Evaluation of Angiotensin Converting Enzyme (Insertion/Deletion) and Angiotensin II Type 1 Receptor (A1166C) Polymorphisms and Renoprotective Response to Angiotensin Receptor Blockers in Iraqi Patients with Diabetic Kidney Disease

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Abstract

Objectives: This study evaluated whether these variants are associated with the renoprotective response to angiotensin II receptor blockers(ARBs) in Iraqi patients with type 2 diabetes mellitus(T2DM).

Methods: a cross-sectional study conducted at the Diabetes and Endocrinology Center, Merjan Medical City, Babylon, Iraq, between March 2022 and January 2023. Eighty-five hypertensive T2DM patients on ARBs (losartan 50–100 mg or candesartan 8–16 mg) were enrolled and grouped by albumin-to-creatinine ratio(ACR)into normoalbuminuric(<30 mg/g; n=32) and DKD(≥30 mg/g; n=53) groups. ACR was measured on random spot urine; eGFR was calculated by the CKD-EPI 2021 creatinine–cystatin C equation. Serum cystatinC CysC), kidney injury molecule-1(KIM-1), ACE1 activity, and ACE2 concentration were quantified by ELISA. ACE (I/D) was genotyped using high-resolution melting real-time PCR; AGTR1(A1166C) by PCR–RFLP. Group comparisons used independent t-test/Mann–Whitney/Kruskal–Wallis and chi-square; logistic regression assessed associations (adjusted for age, sex, DKD) with significance at $p<0.05$.

Results: Compared with normoalbuminuric patients, the DKD group had higher HbA1c(P=0.017), urine albumin and ACR, higher serum CysC, and lower eGFR; KIM-1 did not differ significantly. Retinopathy was more frequent in DKD (P<0.001). Genotype distributions did not differ between groups (ACE (I/D) P=0.146; AGTR1 (A1166C) P=0.501). Across ACE (I/D) and AGTR1 genotypes, renal biomarkers (including eGFR and ACR) and ACE1,ACE2 levels showed no significant differences, indicating similar renoprotective response to ARBs; in adjusted analysis. ACE (I/D) and AGTR1 (A1166C) did not predict ACE1 level with OR=0.442, and P=0.205.

Conclusions: In ARB-treated Iraqi T2DM patients, ACE(I/D) and AGTR1(A1166C) polymorphisms were not associated with differential renoprotective response. Among the evaluated clinical variables, poor glycemic control and coexisting microvascular complications remained the most prominent clinical correlates of DKD status, highlighting their continued clinical relevance in the assessment of patients with diabetic kidney disease .

Keywords: diabetic kidney disease, ACE (I/D), AGTR1 (A1166C), angiotensin receptor blockers, albumin creatinine ratio.

Introduction

Diabetic kidney disease (DKD) is one of the most common and serious microvascular complications of diabetes mellitus, which defined by persistent abnormalities in kidney structure or function, including albuminuria and/or reduced estimated glomerular fiterationrate (eGFR), for at least 3 months. According to KDIGO, albuminuria is categorized as A1 (<30 mg/g), A2 (30–300 mg/g), and A3 (>300 mg/g) using the urine albumin-to-creatinine ratio(ACR), it represents the leading cause of end-stage renal disease (ESRD) worldwide, which has been associated with a higher incidence of mortality and morbidity among diabetic patients.[1,2] The pathogenesis of DKD involve a number of pathways, including metabolic, inflammatory, and hemodynamic pathways, leading to initiation of a complicated cascade of pathological events.[1] The renin-angiotensin-aldosterone system (RAAS) is a multifaceted system that controls internal glomerular pressure in addition to blood pressure, moreover hypertension consider as an independent risk factor for DKD. Hence RAAS blockade is the cornerstone of DKD treatment.[3,4] A lot of interest has been gained for epigenetic role in DKD pathogenesis. One of the mostly expressed gene in the kidney was angiotensin-converting enzyme (ACE) gene and because of ACE's critical function in the RAAS, in addition to several research on its polymorphisms relations to the development of diabetic microvascular complication such as DKD.[5] Thus, polymorphisms in RAAS-related genes such ACE and Ang II receptor type 1 (AGTR1) are linked to development of DKD[6] because ACE (I/D) is the most DKD vulnerable locus, It has been assumed that the ACE (I/D) polymorphisms represent a functional polymorphism which affects activity of both tissue and circulating ACE1. The differences in plasma and tissues ACE1 activity associated with the ACE (I/D) genotype are likely to affect the antiproteinuric (renoprotective) responses to inhibit RAAS.[7] A growing evidence of data suggests a link between the occurrence of DN and AGTR1 (A1166C) single nucleotide polymorphism (SNP).[8]

Traditionally, ACEIs and ARBs have been routinely prescribed in daily clinical nephrology practice to reduce proteinuria in patients with diverse renal disorders, including diabetes. However, the antiproteinuric impact of ACEIs on proteinuria is varied, with reductions in proteinuria ranging from 20 to 80% in a variety of renal diseases.[9]

