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Original Article

Association of cannabinoid gene polymorphism with neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1) in type 2 diabetes mellitus with chronic kidney disease

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Abstract

Diabetic kidney disease (DKD) is a common microvascular complication of type 2 diabetes mellitus (T2DM) and a leading cause of end-stage renal disease (ESRD). Genetic factors, including polymorphisms in the cannabinoid receptor 1 (CNR1) gene, may influence the risk and progression of chronic kidney disease (CKD) in diabetic patients. This study aimed to investigate the association of CNR1 gene polymorphisms, specifically single-nucleotide polymorphisms (SNPs) rs1049353 and rs1776966256, with serum levels of kidney injury biomarkers neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1) in Iraqi patients with T2DM, with and without CKD. A total of 120 subjects were enrolled and divided into three groups: 40 T2DM patients with CKD, 40 T2DM patients without CKD, and 40 healthy controls. Genotyping was performed using conventional polymerase chain reaction followed by Sanger sequencing. Serum NGAL and KIM-1 levels were measured by ELISA. Multiple novel CNR1 gene variants were detected and submitted to the NCBI database. The heterozygous GA genotype of rs1049353 was more prevalent in the CKD group compared to others, although not statistically significant. The rs773947953 (G>A) variant showed significant association with CKD, where the A allele appeared protective. Significant correlations were also observed between NGAL, KIM-1 levels, and specific SNP genotypes, including rs773947953 and new variations at positions 4217 (G>A) and 4224 (rs2481890897). These findings suggest that CNR1 gene polymorphisms influence susceptibility to diabetic kidney injury and are associated with elevated tubular injury markers. Identification of these genetic variations may help in early prediction and personalized management of DKD.

Keywords: Kidney disease; Chronic kidney disease; Diabetic kidney disease; Neutrophil gelatinase-associated lipocalin (NGAL); Kidney injury molecule-1(KIM-1).

1. Introduction

Diabetes is a chronic, heterogeneous metabolic disease characterized by a complex pathogenesis. It is defined by elevated blood glucose levels, or hyperglycemia, due to irregularities in insulin secretion, insulin action, or both. Hyperglycemia presents in multiple types with diverse manifestations, leading to metabolic dysfunctions of carbohydrates, fats, and proteins [1]. Diabetic kidney disease (DKD) is a microvascular complication of diabetes and a primary contributor to end-stage renal disease (ESRD), as- sociated with rising morbidity levels and death rates [2]. It is marked with enhance deterioration of renal function [3]. Diabetic nephropathy has been identified as one of the high adverse reaction, affecting 20% to 40% of people with diabetes, predominantly those with type 2 diabetes mellitus [4]. The primary etiologies of kidney disease progression in diabetic kidney disease (DKD) are glomerular microangiopathy and prolonged diabetes duration, both of which significantly increase disease and death risk in Type II diabetes mellitus (T2DM) [5]. The disease develops silently, deteriorates, and results in irreversible harm [4]. The pathogenesis of disease frequently correlates with alterations in the structure and function of renal cells due to persistent hyperglycemia, the activation of metabolic pathways associated with redox imbalance, and the inflammatory response [6]. Early detection and treatment are critical in diabetic nephropathy. The urine albumin creatinine ratio (ACR) and estimated glomerular filtration rate (eGFR) are the two primary indicators used to identify diabetic kidney disease (DKD) [7]. Numerous studies on the ACR value have demonstrated that not all DKD patients exhibit increased levels in the initial phases of the disease, suggesting that ACR is inadequately sensitive to function as an indicator for the early stages of DKD [8,9]. The estimated glomerular filtration rate (eGFR) based on serum creatinine (SCr) can only be verified when the eGFR value is less than 60 mL/min/1.73 m², indicating that about fifty percent of renal function has already been lost [10]. Tubulo-interstitial damage, characterized by complex structural changes such as glomerular and tubular

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hypertrophy, is linked to all types of chronic kidney disease (CKD), including diabetic nephropathy (DN) [5]. Tubular involvement occurs before glomerular involvement, as evidenced by the presence of numerous tubular proteins and enzymes prior to the onset of microalbuminuria and a rise in serum creatinine SCr [11]. Neutrophil gelatinase-associated lipocalin (NGAL) is a 25 kDa structural tubular marker that belongs to the lipocalin superfamily and is significantly elevated in the blood of patients within hours following ischemia-reperfusion injury [12]. NGAL functions as a binder and transporter of small hydrophobic molecules, as well as a factor of innate antibacterial responses [13]. Neutrophil gelatinase-associated lipocalin (NGAL), a 25 kDa protein synthesized by damaged nephron epithelia, is a highly promising biomarker for renal epithelial injury. Unlike serum creatinine and urinary output, which measure kidney function, NGAL is particularly induced in injured nephrons and then released into the bloodstream and urine, allowing for easy measurement [14].

