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RESEARCH ARTICLE OPEN ACCESS

Influence of the angiotensin-converting enzyme insertion/deletion polymorphism and oxidative stress on type 2 diabetic retinopathy

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Abstract
Objective: This study aimed to investigate the association of the angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism with glycaemic parameters and reactive oxygen species (ROS) levels in patients with type 2 diabetic retinopathy.
Methods: A case-control study was conducted involving 32 patients clinically diagnosed with diabetic retinopathy and 31 age-matched healthy controls. ACE I/D genotyping was performed using the polymerase chain reaction. Glycaemic markers, including fasting blood glucose, HbA1c, insulin, HOMA-IR, and HOMA-IS, were measured alongside ROS levels. Statistical analyses included t-tests, chi-square tests, and correlation matrices to evaluate associations between variables.
Results: Patients with diabetic retinopathy demonstrated significantly elevated glycaemic parameters compared to controls: fasting blood glucose (187.47±77.58 vs 86.68±17.86 mg/dL, p<0.001), HbA1c (8.34±2.13 vs 5.15±0.49%, p<0.001), insulin (23.12±10.0 vs 6.08±1.51 µIU/mL, p<0.001), and HOMA-IR (12.81±11.24 vs 1.29±0.37, p<0.001). ROS concentrations were markedly higher in the patient group (34.51±21.36 vs 15.36±6.74, p<0.001). ACE genotyping revealed only DD and DI genotypes across both groups, with the II genotype absent in this cohort. No significant association was detected between ACE genotype distribution and diabetic retinopathy susceptibility (p=0.701). Sex-stratified analysis showed no significant differences in genotype distribution (p=0.819) or allele frequency (p=0.528). Comparison of biochemical and oxidative stress markers between DD and DI genotypes revealed no significant genotype-dependent variations.
Conclusions: The ACE I/D polymorphism does not appear to contribute to diabetic retinopathy susceptibility in this Iraqi cohort. Despite significant metabolic dysregulation and elevated oxidative stress among patients, this genetic variant demonstrated no direct influence on disease pathogenesis.

Keywords: Diabetic Retinopathy, Angiotensin-Converting Enzyme, ACE I/D Polymorphism, Oxidative Stress, Reactive Oxygen Species, Genetic Association

Plain English Summary

Diabetic retinopathy is an eye condition that affects people with diabetes and can lead to blindness if not treated. Scientists believe that both genes and environmental factors play a role in who develops this complication. This study looked at whether a common variation in the ACE gene, which helps control blood pressure, influences the risk of developing diabetic eye disease. We compared 32 patients with diabetic retinopathy to 31 healthy individuals. We measured their blood sugar levels, insulin, and

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a marker of cell damage called reactive oxygen species. We also analysed their DNA to determine which version of the ACE gene they carried. As expected, patients with eye disease had much higher blood sugar and more cell damage than healthy individuals. However, the version of the ACE gene they carried did not affect their risk of having eye disease. This suggests that for this specific group of Iraqi patients, this particular gene variation is not a major factor in developing diabetic retinopathy. The main drivers appear to be poor blood sugar control and the resulting oxidative stress. These findings add to the ongoing scientific discussion about which genes truly matter for diabetes complications and highlight the importance of maintaining good blood sugar control to prevent eye problems.

Introduction

Diabetic retinopathy (DR) represents one of the most significant microvascular complications resulting from chronic hyperglycaemia and remains a leading cause of visual impairment and blindness among working-age adults worldwide (1). This progressive disorder affects the retinal microvasculature through complex pathological mechanisms that continue to be intensively investigated. In its early stages, DR often remains asymptomatic, making regular ophthalmologic examinations essential for timely diagnosis and appropriate clinical management (2).

The pathogenesis of diabetic retinopathy follows a characteristic progression. In non-proliferative diabetic retinopathy, the loss of capillary pericytes, increased vascular permeability, and vascular endothelial alterations lead to the formation of microaneurysms and dot-blot intraretinal haemorrhages. As the disease advances, vasoconstriction and capillary occlusion result in tortuous capillaries, retinal ischemia, and the appearance of cotton-wool spots. The advanced proliferative stage is characterised by severe hypoxia that stimulates pathological neovascularisation, which may ultimately lead to vitreous haemorrhage and retinal detachment (2, 3).

Diabetic retinopathy exemplifies a multifactorial and complex disease influenced by the interplay of environmental factors, metabolic control, and polygenic susceptibility. Although diabetes mellitus has well-established risk factors, including disease duration, hyperglycaemia, and hypertension, not all individuals with diabetes develop retinopathy. This clinical heterogeneity suggests that genetic variation contributes substantially to disease susceptibility and progression (4, 5, 6, 7). Identifying these genetic determinants remains a priority for understanding disease mechanisms and developing targeted preventive strategies.