A study on the effect of ACE (I/D) on ARB response found that comparing to placebo, ARB is useful in reducing proteinuria in T2DM patients with nephropathy and appears to be effective in patients carrying the II and DD genotypes of the ACE gene.[10] Whereas another study on a larger population discovered that the deletion allele of the ACE gene had a harmful impact on the composite endpoint. The study's unique clinical significance is that losartan had the highest effect on individuals with the greatest need for renoprotective medication (DD and ID genotype). Patients with a better renal prognosis (II genotype) also benefited from renoprotection.[11] Another study found that ACE DD genotype and a genetic risk score of >6 were linked with greater renoprotective response to ARB in T2DM with nephropathy.[12]

Many investigations have found a link between ARB responsiveness and the AGTR1 (A1166C) polymorphism. More recent data indicate that carriers of the C allele of the AGTR1 (A1166C) polymorphism show a more favorable antihypertensive response to valsartan, while earlier studies also suggested that this polymorphism may affect renal and humoral hemodynamic responses to AGTR1 blockade.[13]

The C allele has been linked with enhanced Ang II activity, increased circulating Ang II levels, or heightened responsiveness to Ang II.[13,14] Consequently, individuals carrying the C allele may have reduced therapeutic effects from ARB medication compared to AA individuals this could be result from inadequate ARB block. This also implies that C carriers may be less vulnerable to the blocked effects of lower doses of ARB.[15] Evidence from another study indicated that losartan therapy significantly reduced daily albuminuria in both AA and AC genotype groups.[16] Following short-term therapy such reductions of albuminuria are a hemodynamic phenomenon reflecting the highest reduction in Filtration Fraction (FF) and intraglomerular pressure at the same genotype.[17] However, other studies failed to demonstrate a link between the AGTR1 (A1166C) polymorphism and albuminuria reduction in patients treated with telmisartan for hypertension or with losartan in non-diabetic individuals with renal disease.[18]

Unlike longitudinal pharmacogenetic studies, the present cross-sectional study evaluates the association of ACE and AGTR1 polymorphisms with current renal and albuminuric status in ARB-treated patients with diabetic kidney disease, rather than changes in ACR or eGFR over time.

The objective of the present study to investigate the association of the ACE (I/D) and AGTR1 (A1166C) gene polymorphisms with renoprotective effect of ARB medications.

Methods

This a cross-sectional study was conducted at the Diabetes and Endocrinology Center, Merjan Medical City in Babylon, Iraq, from March\2022 through January\2023. The study enrolled 85 individuals with a confirmed history of both type 2 diabetes mellitus (T2DM) and hypertension, with a minimum diabetes duration of five years and ongoing treatment with angiotensin II receptor blockers (ARBs). Among these participants, 47 patients were receiving losartan at doses ranging between 50 and 100 mg, while 28 were administered candesartan in doses between 8 and 16 mg. Adherence to ARB therapy was assessed using the Morisky Medication Adherence Scale and any patient have less than 6 score excluding from the study. Additionally, patients using other medications known to influence albuminuria, including SGLT2 inhibitors and calcium channel blockers, also, patients with other clinical conditions that could affect albuminuria such uncontrolled hypertension, urinary tract infection, inflammatory renal disease and autoimmune renal disease were also excluded from the study.

Based on urinary albumin-to-creatinine ratio (ACR), patients were categorized into two groups:

1. Group 1: comprised 32 T2DM patients using ARB medications and ACR < 30mg\g.

2. Group 2: comprised 53 T2DM patients using ARB medications and ACR ≥ 30mg\g.

To assess ACR, a random urine sample was collected from each participant. Urinary creatinine was quantified using a kinetic colorimetric method (Jaffe reaction) involving alkaline picrate and measured at a wavelength of 490 nm (Biolab kit),[19] while urinary albumin levels were determined using the Abnova BCG Albumin kit, which employs bromocresol green dye to form a specific color complex with albumin, measured at 620 nm.[20] Detailed methodology for these assays is available in reference.[21] ACR values were calculated by dividing urine albumin (mg/L) by urine creatinine (g/L). Blood pressure measurements were taken using a standard manual sphygmomanometer at the same clinical center.

Ethical approval for this study was obtained from the Research Ethics Committee of the University of Baghdad – College of Pharmacy (Approval No. RECAUBCP4102021A, dated 4/10/2021). Additional approval was secured from the relevant medical institutions, with official acceptance granted by the Research Unit of the Center for Training and Human Development, Babylon Health Directorate, Babylon Province (Decision No. 26, dated 8/3/2022). Verbal informed consent was obtained from all participating patients prior to specimen collection.

Following an overnight fast, venous blood samples were collected from patients while seated, using sterile, disposable syringes. A total of 10 mL of blood was drawn from each patient. Of this volume, 2 mL were transferred to EDTA tubes—1 mL was analyzed immediately for glycated hemoglobin (HbA1c) levels using the Cobas e411 analyzer, while the second 1 mL aliquot was stored at -80°C for subsequent genotyping. The remaining 8 mL were dispensed into a clot-activator tube without anticoagulant, allowed to clot for 10–15 minutes, and then centrifuged to isolate serum. A portion of this serum was directly analyzed for fasting blood glucose (FBS), serum creatinine, and lipid profile using a fully automated Kromo Linear analyzer operated by trained staff at the Diabetes and Endocrinology Center.