Moreover, NGAL has been shown to enhance nephrogenesis and to aid in the transformation of mesenchymal cells into renal epithelial cells. In kidney tubules, the synthesis of NGAL increases in response to various harmful stimuli, such as ischemia–reperfusion injury, si- gnifying the protein's crucial role in tubular regeneration and repair [15]. Kidney injury molecule-1 (KIM-1), a type I transmembrane glycoprotein, is significantly synthe- sized at the apical membranes of proximal tubular epi- thelial cells during tissue regeneration following toxic or ischemic acute kidney injury, as well as during the dediffe- rentiation of tubular epithelial cells [10]. The CB1 receptor enhance death of cell, oxidative and damage, and inflammation through the activation of p38-MAPK [16]. The CB1 cannabinoid receptor, a polypeptide comprising four hundred seventy-three amino acids, is expressed from CNR1 gene, which is part of the G protein-associated receptor suppressor family in chromosome six [17]. This gene exhibits many variations identified in distinct population groupings. The new research has identified a relationship between CNR1 gene polymorphisms and diabetic nephropathy with metabolic syndrome [18]. This study aimed to investigate presence of SNPs Rs1776966256 and Rs1049353 in CB1 gene of Iraqi samples with Type 2 Diabetic Mellitus with and without chronic kidney disease.

2. Materials and methods

2.1. Study design

A cross-sectional study was conducted on patients who had attended Baghdad Teaching Hospital and Ghazi al-Hariri Surgical Specialties Hospital Kidney Diseases and Transplantation Center. The study was carried out on one hundred twenty control and patients with age from 35 to 80 years, already-diagnosed T2DM patients collected from the Hospital During the period from December 2024 to March 2025. The study included three groups divided to Group1: forty patients with type 2 diabetes with chronic kidney disease, Group 2: forty patients type 2 diabetes, Control group forty patient not receiving any medication and did not have a history of any chronic or acute disease and his- tory of diabetes were selected with age between (35-80) years old. Diagnosis of diabetic kidney disease was established by a broad in scope, many question to patient, medical evaluation, biochemical investigation, and Anthropometric measurements, including height, weight, and body mass index (BMI), were recorded. Blood samples were collected to measure kidney test, lipid profile, (eGFR), sugar test.

2.2. Sample collection

Approximately 2 ml of venous blood was drawn from each participant and collected into an ethylene diamine tetraacetic acid (EDTA) tube for DNA extraction fol- lowing the standard protocol. Conventional polymerase chain reaction (PCR) was intensify DNA sequence efficiently of small sample volume. The amplified products were sequenced using the Sanger method to assess the frequency of cannabinoid gene poly- morphisms, and the resulting sequences were submitted to NCBI for accession numbers. The remaining 5 ml of blood was collected to obtain serum. Serum levels of neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1) were subsequently detect using ELISA techniques.

2.3. Ethical statement

Verbal informed consent from patients was obtained in order to take sample. The study designe, participant data, and permission form were examined and approved by the local ethics committee. The study objectives were described to all volunteers.

2.4. DNA extraction and gene genotyping

Specimens were collected by drawing 1.5 ml blood sample kept at -80°C. Variations in the CNR1 gene were identified using conventional polymerase chain reaction (PCR) to amplify regions containing single-nucleo- tide polymorphisms (SNPs). The amplified fragments were separated by electrophoresis on a 2% agarose gel stained with ethidium bromide to determine genotype patterns for both polymorphic sites. PCR products and primers from this study were subjected to Sanger DNA sequencing to identify any polymorphisms. Newly discovered variations were submitted and recorded in the NCBI database.

2.5. Statistical analysis

Data was analyzed using IBM SPSS Statistics (Version 23, IBM Corp., Armonk, NY, USA). Continuous variables (KIM and NGL levels) were expressed as mean ± standard error (SE). A one-way analysis of variance (ANOVA) was performed to assess the chemical overall differences KIM and NGL levels across different genotypes within and between groups (CKD, DM, and Control). Following ANOVA, Tukey's Honest Significant Difference (HSD) to determine pairwise differences between genotypes.