Among the genes implicated in diabetic complications, the angiotensin-converting enzyme gene has attracted considerable research attention. ACE encodes the enzyme responsible for converting angiotensin I to angiotensin II, a potent vasoconstrictor that promotes vascular endothelial growth factor (VEGF) production and triggers pro-angiogenic cascades. Elevated intraocular angiogenesis arising from this pathway contributes directly to proliferative diabetic retinopathy and diabetic macular oedema (8). The ACE gene, located on chromosome 17q23.3, comprises 25 introns and 26 exons with approximately 160 reported variants (9). The most extensively studied polymorphism is the insertion/deletion (I/D) variant within intron 16 (rs1799752), represented by a 287-base pair Alu repetitive sequence. This polymorphism significantly influences circulating ACE concentrations and enzymatic activity, with the deletion (D) allele consistently associated with higher ACE levels compared to the insertion (I) allele (10, 11, 12).

The renin-angiotensin system (RAS) comprises angiotensinogen, angiotensin I and II, renin, angiotensin-converting enzymes 1 and 2 (ACE1 and ACE2), and angiotensin receptors type 1 and 2. Hyperactivation of ACE drives synthesis of the potent vasoconstrictor angiotensin II (13). Elevated angiotensin II disrupts glucose homeostasis by interfering with insulin signal transduction, limiting glucose uptake, increasing insulin resistance, and promoting pancreatic β-cell dysfunction through oxidative stress-mediated mechanisms (14). Hyperglycaemia in diabetic individuals further increases angiotensin II levels, which in turn trigger oxidative stress and glomerular hyperfiltration, ultimately contributing to thrombosis, endothelial injury, inflammation, and vascular remodelling (15).

Oxidative stress, characterised by excessive reactive oxygen species generation and depletion of antioxidant defences, represents a fundamental cellular mechanism implicated in the development of numerous diseases, including diabetes mellitus and its microvascular complications. In diabetic retinopathy, oxidative stress has been extensively documented as a significant contributor to disease progression through multiple pathways, including mitochondrial dysfunction, inflammatory activation, and apoptotic cell death (16). The retinal tissue is particularly vulnerable to oxidative damage due to its high metabolic rate, abundant polyunsaturated fatty acids, and constant exposure to light-induced photo-oxidation.

Despite accumulating evidence linking the RAS pathway to diabetic complications, the role of the

ACE I/D polymorphism in diabetic retinopathy remains controversial, with studies reporting conflicting findings across different populations (17, 18, 19). Some investigations have demonstrated significant associations between the D allele and increased retinopathy risk, while others have found no such relationship. These discrepancies may reflect genuine population-specific genetic effects, differences in study design, or insufficient statistical power. Furthermore, the potential interaction between ACE genetic variation and oxidative stress markers in DR pathogenesis has received limited attention.

Given this background, the present study was designed to investigate the association between the ACE I/D polymorphism, glycaemic control parameters, and circulating ROS levels in Iraqi patients with type 2 diabetic retinopathy. Understanding these relationships may contribute to the growing body of knowledge regarding genetic determinants of diabetic complications in understudied populations and potentially inform personalised risk assessment strategies.

Materials and Methods

Study Design and Participants

A case-control study design was employed to investigate the association between the ACE I/D polymorphism and diabetic retinopathy. The study population consisted of 32 patients with type 2 diabetes mellitus complicated by retinopathy and 31 apparently healthy control individuals. All patient participants were recruited from Marjan Medical City, where they had received clinical diagnoses of diabetic retinopathy, confirmed by specialist ophthalmologists following comprehensive ocular examinations, including fundoscopy. The control group comprised healthy volunteers with no personal or family history of diabetes mellitus, normal fasting blood glucose levels, and no evidence of chronic disease. Inclusion criteria for the patient group were: a confirmed diagnosis of type 2 diabetes mellitus for at least 5 years, clinical evidence of diabetic retinopathy at any stage, and age greater than 35 years. Exclusion criteria encompassed: type 1 diabetes mellitus, other retinal pathologies unrelated to diabetes, active infectious diseases, malignancy, and refusal to provide informed consent. Control participants were required to have fasting blood glucose below 100 mg/dL, HbA1c below 5.7%, and no history of chronic medical conditions.