Residual serum was stored in eppendorf tubes at –80°C for later measurement of serum cystatin C (CysC), kidney injury molecule-1 (KIM-1), angiotensin-converting enzyme 1 (ACE1) activity, and ACE2 concentration. These biomarkers were quantified using commercially available ELISA kits (BT Laboratory, China).[22-25] The estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI Creatinine–Cystatin C equation (2021) via an online calculator.[26]

Genomic DNA was isolated from peripheral blood leukocytes obtained from frozen venous blood samples using the FavorPrep Blood Genomic DNA Extraction Mini Kit. The purity and concentration of the extracted DNA were

assessed with a NanoDrop spectrophotometer, while DNA integrity and molecular weight were verified by 1% agarose gel electrophoresis.

Detection of the ACE (I/D) polymorphism was performed using high-resolution melting real-time PCR (HRM-RT). The amplification reaction was conducted on a Rotor-Gene Q thermal cycler (Qiagen®) with three specific primers[27]:

Primer 1ACE1 CATCCTTTCTCCCATTTCTC

Primer2 ACE2 TCGGATTACAGCCCTGATACAG

Primer3 ACE3 ATTTCAGAGCTGGAATAAAATT

PCR amplification was carried out in a 50 µl reaction volume containing 20 µl SYBR Green PCR Master Mix, 3 µl of primers, 2 µl genomic DNA, 1 µl Mgcl2, and 24 µl DNAse-free water. The cycling protocol included an initial denaturation, followed by 35 cycles of denaturation, annealing, and extension. Genotyping was performed through high-resolution melting curve analysis, where fluorescence changes were monitored during gradual heating, and dissociation peaks (−dF/dT vs. T) were generated for allele discrimination.[28] The dissociation curve was then visually inspected for genotyping, and the results from selected samples of the present study are illustrated in Figures 1, and 2.

The AGTR1 (A1166C) polymorphism was identified using polymerase chain reaction followed by restriction fragment length polymorphism analysis (PCR–RFLP).[29] Specific primers were designed through the NCBI Primer-BLAST tool,[30] yielding an amplified fragment of 316 bp.

Forward primer ATGAGCACGCTTTCCTACCG

Reverse primer TTCTTCGAGCAGCCGTCATC

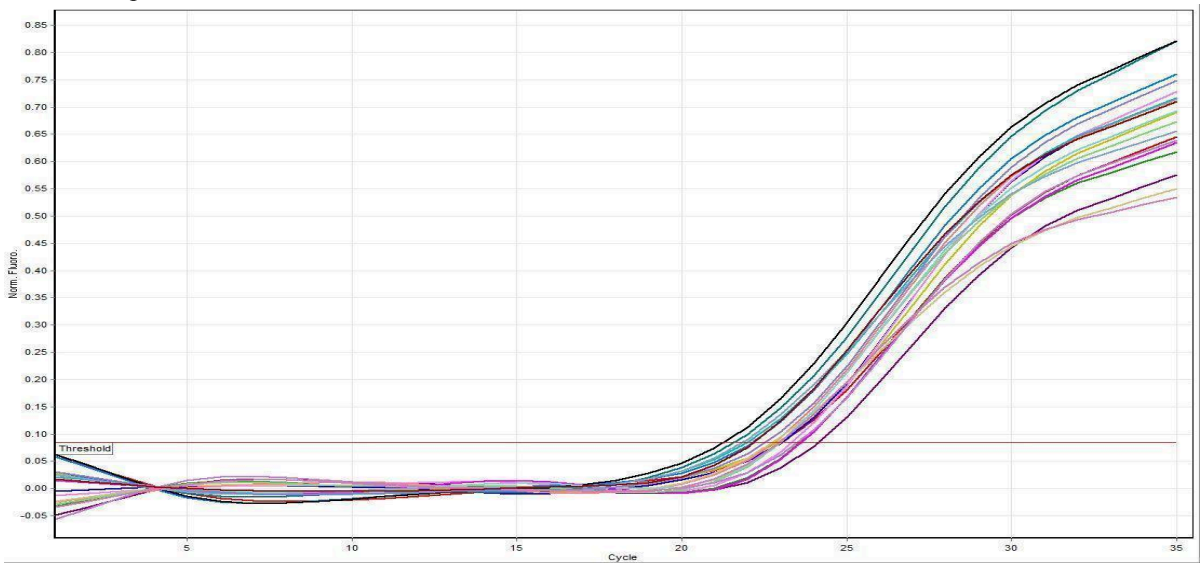

Figure 1: HRM-RT raw data of amplification and melting curves.