3. Result

3.1. Genetic analysis

Genomic DNA was extracted from diabetic mellitus (DM) patients, both with and without chronic kidney disease (CKD), as well as from healthy controls. The concentration of the extracted DNA ranged from 10 to 22 ng/μL. As shown in Figure 1, distinct DNA bands of approximately 667 base pairs were obtained following PCR amplification and electrophoresis on agarose gel. Selected samples were sequenced using the Sanger method, revealing several novel variations that were sub- sequently registered in the NCBI database.

The results revealed that many samples with high genetic variation were submitted to the National Center for

Biotechnology Information (NCBI) and assigned new accession numbers, including BankIt2957847 CNR1_IQ_6 (PV650357), CNR1_IQ_7 (PV650358), CNR1_IQ_9 (PV650359), CNR1_IQ_11 (PV650360), CNR1_IQ_12 (PV650361), CNR1_IQ_15 (PV650362), CNR1_IQ_19 (PV650363), CNR1_IQ_20 (PV650364), CNR1_IQ_21 (PV650365), CNR1_IQ_23 (PV650366), CNR1_IQ_24 (PV650367), CNR1_IQ_25 (PV650368), CNR1_IQ_26 (PV650369), CNR1_IQ_27 (PV650370), CNR1_IQ_28 (PV650371), CNR1_IQ_29 (PV650372), CNR1_IQ_33 (PV650373), and CNR1_IQ_34 (PV650374).

3.2. Genotype distribution and allele frequency of rs1049353 in different groups

The results of the CNR1 cannabinoid gene polymorphism SNP at position 4,053, presented in Table 1, showed a nonsense substitution. The heterozygous GA genotype frequency was high for DM with CKD group (30%) in accordance with DM without CKD group (10%) and the control group (25%), although the differences were not statistically significant. The homozygous GG genotype frequency was 70% in the DM with CKD group, 90% in the DM without CKD group, and 75% in the control group. The frequency of the G allele was 85% in the DM with CKD group, 95% in the DM without CKD group, and 84% in controls.

The rs773947953 SNP of the CNR1 cannabinoid gene, located at position 4,027, results in a missense substitution (Serine > Phenylalanine, S>F). As shown in Table 2, the AA genotype was present in 10% of the DM with CKD group, a higher than in the DM without CKD and control groups. The heterozygous GA genotype frequency was 22.5% in the DM with CKD group, while it was 0% in both the DM without CKD and control groups. The homozygous GG genotype frequency was 67.5% in the DM with CKD group and 100% in the DM without CKD and control groups. The G allele frequency in the DM with CKD group (79%) was lower than in the DM without CKD and control groups, whereas the mutant A allele appeared more frequently in the DM with CKD group (21%), with a significant p-value.

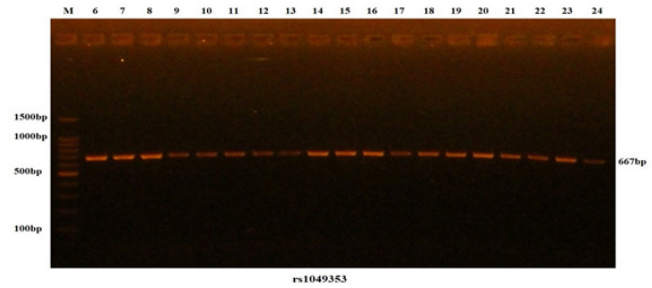

Fig. 1. Results of amplification of the cannabinoid receptor (CNR1) gene from human samples. PCR products were separated by agarose gel electrophoresis and stained with ethidium bromide. M represents the 100 bp DNA ladder marker.

For the CNR1 cannabinoid gene, a new genetic polymorphism was identified at position 4,217 (G>A). The heterozygous GA genotype frequency in the DM with CKD group was 70%, significantly higher than the 30% observed in the DM without CKD(Table 3). The AA genotype frequency was 17.5% in the DM with CKD group, compared to 60% in the DM without CKD group. The G allele frequency was higher in the DM with CKD group (48%) than in the DM without CKD group (25%) and was absent in the control group (0%). Conversely, the mutant A allele was most frequent in the control group (100%), compared to 52% in the DM with CKD group and 75% in the DM without CKD group. These differences were highly significant ($p< 0.01$).

The results demonstrated a strong association between the sense (G>A) mutation and diabetes mellitus (DM) with and without chronic kidney disease (CKD). The A allele was identified as a risk factor linked to disease occurrence, with patients carrying the GA genotype showing a higher susceptibility to disease development (Figure 2).

