. These results are consistent with previous reports [32], which highlight BMI, FBG, and RBG as key risk factors for DN; however, our finding regarding age differs from studies linking older age to increased DN susceptibility.

Genetic analysis revealed a strong association between the VEGF 18bp indel variant (rs35569394) at the −2549 promoter region and DN in individuals with type 2 diabetes mellitus (T2DM) from the Pakistani population. Significant associations were observed under both dominant and recessive models, suggesting that mutant genotypes confer altered susceptibility to DN. Although VEGF mRNA or protein levels were not assessed, these results indicate a genetic association, with no direct conclusions regarding functional effects. Patients with DN also showed elevated HbA1c, FBG, RBG, serum creatinine, and reduced bilirubin, consistent with previous reports [33], highlighting the role of poor glycemic control in microvascular complications. The observed reduction in bilirubin should be considered exploratory. Genotypic distribution differed between DN patients and controls, with the heterozygous I/D genotype more frequent among patients, suggesting a potential risk factor, while the homozygous D/D and wild-type I/I genotypes were more common in controls, indicating possible protective effects. Allelic analysis demonstrated a higher frequency of the I allele in DN patients and predominance of the D allele in controls. Co-dominant and recessive models supported these associations, suggesting a protective role of the D allele. This effect may result from functional changes in the VEGF promoter region, as the 18bp deletion can alter transcriptional activity, potentially modifying VEGF expression [34]. Given VEGF's role in vascular permeability, angiogenesis, and cell proliferation—processes implicated in diabetic microvascular complications—variants influencing VEGF expression may affect DN susceptibility [35]. The impact of VEGF promoter polymorphisms may vary across populations due to ethnic genetic differences and environmental factors, warranting further investigation into the molecular mechanisms underlying these associations.

Multivariate regression analysis demonstrated significant associations between VEGF genotypes and several clinical parameters, including systolic blood pressure (SBP), DBP, FBG, RBG, urea, HDL, TG, GFR, and HbA1c, indicating genotype-specific differences in metabolic, renal, and cardiovascular profiles. Iron levels, age, and BMI were not associated with genotype. Both I/I and

I/D genotypes showed significant associations with SBP and DBP ($p < 0.001$), suggesting higher blood pressure burden in these groups. Glycemic markers, including HbA1c, FBG, and RBG, were strongly associated with genotype ($p < 0.001$), whereas the D/D genotype showed largely non-significant associations, indicating a distinct risk profile. Renal function markers (urea and GFR) and lipid indices (HDL and TG) were also genotype-dependent. Overall, these findings suggest that VEGF genotypes influence metabolic, renal, and cardiovascular parameters, consistent with previous literature [36]. VEGF genotypes were significantly associated with FBG, RBG, HbA1c, albumin, HO-1, and renal function markers, indicating that I/D and I/I genotypes may modulate DN-related clinical traits. Although the higher frequency of the I/D genotype indicates a statistical association, the biological basis of this heterozygote effect remains unclear. Literature supports that VEGF polymorphisms contribute to diabetic complications by increasing vascular permeability and promoting angiogenesis [37]. VEGF is expressed in podocytes, mesangial cells, and tubular cells of the kidney, highlighting its role in renal physiology [38]. In diabetes, VEGF expression is reportedly increased via protein kinase C pathway activation [39]. Genetic variation in VEGF has been linked to DN susceptibility across diverse populations [40–42], and elevated renal and urinary VEGF levels correlate with disease progression, proteinuria, and declining renal function [43]. Dysregulated VEGF may contribute to endothelial dysfunction and inflammation, key features of DN [44]. Collectively, these findings support the contribution of cytokine-related genetic factors to DN pathogenesis and suggest that the VEGF 18bp indel variant may influence susceptibility, though its prognostic relevance requires further investigation.

This study has several limitations. Exclusion of T2DM patients without nephropathy limits the ability to identify DN-specific genetic variants. Multivariable regression adjusting for clinical variables was not performed, as the focus was primarily on genetic associations, and residual confounding cannot be excluded. Only a single VEGF variant was analyzed, whereas other polymorphisms may also affect DN risk. While Hardy–Weinberg equilibrium was satisfied in controls, associations involving HDL, GFR, and bilirubin should be considered hypothesis-generating and require validation in larger cohorts with appropriate statistical adjustments. Finally, due to the exploratory design and relatively modest sample size, formal correction for multiple testing was not applied; results were interpreted cautiously, focusing on strongly significant associations.

Conclusions

In this study, the VEGF 18 bp insertion/deletion variant rs35569394, located in the −2549 promoter region of the VEGF gene, showed a significant association with diabetic nephropathy (DN) in individuals with type 2 diabetes mellitus (T2DM) from the Pakistani population, supporting the involvement of cytokine-related genetic factors in the pathogenesis of DN. These findings suggest that this variant may influence susceptibility to DN; however, its prognostic significance cannot be established due to the cross-sectional design of the study. Validation in larger cohorts and ethnically diverse populations is therefore warranted. Moreover, this study evaluated only a single VEGF variant; other polymorphisms within the VEGF gene or related biological pathways may also contribute to DN risk and should be investigated in future studies.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Karachi Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi. All methods were conducted in accordance with the regulations of the University of Karachi and the principles outlined in the Declaration of Helsinki. Participants were fully informed about the study objectives and the implications of their involvement, and written informed consent was obtained via email. To ensure confidentiality, pseudonyms were used when referring to individual participants.

Human and Animal Rights

No animals were used in this study. The study on humans was conducted in accordance with the ethical rules of the Helsinki Declaration and Good Clinical Practice.

Consent for Publication

The authors confirm that this manuscript is original, unpublished, and not under review elsewhere. Written informed consent for publication was obtained from all participants where applicable, and consent documents are available upon request.

Availability of Data and Materials

All data analysed during this study are included in this published article. Associated raw data are available from the corresponding author upon reasonable request.

Funding

This study did not receive any financial support.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

The authors acknowledge the support of the Research Department at the Baqai Institute of Diabetology and Endocrinology (BIDE), Karachi, Pakistan, for their active collaboration and provision of resources for this study.

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