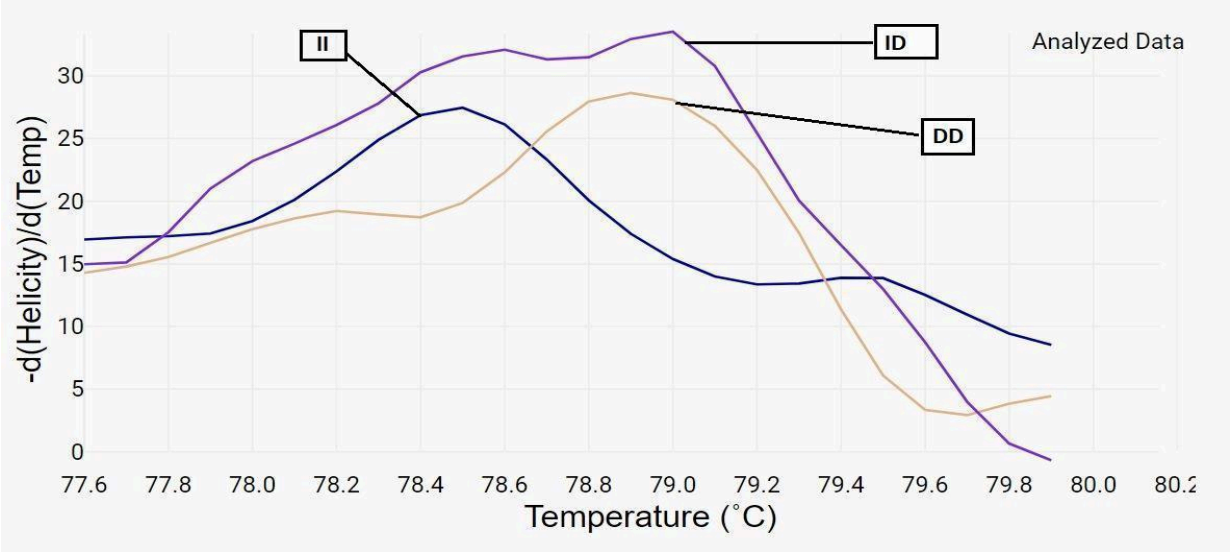

Figure 2: Analyzed HRM-RT data showing separation of three genotypes (II, ID, DD) according to melting temperature.

PCR amplification was performed in a 20 µl reaction mixture containing 2 µl of genomic DNA, 1 µl of each primer, 8 µl master mix, 0.5 µl MgCl2, and 7.5 µl DNAse-free water. The cycling program including an initial denaturation, followed by 35 cycles of denaturation, annealing, and extension, with a final extension step. Amplified products were visualized by 2% agarose gel electrophoresis with EtBr staining under UV light. For genotyping, PCR products were digested with the restriction enzyme **DdeI**, selected using SnapGene viewer software (V6.0.5). The restriction reaction is carried out by using a mixture of 3 µl of PCR product, 0.25 µl of the selected restriction enzyme (DdeI), restriction buffer 1.5 µl which was then completed to 13 µl by 8.5 µl DNAse-free water, vaseline oil was added to reduce evaporation since the mixture was incubated overnight at 60 °C, and the resulting fragments were analyzed on 2% agarose gels to confirm product length, specificity, and digestion pattern.[28,29] Representative gel electrophoresis results are presented in Figure 3.