Sample Collection and Processing

Peripheral venous blood samples (5 mL) were collected from all participants under standardised conditions following an overnight fast of 10-12 hours. Blood samples were divided into three aliquots: one containing EDTA for genomic DNA extraction, one containing fluoride oxalate for glucose estimation, and one plain tube for serum separation. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -20°C until biochemical analysis.

Biochemical Measurements

Fasting blood glucose concentrations were determined using the glucose oxidase method employing commercial kits (BioSystems, Spain) following manufacturer instructions. Glycated haemoglobin (HbA1c) was measured by ion-exchange chromatography using commercially available kits. Serum insulin levels were quantified by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Monobind, USA) with an analytical sensitivity of 0.75 µIU/mL and an intra-assay coefficient of variation below 10%.

Insulin resistance and insulin sensitivity were calculated using the homeostatic model assessment (HOMA) approach according to standard formulas (20):

HOMA-IR (insulin resistance) = [fasting insulin (µIU/mL) × fasting glucose (mg/dL)] / 405

HOMA-IS (insulin sensitivity) = 1 / HOMA-IR

Reactive oxygen species levels were determined using a colourimetric assay based on the method described by Erel (21). This method measures total oxidant status through the oxidation of ferrous ions to ferric ions in the presence of oxidants in the sample. Results were expressed in arbitrary units (µmol H_2O_2 equivalent/L).

DNA Extraction and ACE Genotyping

Genomic DNA was extracted from whole blood samples using a commercial extraction kit (Cat. No. FABGK100, Favorgen Biotech Corp., Taiwan) according to the manufacturer's protocols. DNA concentration and purity were assessed by spectrophotometry at 260/280 nm, with samples showing ratios between 1.8 and 2.0 considered acceptable for downstream applications.

The ACE I/D polymorphism (rs1799752) was genotyped using polymerase chain reaction with primer sequences previously described (22). The primer set consisted of forward primer (F): 5′-CCCATCCTTTCTCCCATTTCTC-3′, and reverse primers: 5′-GGTTTCACCGTTTTAGCCGGGA-3′ and 5′-CCATGCCCATAACAGGTCTTCA-3′. The inclusion of two reverse primers enables confirmation of the DD genotype and reduces the risk of mistyping heterozygous samples. PCR amplification was performed in a 25 µL reaction volume containing 100 ng genomic DNA, 10 pmol of each primer, 200 µM dNTPs, 1.5 mM $MgCl_2$,

and 1 U Taq DNA polymerase in standard buffer. Thermal cycling conditions included initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 40 seconds, extension at 72°C for 45 seconds, and final extension at 72°C for 7 minutes.

PCR products were separated by electrophoresis on 1.5% agarose gels stained with ethidium bromide, run at 70 V for 40 minutes, and visualised under ultraviolet transillumination. The insertion allele (I) produced a 490 bp fragment, while the deletion allele (D) produced a 190 bp fragment. Samples showing only the 190 bp fragment were classified as DD genotype, those with both 190 bp and 490 bp fragments as DI genotype, and those with only the 490 bp fragment as II genotype. To confirm DD genotyping results and exclude potential mistyping of DI as DD due to preferential amplification of the smaller D allele, all samples initially typed as DD underwent a second independent PCR amplification and analysis.

Statistical Analysis

Data were analysed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation. Normality of distribution was assessed using the Shapiro-Wilk test. Comparisons between patient and control groups were performed using an independent samples t-test for normally distributed variables and a Mann-Whitney U test for non-normally distributed variables. Categorical variables, including genotype distributions and allele frequencies, were compared between groups using chi-square (χ^2) tests or Fisher's exact test where appropriate.

Odds ratios (OR) with 95% confidence intervals (CI) were calculated to estimate the strength of association between ACE genotypes and diabetic retinopathy risk. Hardy-Weinberg equilibrium was assessed in the control group using the chi-square goodness-of-fit test. Correlation analyses were performed using Pearson's correlation coefficient for normally distributed variables and Spearman's rank correlation for non-normally distributed variables. A p-value less than 0.05 was considered statistically significant for all analyses.