The genetic polymorphism showing a new variation at position 3,998 (G>A) revealed that the heterozygous GA genotype frequency in the DM with CKD group was 82.5%, slightly higher than 80% in the DM without CKD group, with no significant difference between groups (Table 4). The GG genotype frequency in the DM with CKD group was 17.5%, similar to 20% in the DM without CKD group and higher than 7.5% in the control group. The G allele frequency was comparable between the DM with CKD group (59%) and the DM without CKD group (60%), and slightly higher than in controls (54%). The mutant A allele appeared more frequently in the DM without CKD group (60%) compared to 41% in the CKD group and 46% in controls. These differences were not statistically significant.

The genetic polymorphism showing a new variation at position 4,350 (G>A) revealed that the heterozygous GA genotype frequency in the DM with CKD group was 75%,

Table 1. Genotype distribution and allele frequency of (G>A) rs1049353:4,053 in different groups.

Genotype/ (G>A) rs1049353:	Genotype Frequency CKD No. (%)	DM No. (%)	Control No. (%)	Chi-Square	P-value
GG	28 (70%)	36 (90.0%)	33 (75%)		
GA	12(30%)	4 (10.0%)	7 (25%)	5.289	0.071 NS
AA	0 (0.00%)	0 (0.00%)	0 (0.00%)		
Total	40	40	40		
Allele Frequency					
G	68(85%)	76 (95%)	73 (84%)	4.71	0.054 NS
A	12 (15%)	4 (5%)	7 (16%)		
Total	80	80	80		

NS: Non-Significant.

Table 2. Genotype distribution and allele frequency of (G>A)4,027 rs773947953 in different groups.

Genotype Frequency					
Genotype/ (G>A) 4,027 rs773947953	**DM with CKD No. (%)**	**DM No. (%)**	**Control No. (%)**	**Chi-Square**	**P-value**
GG	27 (67.5%)	40 (100%)	40 (100%)		
GA	9 (22.5%)	0 (0.00%)	0 (0.00%)	29.159	0.000**
AA	4 (10.0%)	0 (0.00%)	0 (0.00%)		
Total	40	40	40		
		Allele Frequency			
G	63 (79%)	80 (100%)	80 (100%)	36.59	0.000**
A	17 (21%)	0 (0%)	0 (0%)		
Total	80	80	80		
		**(P<0.01)			

Table 3. Genotype distribution and allele frequency of (G>A) 4,217 in different groups.

Genotype Frequency					
Genotype/ (G>A) 4,217	**DM with CKD No. (%)**	**DM No. (%)**	**Control No. (%)**	**Chi-Square**	**P-value**
GG	5 (12.5%)	4 (10.0%)	0 (0.00%)		
GA	28 (70.0%)	12 (30.0%)	0 (0.00%)	55.434	0.000**
AA	7 (17.5%)	24 (60.0%)	40 (100%)		
Total	40	40	40		
		Allele Frequency			
G	38 (48%)	20 (25%)	0 (0%)	49.342	0.000**
A	42 (52%)	60 (75%)	80 (100%)		
Total	80	80	80		
		**(P<0.01)			

which was lower than the 90% observed in the DM without CKD group (Table 5). The GG genotype frequency was 25% in the DM with CKD group, compared to 10% in the DM without CKD group and 0% in the control group. The G allele frequency was higher in the DM with CKD group (63%) than in the DM without CKD group (55%) and the control group (50%). Conversely, the mutant A allele was more frequent in the control group (50%) than in the DM with CKD (37%) and DM without CKD groups (45%). These differences were not statistically significant.

Additionally, a new variation located at position 4,148 (G>A) showed that the heterozygous GA genotype frequency in the DM with CKD group was 87.5%, which was higher than the 70.0% observed in the DM without CKD group (Table 6). The GG genotype frequency in the DM with CKD group was 12.5%, compared to 30% in the DM without CKD group. The mutant A allele appeared more frequently in the control group (46%) than in the DM with CKD (44%) and DM without CKD groups (35%). These differences were not statistically significant.

For the CNR1 cannabinoid gene, the genetic polymorphism showed a new variation at position 4,337 (G>A). The heterozygous GA genotype frequency in the DM with CKD group was 67.5%, which was lower than 100% in the DM without CKD group, with a significant difference between the groups (Table 7). The GG genotype frequency was 32.5% in the DM with CKD group and 0% in the DM without CKD group. The G allele frequency was higher in the DM with CKD group (66%) compared to the DM without CKD group (50%) and the control group (54%). Conversely, the mutant A allele was more frequent in the DM without CKD group (50%) than in the DM with CKD group (34%). These differences were statistically significant.