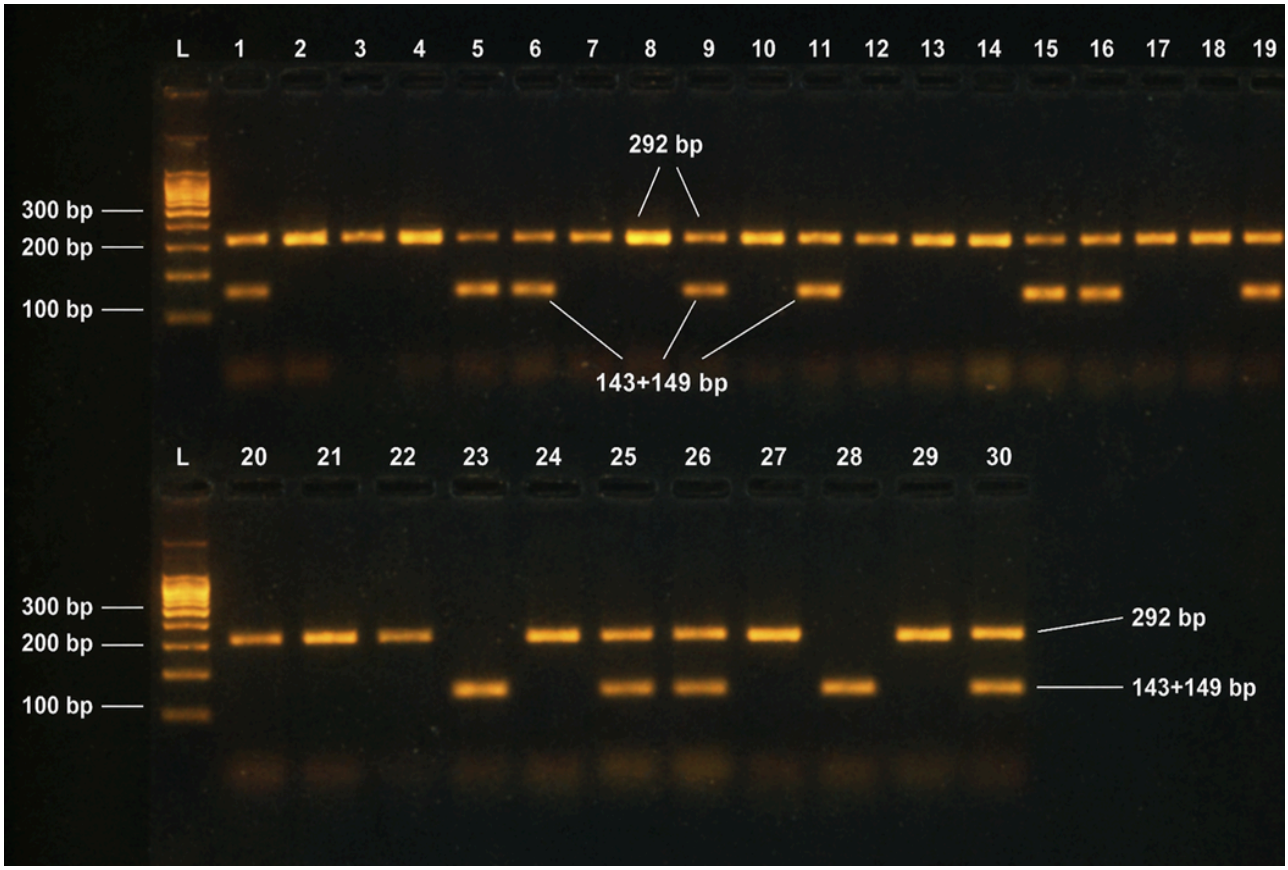

Figure 3: RFLP-PCR analysis of AGTR1 A1166C gene fragments on 2% agarose gel (EtBr stained, 70 V, 1 h). 1,000 bp DNA ladder Lane L: 100 bp DNA ladder; lanes 1, 5, 6, 9, 11, 15, 16, 19, 25, 26, 30: AC genotype; lanes 23, 28: CC genotype; other lanes: AA genotype

All statistical analyses were performed using SPSS software, version 26.0 for Windows (IBM Corp., Armonk, NY, USA). The distribution of continuous variables was examined with the Shapiro–Wilk test. Data that followed a normal distribution are reported as mean ± standard deviation (SD), whereas non-normally distributed data are presented as median with interquartile range (IQR). Group comparisons were made using the independent t-test for normally distributed data, while the Mann–Whitney U test and Kruskal–Wallis H test were applied for non-parametric variables. Categorical variables are expressed as frequencies and percentages, and differences were assessed using the chi-square test. To evaluate the predictive relationship between ACE1 levels and the studied polymorphisms, binary logistic regression was applied, controlling for age, sex, and diabetic kidney disease (DKD) status. For binary logistic regression analysis, serum ACE levels were dichotomized as normal (≤ 40 U/L) and abnormal (> 40 U/L). This cutoff was selected for analytical purposes and in light of published laboratory references indicating that serum ACE reference intervals are method-dependent, with some sources reporting an upper normal limit around 40 U/L(31). Statistical significance when a *P*-value <0.05.

Results

The demographic, clinical, genetic, and laboratory characteristics of the study groups are summarized in Table 1. Significant differences were observed only in retinopathy, HbA1c, urinary albumin, urinary creatinine, ACR, and GFR. In addition, serum CysC levels were significantly higher in the DKD group compared to the normalbuminuric group, also TC was higher in DKD patient group but this elevation was marginally significant.

Genetic analysis revealed no significant intergroup differences for either gene. For ACE (I/D) polymorphism, the ID genotype was most prevalent among DKD patients, followed by DD and then II. For the AGTR1 (A1166C) polymorphism, the AA genotype showed the highest frequency, followed by AC, while the mutant CC genotype was detected in only three patients.

Table 1: Demographic, clinical, genetic and laboratory data of the study groups.

Parameters	**Patients groups**		**p-value**
	DKD ***N*** **= 53**	**normalbuminuric** ***N*** **= 32**	
Demographic, clinical and genetic			
Age (years)	60.98 ± 8.41	58.56 ± 7.44	0.171
Gender male: female	19:34	18:14	0.180
BMI (kg\m2)	30.34 ± 4.61	30.78 ± 4.0	0.863
FBS (mmol\l)	10.1 (7.7–12.5)	8.75 (7.73–10.3)	0.207
HbA1C (%)	8.9 (7.85–10.35)	8.0 (6.9–9.2)	0.017*
TC (mmol\l)	4.6 (3.8–5.15)	4.1 (2.85–4.88)	0.046
TG (mmol\l)	1.9 (1.5–2.7)	1.5 (1.22–2.68)	0.202
HDL (mmol\l)	0.9 (0.9–1.1)	0.9 (0.8–1.0)	0.671
SBP (mmHg)	128.96 ± 16.50	128.44± 13.70	0.880
DBP (mmHg)	77.45 ± 9.44	81.09 ± 6.69	0.060
Living place			
Urban	29 (54.7%)	17 (53.1%)	0.881
Rural	24 (45.3%)	15 (46.9%)	
Retinopathy	48(90.6%)	11 (34.4%)	0.000*
CHD	19 (35.8%)	9 (28.1%)	0.672
ACE (I\D)			
II	12 (22.6%)	12 (37.5%)	0.146
DD	16 (30.2%)	10 (31.3%)	
ID	25 (47.2%)	10(31.3%)	
AGTR1 (A1166C)			
AA	32 (60.4%)	22 (68.8%)	0.501
AC	19 (35.8%)	9 (28.1%)	
CC	2 (3.8%)	1 (3.1%)	
Laboratory biomarkers			
Urine albumin (mg\l)	7.9 (5.5–22.5)	4.45 (3.8–5.2)	0.000*
Urine creatinine (mg/dl)	102.6 (76–146.6)	185.25 (148.93–250.05)	0.000*
ACR (mg\g)	81.5 (52.3–155.5)	25.7 (20.5–29.0)	0.000*
S.Cr. (mmol\l)	85.2 ± 25.89	77.84 ± 19.6	0.142
GFR (ml.minute.1.73 m2)	87 (72.5–100)	97.5 (87.5–110.5)	0.005*
KIM1 (ng\ml)	1.39 ± 1.13	1.15 ± 0.48	0.182
CysC (mg\dl)	1.05 ± 0.89	0.7 ± 0.23	0.038*