Results

Baseline Characteristics of Study Participants

The demographic and clinical characteristics of the study population are presented in Table 1. The patient group comprised 32 individuals with type 2 diabetic retinopathy, while the control group included 31 healthy participants. Significant differences were observed between groups across all measured parameters. Patients were older than controls (56.43±9.75 vs 40.03±9.85 years, p<0.001) and had higher body mass index (30.36±5.53 vs 27.40±4.54 kg/m², p=0.023), reflecting the association between obesity and type 2 diabetes. All glycaemic parameters differed markedly between groups. Patients demonstrated substantially elevated fasting blood glucose (187.47±77.58 vs 86.68±17.86 mg/dL, p<0.001) and HbA1c levels (8.34±2.13 vs 5.15±0.49%, p<0.001), indicating poor long-term glycaemic control. Fasting insulin concentrations were significantly higher in the patient group (23.12±10.0 vs 6.08±1.51 µIU/mL, p<0.001), consistent with the insulin resistance characteristic of type 2 diabetes. Consequently, HOMA-IR was markedly elevated in patients (12.81±11.24 vs 1.29±0.37, p<0.001), while HOMA-IS was correspondingly reduced (0.29±0.09 vs 0.37±0.01, p<0.001). The mean duration of diabetes among patients was 10.22±6.56 years.

Notably, reactive oxygen species levels were significantly higher in the patient group compared to controls (34.51±21.36 vs 15.36±6.74, p<0.001), confirming the presence of enhanced oxidative stress in individuals with diabetic retinopathy.

Table 1: Demographic and Biochemical Characteristics of Study Groups

Variables	Control Group (n=31) Mean ± SD	Patient Group (n=32) Mean ± SD	t-value	p-value
Age (years)	40.03±9.85	56.43±9.75	6.64	<0.001
BMI (kg/m²)	27.40±4.54	30.36±5.53	2.31	0.023
HbA1c (%)	5.15±0.49	8.34±2.13	8.1	<0.001
FBG (mg/dL)	86.68±17.86	187.47±77.58	7.02	<0.001
Insulin (µIU/mL)	6.08±1.51	23.12±10.0	9.37	<0.001
HOMA-IS	0.37±0.01	0.29±0.09	4	<0.001
HOMA-IR	1.29±0.37	12.81±11.24	5.7	<0.001
ROS (µmol H_2O_2 eq/L)	15.36±6.74	34.51±21.36	4.3	<0.001
Diabetes Duration (years)	—	10.22±6.56	—	—

BMI: Body Mass Index; FBG: Fasting Blood Glucose; HOMA-IS: Homeostatic Model Assessment-Insulin Sensitivity; HOMA-IR: Homeostatic Model Assessment-Insulin Resistance; ROS: Reactive Oxygen Species

Correlation Analysis of Study Variables

Correlation analyses revealed distinct patterns of association among study variables between the two groups, as illustrated in Table 2. The patient group demonstrated a tightly integrated pattern among glycaemic parameters reflecting the profound metabolic dysregulation characteristic of diabetic retinopathy. In patients, strong positive correlations were observed between fasting blood glucose and insulin (r=0.745, p<0.001), and between insulin and HOMA-IR (r=0.949, p<0.001). Conversely, strong negative correlations were evident between fasting blood glucose and HOMA-IS (r=-0.925, p<0.001), and between HOMA-IS and HOMA-IR (r=-0.906, p<0.001). These relationships confirm the expected pathophysiological connections among hyperglycaemia, compensatory hyperinsulinemia, and insulin resistance in the diabetic state.

Age in the patient group correlated positively with HOMA-IS (r=0.564, p=0.002), an unexpected finding that may reflect survival bias or treatment effects in older patients with longer disease duration. No significant correlations were detected between ROS levels and any glycaemic parameters in either group, suggesting that oxidative stress, while elevated in patients, operates partially independently of the measured glycaemic indices.

In contrast, the control group displayed more modest and physiologically expected correlations. Age correlated positively with BMI (r=0.391, p=0.029) and HbA1c (r=0.474, p=0.007), reflecting the gradual decline in glucose tolerance with advancing age even in healthy individuals. Fasting blood glucose correlated negatively with HOMA-IS (r=-0.591, p<0.001) and positively with HOMA-IR (r=0.566, p=0.001), maintaining the normal inverse relationship between insulin sensitivity and glucose regulation. Insulin correlated strongly with HOMA-IR (r=0.778, p<0.001) and negatively with HOMA-IS (r=-0.728, p<0.001), as expected.

The correlation matrix highlights the fundamental differences in metabolic regulation between healthy individuals and those with diabetic retinopathy, with the patient group exhibiting amplified relationships consistent with severe insulin resistance and β-cell stress.