For the variation located at 4,171 (G>A) with a missense mutation, the heterozygous GA genotype frequency in the DM with CKD group was 75%, higher than 60% in the DM without CKD group and 45% in the control group,

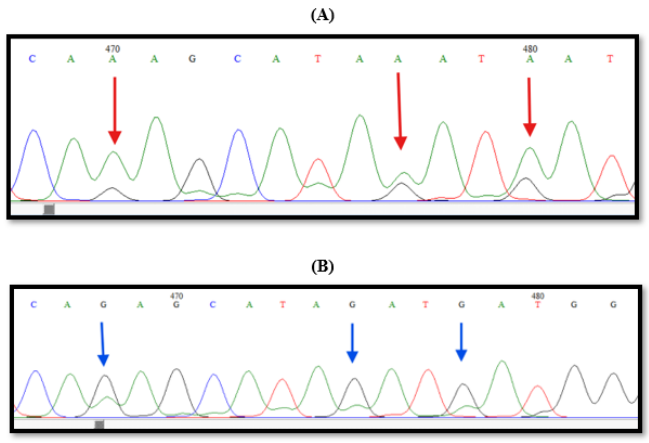

Fig. 2. Nucleotide variations (G>A) at position 4,217, rs2481890897 at 4,224. (A) SNP location – Red Arrow. (B) Wild nucleotide – Blue Arrow.

Table 4. Genotype distribution and allele frequency of (G>A)3,998 in different groups.

Genotype Frequency					
Genotype/ (G>A) 3,998	**CKD No. (%)**	**DM No. (%)**	**Control No. (%)**	**Chi-Square**	**P-value**
GG	7 (17.50%)	8 (20.0%)	3 (7.5%)	2.604	0.272 NS
GA	33 (82.5%)	32 (80.0%)	37 (92.5%)		
AA	0 (0.00%)	0 (0.00%)	0 (0.00%)		
Total	40	40	40		
Allele Frequency					
G	47 (59%)	48 (60%)	43 (54%)	0.7161	0.397 NS
A	33 (41%)	32 (60%)	37 (46%)		
Total	80	80	80		
NS: Non-Significant.					

Table 5. Genotype distribution and allele frequency of (G>A): 4,350 in different groups.

Genotype Frequency					
Genotype/ (G>A): 4,350	**CKD No. (%)**	**DM No. (%)**	**Control No. (%)**	**Chi-Square**	**P-value**
GG	10 (25.0%)	4 (10.0%)	0 (0.00%)	8.881	0.012*
GA	30 (75.0%)	36 (90.0%)	40 (100%)		
AA	0 (0.00%)	0 (0.00%)	0 (0.00%)		
Total	40	40	40		
Allele Frequency					
G	50 (63%)	44 (55%)	40 (50%)	2.5684	0.1090 NS
A	30 (37%)	36 (45%)	40 (50%)		
Total	80	80	80		
* (P≤0.05), NS: Non-Significant.					

Table 6. Genotype distribution and allele frequency of (G>A)4,148 in different groups.

Genotype Frequency					
Genotype/ (G>A) 4,148	**DM with CKD No. (%)**	**DM No. (%)**	**Control No. (%)**	**Chi-Square**	**P-value**
GG	5 (12.5%)	12 (30.0%)	3 (7%)	8.04	0.01*
GA	35 (87.5%)	28 (70.0%)	37 (93%)		
AA	0 (0.00%)	0 (0.00%)	0 (0.00%)		
Total	40	40	40		
Allele Frequency					
G	45 (56%)	52 (65%)	43 (54%)	2.297	0.1296 NS
A	35 (44%)	28 (35%)	37 (46%)		
Total	80	80	80		
* (P≤0.05),					

with no significant difference between groups (Table 8). The GG genotype frequency in the DM with CKD group was 25%, which was lower than 40% in the DM without CKD group and 55% in the control group. The G allele frequency was 62.5% in the DM with CKD group, lower than 70% in the DM group and controls.

Additionally, a new variation located at 4,009 (G>A) showed that the heterozygous GA genotype frequency in the DM with CKD group was 82.5%, slightly higher than 80% in the DM without CKD group, with no significant difference between groups (Table 9). The GG genotype frequency was 17.5% in the DM with CKD group, 20% in the DM without CKD group, and 17.5% in the control group. The G allele frequency was similar among the DM with CKD (59%), DM without CKD (60%), and the control groups (59%). The mutant A allele frequency was also similar between the DM with CKD and control groups (41%) and slightly lower in the DM without CKD group (40%) variation was missense mutation.