Values for continuous variables were calculated using the two-sided *t*-test or Mann–Whitney test, while the Pearson χ^2 test was applied for clinical and genetic variables. Proportional data are presented as counts and percentages. Normally distributed variables (age, BMI, SBP, DBP, S.Cr, CysC, KIM-1) are expressed as mean ± SD, and skewed variables as median (IQR)." DKD, diabetic kidney disease; BMI, body mass index; CHD, chronic heart disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1C,glycated hemoglobin; FBS, fasting blood sugar; Hb, hemolglobin; TC total cholesterol ; HDL, high-density lipoprotein; TG, triglyceride; ACR, albumin creatinine ratio; GFR: glomerular filtration rate; KIM1,kideny injury molecule1.*significant ($p< 0.05$)"

The renoprotective effect of ARBs was determined by comparing serum levels of renal-related markers in different genotypes. Table 2 shows the differences in the levels of biomarkers according to different ACE (I\D) genotypes, which revealed no significant difference in all biomarkers. Additionally, DD carriers had a lower eGFR

in comparison with other genotypes, however, the differences were not significant. This means that the renoprotective effect of ARBs does not differ among T2DM patients according to ACE (I\D) genotypes.

Table 2: Comparison of biomarker levels across ACE (I/D) genotypes.

Parameters	**Patients groups**			**P-value**
	DD N=26	**ID N=35**	**II N=24**	
S.ACE1(U\L)	56.25(38.43-84.94)	53.69(47.06-67.66)	56.95(42.09-70.45)	0.972
S.ACE2(ng\ml)	2.77(2.43-4.29)	2.77(2.36-3.67)	2.78(2.22-3.42)	0.830
ACR(mg\g)	50.7(27.2-100.3)	51.1(29.2-111.4)	34.6(25.25-138.33)	0.754
eGFR(ml\min\1.73m2)	82.5(69-100.25)	95(81-107)	95.5(87-105.5)	0.101

Data presented as median (IQR), independent sample Kruskal-Wallis Test

"ACE1: angiotensin converting enzyme1, ACE2: angiotensin converting enzyme2, ACR: albumin creatinine ratio, GFR: glomerular filtration rate"

The binary logistic regression analysis of S.ACE1 levels (categorized as normal ≤ 40 U/L and abnormal > 40 U/L) presented in Table 3 demonstrated no significant association with ACE (I/D) genotypes. This indicates that ACE (I/D) genotypes do not influence the response to ARB therapy.

Table 3: Association of S.ACE1 with ACE (I/D) genotypes in ARB-treated T2DM (binary logistic regression).

Independent variables	**Beta**	**OR (CI 95%)**	**P-value**
Age	-0.014	0.986 (0.918-1.059)	0.698
Gender	-0.194	0.824 (0.256-2.652)	0.745
DKD state	-0.354	0.702 (0.201-2.454)	0.579
*ACE(I/D)*genotype	-0.816	0.442 (0.125-1.562)	0.205

DKD: *diabetic kidney disease,* ***ACE:*** *angiotensin converting enzyme,* ***OR:*** *odd ratio,* ***CI:*** *confidence interval.*

The serum ACE1 and ACE2 levels were higher in DKD patients and lower in normalbuminuric patients; however, the differences were not significant. The S.ACE1 activity was higher in DKD patients with DD genotypes, followed by the II genotype and lower for ID genotype carriers, while in normalbuminuric patients, the lower level was in DD carriers, subsequent by the ID genotype and the II carriers had the highest level; however, the differences were not significant for S.ACE1 in all studied genotypes.

Regarding S.ACE2 level in DKD patients which was higher in II, followed by the ID and DD genotypes, while in normalbuminuric patients, a higher level was seen in DD, followed by the ID carriers and the II carriers had the lowest level. However, these differences were not significant, as demonstrated in Table 4.

Table 4: S.ACE1 activity and S.ACE2 concentration in relation to ACE genotypes among ARB-treated DKD and normalbuminuric T2DM groups.

Parameters	**ARB groups**		**P-value**
	normabuminuric	**DKD**	
	N =32	N =53	
S.ACE1(U\L)	53.84(41.7-70.22)	56.16(42.59-69.26)	0.618
S.ACE2(ng\ml)	2.65(2.21-3.90)	2.77(2.44-3.34)	0.838
II	N =12	N =12	
S.ACE1	56.95(43.1-126.73)	57.02(42.09-70.45)	0.908
S.ACE2	2.51(2.2-3.88)	2.81(2.35-3.37)	0.751
ID	N =10	N =25	
S.ACE1	52.72(46.35-69.75)	53.69(48.08-66.4)	0.971
S.ACE2	2.53(2.05-3.90)	2.77(2.46-3.45)	0.571
DD	N =10	N =16	

S.ACE1	43.49(39.84-77.49)	62.11(36.3-108.59)	0.493
S.ACE2	2.88(2.44-5.11)	2.73(2.22-4.18)	0.562

Data presented as median (IQR), Mann-Whitney U test. "DKD: diabetic kidney disease, S.ACE1: serum angiotensin converting enzyme1, S.ACE2: serum angiotensin converting enzyme2".