Table 2: Correlation Matrix for Study Variables in Patient and Control Groups

Variable	Group	AGE	BMI	HbA1c	FBG	IN	HOMA-IS	HOMA-IR	ROS
AGE	Patient	1	0.057	0.182	0.183	0.017	0.564**	0.114	0.02
	Control	1	0.391*	0.474**	0.385*	-0.088	-0.207	0.161	-0.073
BMI	Patient		1	0.007	0.208	0.314	-0.303	0.21	0.216
	Control		1	0.312	0.202	0.136	-0.24	0.248	0.15
HbA1c	Patient			1	0.003	-0.082	0.034	-0.183	-0.084
	Control			1	0.349	-0.068	-0.224	0.174	-0.256
FBG	Patient				1	0.745**	-0.925**	0.883**	-0.06
	Control				1	-0.074	-0.591**	0.566**	0.244
IN	Patient					1	-0.295	0.949**	-0.065
	Control					1	-0.728**	0.778**	0.133
HOMA-IS	Patient						1	-0.906**	-0.171
	Control						1	-0.967**	-0.199
HOMA-IR	Patient							1	-0.063
	Control							1	0.26
ROS	Patient								1
	Control								1

*Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level

Patient group correlations are presented in the upper row for each variable; Control group correlations
in the lower row

ACE I/D Genotype Distribution

Genotyping of the ACE I/D polymorphism revealed the presence of DD and DI genotypes in both study groups, while the II genotype was not detected in any participant (Figure 1). This absence of the II genotype represents an important observation that may reflect population-specific allele frequencies or the relatively modest sample size.

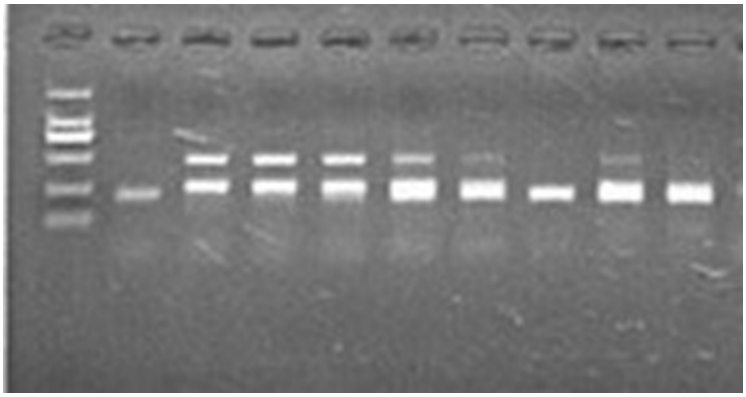


Figure 1: Agarose Gel Electrophoresis of ACE I/D Polymorphism PCR Products
Lane M: 100 bp DNA ladder. Lanes 1, 2, 4, 7, 8, 12, and 13 show a single band at 190 bp, representing the DD genotype. Lanes 3, 9, 10, and 11 show two bands at 490 bp and 190 bp, representing the DI genotype. No II genotype (single 490 bp band) was observed in this study

The distribution of ACE genotypes and alleles between patient and control groups is presented in Table 3. Among patients, 16 individuals (50.0%) carried the DD genotype, and 16 (50.0%) carried the DI genotype. In the control group, 17 individuals (54.8%) had the DD genotype and 14 (45.2%) had the DI genotype. Statistical analysis revealed no significant difference in genotype distribution between the two groups (χ^2=0.148, p=0.701). The odds ratio for diabetic retinopathy associated with the DD genotype compared to the DI genotype was 1.21 (95% CI: 0.45-3.27), indicating no significant increase in disease risk.

Allele frequency analysis demonstrated that the D allele was present at frequencies of 0.75 in patients and 0.77 in controls, while the I allele frequencies were 0.25 and 0.23, respectively. This difference was not statistically significant (χ^2=0.051, p=0.822). The control group genotype distribution was consistent with Hardy-Weinberg equilibrium expectations (p>0.05).

Table 3: ACE Genotype and Allele Distribution in Study Groups

ACE Genotype/Allele	Patient Group n=32 (%)	Control Group n=31 (%)	Odds Ratio (95% CI)	p-value
Genotype				
DD	16 (50.0)	17 (54.8)	1.21 (0.45-3.27)*	0.701
DI	16 (50.0)	14 (45.2)	Reference	
II	0 (0.0)	0 (0.0)	—	
Allele				
D	48 (75.0)	48 (77.4)	0.87 (0.30-2.52)**	0.822
I	16 (25.0)	14 (22.6)	Reference	

*Odds ratio for DD vs DI genotype; **Odds ratio for D vs I allele

Sex-Stratified Analysis of Genotype Distribution

Sex-stratified analysis was performed to evaluate potential gender-specific effects of the ACE polymorphism, with results presented in Table 4. In the patient group, the DD genotype was equally distributed between males and females (8 individuals each, representing 25% of the total patient group). Similarly, the DI genotype showed equal distribution between sexes (8 males, 8 females). In the control group, the DD genotype was observed in 7 males (22.6%) and 10 females (32.3%), while the DI genotype was present in 8 males (25.8%) and 6 females (19.4%).