3.3. Correlation of serum KIM 1, NGAL and genotype distribution of (G>A) at 4,027 rs773947953 for CNR gen in different groups

The results in Table 10 indicate the relationship between the (G>A) missense mutation at position 4,027 (rs773947953) and the levels of NGAL and KIM-1. In the DM with CKD group, individuals with the GG genotype showed the highest levels of NGAL (1214.29 ± 62.1) and KIM-1 (354.77 ± 20.1), while those with the AA genotype had lower levels of NGAL (656.01 ± 21.1) and KIM-1

Table 7. Genotype distribution and allele frequency of G>A 4,337 in different groups

Genotype Frequency					
Genotype/ (G>A): 4,337	**CKD No. (%)**	**DM No. (%)**	**Control No. (%)**	**Chi-Square**	**P-value**
GG	13 (32.5%)	0 (0.00%)	3 (5%)		
GA	27 (67.5%)	40 (100%)	37 (95%)	15.68	0.00**
AA	0 (0.00%)	0 (0.00%)	0 (0.00%)		
Total	40	40	40		
Allele Frequency					
G	53 (66%)	40 (50%)	43 (54%)	5.112	0.029*
A	27 (34%)	40 (50%)	37 (46%)		
Total	80	80	80		
*(P≤0.05), **(P≤0.01)					

Table 8. Genotype distribution and allele frequency of (G>A): 4,171 in different groups.

Genotype Frequency					
Genotype/ (G>A): 4,171	**CKD No. (%)**	**DM No. (%)**	**Control No. (%)**	**Chi-Square**	**P-value**
GG	10 (25.0%)	16 (40.0%)	22 (55%)		
GA	30 (75.0%)	24 (60.0%)	18 (45%)	5.211	0.07 NS
AA	0 (0.00%)	0 (0.00%)	0 (0.00%)		
Total	40	40	40		
Allele Frequency					
G	50 (62.5%)	56 (70%)	62 (77%)	4.282	0.052 NS
A	30 (37.5%)	24 (30%)	18 (23)		
Total	80	80	80		
NS: Non-Significant.					

Table 9. Genotype distribution and allele frequency of (G>A) 4,009 in different groups.

Genotype Frequency					
G Genotype/ (G>A) 4,009	**CKD No. (%)**	**DM No. (%)**	**Control No. (%)**	**Chi-Square**	**P-value**
GG	7 (17.5%)	8 (20.0%)	7 (17.5%)		
GA	33 (82.5%)	32 (80.0%)	33 (82.5%)	0.769	0.68 NS
AA	0 (0.00%)	0 (0.00%)	0 (0.00%)		
Total	40	40	40		
Allele Frequency					
G	47 (59%)	48 (60%)	47 (59%)	0.038	0.897 NS
A	33 (41%)	32 (40%)	33 (41%)		
Total	80	80	80		
NS: Non-Significant.					

(248.53 ± 16.1). In the DM without CKD group, the GA genotype exhibited higher levels of NGAL (767.19 ± 36.6) and KIM-1 (233.57 ± 32.6), whereas the GG genotype showed lower levels of NGAL (361.23 ± 20.4) and KIM-1 (105.57 ± 22.1). In the control group, the GG genotype corresponded to NGAL levels of 171.08 ± 12.2 and KIM-1 levels of 70.96 ± 8.7. The presence of the G allele was associated with disease manifestation and higher levels of NGAL and KIM-1.

The rs2481890897 SNP of the CNR1 cannabinoid gene, located at position 4,224, showed that the heterozygous GA genotype in the DM with CKD group had mean KIM-1 and NGAL levels of 371.18 ± 1.96 and 1226.07 ± 2.48, respectively, which were significantly higher than those in the DM without CKD group. There was a statistically significant association between rs2481890897 and serum levels of KIM-1 and NGAL. The GG genotype frequency in the DM with CKD group had a mean KIM-1 level of 337.79 ± 4.94, which was also higher than the means for the DM without CKD and control groups (103.71 ± 3.94 and 74.49 ± 1.66, respectively). Similarly, the NGAL mean in the DM with CKD group with the GG genotype was 1021.73 ± 4.49, exceeding that of the DM without CKD and control groups (371.28 ± 6.1 and 169.83 ± 0.87, respectively) (Table 11).