Table 5 presents the differences in biomarker levels assessing the antiproteinuric effect of ARBs according to AGTR1 (A1166C) genotypes. No significant differences were observed between the wild-type AA and the heterozygote AC + mutant CC genotypes across all measured biomarkers. However, the AA genotype was associated with lower S.ACE1, eGFR, and ACR levels, and with higher S.ACE2 levels.

Table 5: Comparison of biomarker levels across AGTR1 (A1166C) genotypes.

Biomarkers	**ARB groups**		**P value**
	AA N=54	**AC+CC N=31**	
S.ACE1(U\L)	54.99(42.89-79.75)	57.21(42.23-70.45)	0.920
S.ACE2(ng\ml)	2.77(2.37-3.71)	2.57(2.28-3.27)	0.520
ACR(mg\g)	43.5(27.58-110.8)	60.7(29.2-120.4)	0.398
eGFR(ml\min\1.73m2)	92.5(78-106.25)	93(83-104)	0.866

Data presented as median (IQR), Mann-Whitney U test "S.ACE1: serum angiotensin converting enzyme1, ACR: albumin creatinine ratio, S.ACE2: serum angiotensin converting enzyme2, eGFR: estimated glomerular filtration rate".

The binary logistic regression analysis of S.ACE1 levels (categorized as normal ≤ 40 U/L and abnormal > 40 U/L), had shown in Table 6, which revealed no significant association with AGTR1 (A1166C) genotypes. This indicates that AGTR1 (A1166C) genotypes do not influence the response to ARB therapy.

Table 6: Association of S.ACE1 with AGTR1 (A1166C) genotypes in ARB-treated T2DM (binary logistic regression).

Independent variables	**Beta**	**OR (CI95%)**	**P-value**
Gender	-0.169	0.844 (0.263-2.705)	0.776
Age	-0.025	0.975 (0.907-1.048)	0.497
DKD state	-0.158	0.854 (0.248-2.942)	0.803
AGTR1(A1166C)	-0.570	0.566 (0.179-1.792)	0.333

DKD: diabetic kidney disease, AGTR1: angiotensin type 1receptor, OR: odd ratio, CI: confidence interval.

Table 7 demonstrates that S.ACE1 and S.ACE2 levels were higher in DKD patients, particularly among AA genotype carriers, compared with normalbuminuric T2DM patients, although the differences were not statistically significant. In contrast, among AC+CC genotype carriers, both markers showed higher levels in the normalbuminuric T2DM group, but again the differences were not significant.

Table 7: S.ACE1 activity and S.ACE2 concentration in relation to AGTR1 genotypes among ARB-treated DKD and normalbuminuric T2DM groups.

Parameters	**ARB groups**		**P-value**
	normalbuminuric	**DKD**	
AA	N=22	N=32	
S.ACE1(U\L)	48.98(41.14-62.44)	56.8(45.05-73.71)	0.208
S.ACE2(ng\ml)	2.53(2.11-4.21)	2.78(2.59-3.64)	0.291
AC+CC	N=10	N=21	
S.ACE1(U\L)	64.51(42.95-79.62)	53.69(39.25-66.57)	0.375
S.ACE2(ng\ml)	3.02(2.4-3.75)	2.52(2.24-3.15)	0.212

Data presented as median (IQR), Mann-Whitney U test.
"DKD: diabetic kidney disease, S.ACE1: serum angiotensin converting enzyme1, S.ACE2: serum angiotensin converting enzyme2".dasx

Discussion

This study compared T2DM patients who developed DKD against those who maintained normoalbuminuria. The findings provide an insights into the clinical and molecular correlates of DKD progression and renoprotective effect of ARB medications.

Consistent with previous studies, glycemic control was significantly poorer among patients with DKD, as reflected by elevated HbA1c levels compared to the normoalbuminuric group and also found a markedly higher prevalence of diabetic retinopathy in the DKD group. Prior meta-analysis demonstrating each 1% rise in HbA1c significantly heightens DKD risk.[32] An Iraqi study similarly found that elevated HbA1c strongly correlates with increased urinary albumin and early nephropathy, reinforcing our findings on local grounds.[33] Internationally, ADA Standards of Care and KDIGO guidance concur: poorer glycemic control is a major accelerant of DKD, and albuminuria/eGFR should be monitored at least annually in T2DM to detect deterioration early.[34] Additionally retinopathy and nephropathy often co-occur—both arising from microvascular endothelial damage and AGEs-induced dysfunction.[35] This co-occurrence is strongly supported by an Iraqi population study from Basrah, which reported diabetic retinopathy prevalence ~30% and showed significant associations with hyperglycemia, dyslipidemia, and nephropathy.[36] Although the difference in total cholesterol was modest, it may still be clinically relevant, as dyslipidemia commonly coexists with diabetic kidney involvement. This higher elevation in total cholesterol in DKD patients, supporting evidence that dyslipidemia accelerates glomerular injury and albuminuria. Mechanistically, KDIGO and contemporary reviews highlight that dyslipidemia adds a "lipotoxic" burden on the kidney and is a rationale for statin therapy in diabetes with CKD.[37-39]