Statistical analysis confirmed no significant difference in genotype distribution between males and females across both groups (χ^2=0.925, p=0.819). Similarly, allele frequency analysis revealed no sex-specific differences in D or I allele carriage (χ^2=2.217, p=0.528). These findings indicate that the ACE I/D polymorphism distribution is independent of sex in this study population, and any potential effect on diabetic retinopathy risk would not be expected to differ between males and females.

Table 4: Sex-Stratified ACE Genotype and Allele Distribution

ACE Genotype/Allele	Patient Group		Control Group			
	Male n=16 (%)	Female n=16 (%)	Male n=15 (%)	Female n=16 (%)	χ^2	p-value
Genotype					0.925	0.819
DD	8 (25.0)	8 (25.0)	7 (22.6)	10 (32.3)		
DI	8 (25.0)	8 (25.0)	8 (25.8)	6 (19.4)		
Allele					2.217	0.528
D	0.75	0.75	0.73	0.81		
I	0.25	0.25	0.27	0.19		

Values for genotypes represent the number of individuals with the percentage of the total group in parentheses; allele values represent frequency

Association Between ACE Genotypes and Biochemical Parameters

To determine whether the ACE genotype influences the biochemical and oxidative stress profiles within each group, we compared all measured parameters between DD and DI genotype carriers (Table 5). In the control group, no significant differences were observed between DD and DI genotypes for any parameter, although insulin approached statistical significance (5.62±0.76 vs 6.64±1.99 µIU/mL, p=0.062) and HOMA-IR showed a trend toward higher values in DI carriers (1.44±0.44 vs 1.17±0.27, p=0.052). These marginal differences may reflect chance findings given the multiple comparisons performed.

In the patient group, no significant differences emerged between DD and DI genotype carriers for any measured parameter. Fasting blood glucose, HbA1c, insulin, HOMA-IS, HOMA-IR, ROS, and BMI were all comparable between the two genotype groups. Notably, ROS levels were nearly identical between DD and DI patients (34.92±17.42 vs 34.10±25.29, p=0.916), indicating that the ACE genotype does not influence the degree of oxidative stress in this patient population.

These findings demonstrate that despite the well-established relationship between ACE I/D polymorphism and circulating ACE enzyme levels, this genetic variation does not translate into detectable differences in glycaemic control, insulin resistance, or oxidative stress markers in either healthy individuals or patients with diabetic retinopathy.

Table 5: Association of ACE Genotypes with Biochemical Parameters

Variable	Control Group (Mean ± SD)			Patient Group (Mean ± SD)		
	DD (n=17)	DI (n=14)	p-value	DD (n=16)	DI (n=16)	p-value
FBG (mg/dL)	85.14±18.22	80.55±17.92	0.606	207.15±84.01	170.61±70.18	0.239
HbA1c (%)	5.19±0.53	5.10±0.45	0.636	8.09±2.62	8.58±1.54	0.537
Insulin (µIU/mL)	5.62±0.76	6.64±1.99	0.062	25.43±12.16	20.81±6.90	0.196
HOMA-IS	0.37±0.014	0.36±0.01	0.11	0.31±0.14	0.28±0.018	0.488
HOMA-IR	1.17±0.27	1.44±0.44	0.052	16.31±14.21	9.81±7.16	0.145
ROS (µmol H_2O_2 eq/L)	14.04±3.80	16.78±8.90	0.319	34.92±17.42	34.10±25.29	0.916
BMI (kg/m²)	28.59±4.91	25.90±3.72	0.109	31.09±5.02	29.63±6.06	0.465

Discussion

The present study investigated the association between the ACE I/D polymorphism, glycaemic parameters, and oxidative stress markers in patients with type 2 diabetic retinopathy compared to healthy controls. Our findings demonstrate that while patients with diabetic retinopathy exhibit profound metabolic dysregulation characterised by hyperglycaemia, hyperinsulinemia, insulin resistance, and elevated oxidative stress, the ACE I/D polymorphism shows no significant association with disease susceptibility or with the measured biochemical parameters.

The marked elevation in glycaemic parameters observed in our patient cohort aligns consistently with previous literature documenting the central role of chronic hyperglycaemia in diabetic retinopathy pathogenesis (23, 24). The significantly higher HbA1c levels in patients (8.34% vs 5.15%) reflect prolonged exposure to elevated glucose concentrations, which initiates multiple biochemical pathways within retinal tissue, including advanced glycation end-product formation, protein kinase C activation, and polyol pathway flux. These metabolic disturbances collectively contribute to retinal ischemia, inflammation, and the creation of a pro-

angiogenic environment that ultimately manifests as the characteristic vascular pathology of diabetic retinopathy (25, 26).