For CNR1 cannabinoid gene, the genetic polymorphism showed new variation located 4,217(G>A), the heterozygous for the G allele GA genotype in DM with CKD

Table 10. Correlation of serum KIM 1, NGAL and genotype distribution of (G>A) at 4,027 rs773947953.

Group	Genotype	KIM (mean ± SE)	NGAL (mean ± SE)
DM with CKD	GG	354.77 ± 20.1	1214.29 ± 62.1
	GA	233.57 ± 32.6	767.19 ± 36.6
	AA	248.53 ± 16.12	656.02 ± 21.1
DM	GG	105.57 ± 22.1	361.23 ± 20.4
	GA	-	-
	AA	-	-
Control	GG	70.96 ± 8.7	171.08 ± 12.2
	GA	-	-
	AA	-	-
P. Value		0.0000***	0.000*

In the DM with CKD group, individuals with the GG genotype have significantly higher levels of KIM and NGAL compared to the GA and AA genotypes. The DM without CKD and control groups show lower KIM and NGAL levels for the GG genotype compared to DM with CKD. The p-values indicate strong statistical significance for differences in KIM and NGAL levels among groups and genotypes

Table 11. Correlation of serum KIM 1, NGAL and genotype distribution of (G>A),4,224 rs2481890897 for CNR gen in different groups:

Group	Genotype	KIM	NGL
DM with CKD	GG	337.79±4.94a	1021.73±4.49a
	GA	371.18±1.96b	1226.07±2.48c
DM	GG	103.71±3.94e	371.28±6.1e
	GA	97.95±3.89d	339.92±1.18d
Control	GG	74.49±1.66d	169.83±0.87d
P. Value		0.0000***	0.0000***

Table 12. Correlation of serum KIM 1, NGAL and genotype distribution of (G>A) at 4,217.

Group	Genotype	KIM	NGL
DM with CKD	GG	356.87±2.66a	935.28±5.26a
	GA	349.15±2.27a	1146.11±4.11a
	AA	361.46±3.08a	1111.72±4.98b
DM	GG	99.66±2.98d	363.62±3.52d
	GA	113.3±3.26c	365.12±7.68c
	AA	108.55±2.08c	355.33±7.44c
Control	AA	76.41±4.09c	171.51±4.84c
P. Value		0.0000***	0.0000***

group according to the Table 12 have mean and SD for KIM1 (349.15±2.27) while NGAL (1146.11±4.11) higher than GA genotype in DM without CKD group respectively (113.3±3.26), (365.12±7.68) there is statistically significant between new variation 4,217 and serum proteins (KIM1 and NGAL), the recessive type for GG genotype frequency in DM with CKD group have KIM mean (356.87±2.66) higher than KIM mean for DM wit- hout CKD (99.66±2.98), while NGAL mean for DM with CKD (935.28±5.26) was higher than NGAL mean for DM without CKD (363.62±3.52), The homozygous for A allele AA genotype in DM with CKD have mean (361.46±3.08) for KIM and NGAL (1111.72±4.98) higher than AA genotype for DM without CKD and control group.

For the CNR1 cannabinoid gene, a new genetic polymorphism was identified at position 4,148 (G>A). In the DM with CKD group, individuals with the heterozygous GA genotype had mean and SD values for KIM-1 of 325.19 ± 3.1 and for NGAL of 1091.68 ± 5.16, which were significantly higher than those with the GA genotype in the DM without CKD group (107.92 ± 2.63 for KIM-1 and 359.07 ± 3.65 for NGAL). There was a statistically significant association between the 4,148 (G>A) variation and serum levels of KIM-1 and NGAL. Additionally, the recessive GG genotype in the DM with CKD group showed higher mean values of KIM-1 (293.97 ± 1.12) and NGAL (788.66 ± 4.28) compared to the DM without CKD group (KIM-1: 100.08 ± 2.41; NGAL: 366.27 ± 4.92) (Table 13).

For the CNR1 cannabinoid gene, a new genetic polymorphism was identified at position 4,337 (G>A). In the DM with CKD group, individuals with the heterozygous GA genotype for the G allele exhibited mean ± SD values of KIM-1 (364.4 ± 2.07) and NGAL (973.17 ± 5.51), which were significantly higher than those with the same genotype in the DM without CKD group (KIM-1: 104.12 ± 2.48; NGAL: 362.9 ± 1.79). There was a statistically significant association between the 4,337 (G>A) variant and serum levels of KIM-1 and NGAL. Additionally, the

recessive GG genotype frequency in the DM with CKD group showed higher mean KIM-1 (350.05 ± 4.47) and NGAL (1148.07 ± 8.65) levels compared to the DM without CKD group (KIM-1: 105.2 ± 1.08; NGAL: 358.51 ± 2.91) (Table 14).