As expected, albuminuria was elevated and eGFR was reduced in DKD patients. Serum CysC was also significantly higher, emphasizing its value as an early indicator of kidney impairment—findings supported by another Iraqi work demonstrating that serum CysC effectively reflects early nephropathic changes.[21,40] Although serum KIM-1 levels were elevated in the DKD group, the difference was not statistically significant in the present study. However, previous studies have reported significant associations between KIM-1, albuminuria, declining eGFR, and diabetic kidney injury, suggesting that variations in disease stage, cohort composition, sample size, and assay methodology may influence its detectability across studies. This interpretation is supported by Iraqi studies with larger sample sizes, which demonstrated significantly elevated serum KIM-1 levels and identified KIM-1 as an early tubular marker for DKD.[21,41,42]

From a genetic perspective, no significant differences in ACE (I/D) or AGTR1 (A1166C) polymorphism distributions between groups was observed. While meta-analysis suggest that the ACE I/D polymorphism, particularly the D allele, contributes to susceptibility to nephropathy through increased angiotensin II activity and intraglomerular hypertension.[43] Within Iraq, Dhumad et al. reported that while the D allele and DD genotype of the ACE I/D polymorphism were more prevalent among T2DM patients, they were not significantly associated with cardiac autonomic neuropathy.[44] More recently, Yahya et al. reported that DD genotype was associated with a higher frequency of DN and conferred about a twofold increased risk relative to the II genotype.[45] Additionally, in our previous study, we demonstrated that these same polymorphisms significantly affected the antiproteinuric response to ACE inhibitors in Iraqi patients with type 2 diabetes mellitus, even if not directly correlated with DKD presence.[28] For the AGTR1 (A1166C) variant showed no significant link with DKD, many other findings from certain populations where this polymorphism was not predictive of renal outcomes[46]. These discrepancies may be attributed to ethnic differences, environmental influences, or gene–environment interactions.

Since ACE activity may represent a rate-limiting step in Ang II formation under disease conditions, the ACE genotype could potentially influence the therapeutic response to ARBs, which act further downstream in the renin–angiotensin pathway compared to ACEIs.[48] The present study revealed no differences in response to ARBs treatment between different ACE (I\D) genotypes as there are no significant difference for all renal parameters between *ACE* genotypes and even after controlling DKD state, age, and sex, the ACE (I\D) was not found to be a significant predictor of ACE1 level. Additionally, DD carriers showed a numerically lower eGFR than ID and II carriers, a trend that may warrant further evaluation in larger studies. These finding was in accordance with previous studies finding[10,48] which found that therapeutic response to ARB regarding proteinuria does not differ between genotypes. The Cheema et al. study revealed that that patients carrying the DD or ID genotypes with proteinuria showed a more favorable response across nearly all endpoints compared with those carrying the II genotype.[12] Also,

a large prospective study on thousands of T2DM from different ethnicities in different countries by Parving et al. found that D allele was associated with a more favorable response to ARBs in T2DM having overt nephropathy.[11] While Felehgari V et al. study found that diabetic patients with nephropathy and DD genotype had a better response to ARBs compared to normoalbuminuric patients with DD genotype; however, this finding was derived from a study that evaluated both captopril and losartan, rather than ARB therapy alone.[49]

For AGTR1 (A1166C), the current study revealed no significant differences for all renal parameters used for detecting response to ARBs between AA and AC+CC genotype carrier's even after controlling other covariates and also after dividing patients according to DKD state. These finding was in accordance with the finding of Cheema et al. study[12] which showed the response to ACEI and ARB therapy was not significantly influenced by AGT or AGTR1 gene polymorphisms. Also, a systematic review on antihypertensive effect of ARB suggested that AGTR1 (A1166C) was not associated with response to RAAS blockage.[50]

Conclusion

In this single-center Iraqi cohort of hypertensive T2DM patients on ARBs, we found no evidence that ACE (I/D) or AGTR1 (A1166C) polymorphisms modify the renoprotective response, as reflected by albuminuria, eGFR, ACE1 activity, or ACE2 concentration. Genotype frequencies were comparable between DKD and normoalbuminuric groups, and biomarker profiles did not differ across genotypes; multivariable analysis likewise did not implicate ACE (I/D) as a predictor of ACE1 levels. These results suggest that routine genotyping of ACE (I/D) and AGTR1 (A1166C) is unlikely to guide ARB therapy selection in similar clinical settings, whereas From a clinical perspective, optimization of glycemic control and surveillance for microvascular complications remain relevant in the broader management of DKD risk.

Limitations

The main limitations of this study are the cross-sectional, single-center design limits causal inference and generalizability, modest sample size and low-frequency variants reduce statistical power, outcomes relied on single spot measurements rather than repeated or 24-h urine assessments, Inability to assess change in ACR or eGFR over time due to cross-sectional design, lack of ARB dose standardization across patients, the lack of subgrouping of patients according to the severity of albuminuria. and group size imbalance may affect precision of comparisons. Another limitation is that multiple biomarkers and genotypes were tested without formal correction for multiple comparisons, and the findings should therefore be interpreted with caution.

Disclosure

No funding was received for this study.

Conflicts of interest

There were no conflicts of interest among the writers.

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