The substantially elevated HOMA-IR values in our patient group (12.81 vs 1.29) confirm the profound insulin resistance characteristic of type 2 diabetes and extend this understanding to the specific context of retinopathy. Insulin resistance contributes to diabetic complications through multiple mechanisms beyond simple hyperglycaemia, including direct effects on endothelial function, inflammatory mediator production, and growth factor signalling (27). The strong correlations observed among FBG, insulin, HOMA-IR, and HOMA-IS in the patient group reflect the integrated nature of these metabolic disturbances, while their absence in controls confirms that these relationships are pathophysiological rather than physiological.

The significantly elevated ROS levels in patients with diabetic retinopathy (34.51 vs 15.36) provide further evidence for the role of oxidative stress in disease pathogenesis. The retina is particularly vulnerable to oxidative damage due to its high oxygen consumption, abundant polyunsaturated fatty acids, and constant light exposure. Hyperglycaemia-induced oxidative stress arises from multiple sources, including mitochondrial electron transport chain dysfunction, NADPH oxidase activation, and uncoupling of nitric oxide synthase (28). The resulting oxidative damage to retinal capillary endothelial cells and pericytes contributes to the characteristic vascular changes of diabetic retinopathy (29). Our finding that ROS levels did not correlate significantly with glycaemic parameters suggests that oxidative stress in diabetic retinopathy may be influenced by additional factors beyond current glycaemic control, potentially including disease duration, genetic determinants of antioxidant capacity, and cumulative glycaemic exposure.

The absence of a significant association between the ACE I/D polymorphism and diabetic retinopathy in our study contributes to an ongoing scientific dialogue characterised by conflicting findings. While some previous investigations have reported positive associations, our results align with a substantial body of evidence suggesting that this variant may not independently influence retinopathy risk, particularly in Asian populations. The meta-analysis by Luo et al. (4) encompassing 10,168 subjects found no overall association between ACE I/D polymorphism and diabetic retinopathy, though subgroup analyses revealed some heterogeneity across ethnic groups. Similarly, Coelho and colleagues (19) in an updated meta-analysis concluded that the ACE I/D polymorphism does not represent a risk factor for diabetic retinopathy in type 2 diabetes patients.

Our findings contrast with several earlier reports from Middle Eastern populations. Feghhi et al. (30) reported a significant association between the II genotype and proliferative diabetic retinopathy in an Iranian cohort (p=0.015), while Nikzamir et al. (31) observed a significant association in the DI versus II comparison (p=0.026). Matsumoto et al. (32) identified a significant correlation between the D allele and proliferative diabetic retinopathy (p=0.041) in a Japanese population. In a Pakistani cohort, Saleem et al. (33) found that the ACE I/D variant was strongly associated with non-proliferative diabetic retinopathy compared to the proliferative form. These discrepancies may reflect genuine population-specific genetic effects, differences in linkage disequilibrium patterns, variation in retinopathy severity classification, or differences in environmental exposures that modify genetic effects.

Several factors may explain our null findings. First, the absence of the II genotype in our study population, while consistent with previous reports of high D allele frequency in Middle Eastern populations, limited our ability to assess the full range of genotype-phenotype relationships. The II genotype has been associated with the lowest ACE activity levels (11), and its absence may have reduced our statistical power to detect genotype-dependent differences. Second, our sample size, while comparable to several published studies, may have been insufficient to detect modest genetic effects. Given the multifactorial nature of diabetic retinopathy, individual genetic variants are likely to exert small effect sizes that require large sample sizes for reliable detection. Third, we did not stratify patients by retinopathy severity, and ACE genotype may influence specific subtypes of retinopathy (e.g., proliferative vs non-proliferative) rather than disease presence per se.

The lack of association between ACE genotype and ROS levels in our study warrants comment. Given the established relationship between angiotensin II and oxidative stress through NADPH oxidase activation (34), we hypothesised that genotype-dependent differences in ACE activity might translate into measurable differences in systemic oxidative stress markers. The absence of such an association suggests that either the relationship between ACE genotype and oxidative stress is tissue-specific rather than systemic, or that other genetic and environmental factors exert dominant effects on circulating ROS levels in this population.

The similar biochemical profiles between DD and DI genotype carriers within both patient and control groups provide important negative evidence. Despite the well-documented effect of

the I/D polymorphism on circulating ACE activity, with DD carriers having approximately twice the ACE levels of II carriers (11, 12), this variation did not translate into detectable differences in glycaemic control or insulin resistance. This finding suggests that the metabolic effects of the RAS system may be mediated through local tissue ACE activity rather than circulating levels, or that compensatory mechanisms buffer the effects of genetically-determined ACE variation on glucose homeostasis.

Study Limitations and Future Directions

Several limitations of this study should be acknowledged when interpreting our findings. First, the relatively small sample size limits statistical power and increases the risk of type II error, particularly for detecting modest genetic effects. The wide confidence intervals around our odds ratio estimates reflect this limited precision. Second, the absence of the II genotype in our study population prevented a complete assessment of the genotype-phenotype relationship and comparison with studies where this genotype is more prevalent. Third, we did not measure circulating ACE levels, which would have confirmed the functional consequences of the I/D polymorphism in our population and allowed direct correlation with biochemical parameters. Fourth, the cross-sectional design precludes assessment of temporal relationships between genotype, oxidative stress, and retinopathy development. Fifth, we did not control for potential confounders, including hypertension, medication use (particularly ACE inhibitors or angiotensin receptor blockers), and duration of diabetes beyond the reported mean. Sixth, retinopathy severity was not systematically graded, potentially masking genotype associations with specific disease stages.

Future research should address these limitations through larger, prospective studies with adequate power to detect modest genetic effects. Measurement of circulating ACE activity alongside genotyping would confirm the functional consequences of the I/D polymorphism in the study population. Comprehensive assessment of retinopathy severity using standardised grading systems (e.g., Early Treatment Diabetic Retinopathy Study criteria) would enable evaluation of genotype effects on disease progression rather than simply disease presence. Inclusion of other RAS pathway genes (AGT, AGTR1, AGTR2, ACE2) in a pathway-based analytic approach may reveal interactive effects that single-variant analyses miss. Finally, longitudinal studies examining the relationship between ACE genotype, oxidative stress trajectories, and incident retinopathy would provide stronger evidence for causal relationships.

Conclusion

The present study demonstrates that patients with type 2 diabetic retinopathy exhibit profound metabolic dysregulation characterised by hyperglycaemia, hyperinsulinemia, insulin resistance, and elevated oxidative stress compared to healthy controls. However, the ACE I/D polymorphism shows no significant association with disease susceptibility, and genotype does not influence the degree of glycaemic disturbance or oxidative stress within either patient or control groups. These findings suggest that despite the established role of the renin-angiotensin system in diabetic complications, the common I/D polymorphism in the ACE gene does not independently contribute to retinopathy risk in this Iraqi cohort. The primary drivers of diabetic retinopathy in this population remain poor glycaemic control and the consequent oxidative stress, reinforcing the clinical importance of intensive glucose management for preventing this sight-threatening complication.

List of Abbreviations

ACE: Angiotensin-Converting Enzyme
AGT: Angiotensinogen
AGTR1: Angiotensin II Receptor Type 1
AGTR2: Angiotensin II Receptor Type 2
BMI: Body Mass Index
CI: Confidence Interval
DNA: Deoxyribonucleic Acid
DR: Diabetic Retinopathy
EDTA: Ethylenediaminetetraacetic Acid
ELISA: Enzyme-Linked Immunosorbent Assay
FBG: Fasting Blood Glucose
HOMA-IR: Homeostatic Model Assessment of Insulin Resistance
HOMA-IS: Homeostatic Model Assessment of Insulin Sensitivity
I/D: Insertion/Deletion
OR: Odds Ratio
PCR: Polymerase Chain Reaction
RAS: Renin-Angiotensin System
ROS: Reactive Oxygen Species
SPSS: Statistical Package for Social Sciences
VEGF: Vascular Endothelial Growth Factor

Declarations

Ethics Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Biology Department, College of Science, University of Babylon (Approval Code: B240502, dated 3 May 2024). Written informed consent was obtained from all individual participants included in the study.

Consent for Publication
All authors have reviewed the final manuscript and given their consent for publication. The work will be published under the Creative Commons Attribution Non-Commercial 4.0 license.

Data Availability
The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests
The authors declare that they have no competing interests.

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Author Contributions
AZMS: Conceptualisation, Investigation, Writing - Original Draft
ANAH: Methodology, Formal Analysis, Investigation
HNM: Investigation, Data Curation, Visualisation
MAH: Resources, Validation, Writing - Review & Editing
AMN: Conceptualisation, Supervision, Project Administration, Writing - Review & Editing

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