4. Discussion

The endocannabinoid system has numerous clinical conditions. Verifiable information about the correlation between the cannabinoid system and itch; this study determines genetic variants in the endocannabinoid receptor 1 (CNR1) gene with diabetic nephropathy. The rs1049553 variant for CNR1 gene in hemodialysis patients and healthy controls. Significant association in patients with and without UP, even after excluding individuals with diabetes and dyslipidemia [19]. The most common G to A transition in rs1049353, involving the A and G alleles, has been strongly associated with type 2 diabetes mellitus, both with and without kidney failure. Doris et al. (2019) [20] demonstrated that this transition is linked to higher body mass index and increased waist circumference. Our study highlights the impact of specific variant within the CNR1 gene, particularly rs1049353, on the development of kidney disease in patients with Type 2 Diabetes Mellitus. In addition, [21] shows the effect of lead, cadmium and arsenic in a population that had been directly exposed to these metals. Significant relationships were also found between the CNR1 rs1049353 gene variants and the lead exposure marker. Statistically significant differences were seen between T2DM individuals with diabetic nephropathy (DN) and those without.

The rs10493353 variant in CNR1 gene with Diabetic nephropathy in Type 2 Diabetic Mellitus patients, and the A allele decreases the risk of DN in T2DM patients. Some environmental conditions affect the gene. The rs10493353 G/A variant was independent marker of DN in T2DM patients. The blockade of CNR1 can be used for treatment of Diabetic nephropathy in Type 2 Diabetic Mellitus patients. The study provides information on the rs10493353 (G/A) variant in the progression of DN. Reportedly, the rs10493353 (also called G1359A) is involved in the mechanism of metabolic syndromes. The [22] indicates that patients with the AA genotype decrease BMI, blood pressure, and insulin resistance. The A allele of the CNR1 gene protect of diabetic nephropathy with type 2 diabetes mellitus. The risk factors of diabetic nephropathy (DN) can protective function of the A allele in the progression of DN, as the A allele diminishes the risk of DN. Some environmental factors can effect of genes for DN. The blockade of CNR1 can be used for treatment of Diabetic nephropathy in Type 2 diabetic mellitus patients [18].

Cannabinoid receptor-1(CNR1) gene polymorphism effect the diabetic nephropathy (DN) in patients with type 2 diabetes mellitus (T2DM). It has been confirmed that CNR1 plays an important role in kidneys, predicted that patients with the result of the rs1776965150 show that genetic polymorphism the A allele frequency with non-significant difference CNR1 genetic variants, specifically the rs750464422 polymorphisms, were correlated with the development of diabetic nephropathy (DN), whereas the rs1776965150 polymorphisms showed no association with DN [23].

The CB1R have a broad role of renal diseases. In metabolic syndrome, obesity and diabetes, CB1R inhibition not only enhance metabolic parameters, but also play a direct role in the protection of renal function, since a large proportion of CKD patients suffer from metabolic syndrome and obesity, recent studies hi- ghlighted that CB1R also promotes renal fibrosis in non- metabolic nephropathies and that its inhibition diminishes the development of renal fibrosis [24].

Hyperglycemia and activation of the renin-angiotensin system are thought to be the two main drivers of this pathology. We have recently shown that selective blockade of peripheral cannabinoid receptor-1 (CB1R) delayed and diminished the onset of type 2 diabetes, involved CB1R activation in glomerular, and suggested that antagonism of peripheral CB1R may serve as a novel, effective, and

Table 13. Correlation of serum KIM 1, NGAL and genotype distribution of (G>A) at 4,148.

Group	Genotype	KIM	NGL
DM with CKD	GA	325.19±3.1a	1091.68±5.16a
	GG	293.97±1.12a	788.66±4.28b
DM	GA	107.92±2.63c	359.07±3.65c
	GG	100.08±2.41c	366.27±4.92c
Control	GA	72.01±2.54d	170.33±3.41d
	GG	58.03±2.98d	180.32±3.72d
P. Value		0.0000***	0.0000***

Table 14. Correlation of serum KIM 1, NGAL and genotype distribution of (G>A) at 4,337.

Group	Genotype	KIM	NGL
DM with CKD	GG	350.05±4.47a	1148.07±8.65b
	GA	364.4±2.07a	973.17±5.51a
DM	GG	105.2±1.08d	358.51±2.91e
	GA	104.12±2.48e	362.9±1.79e
Control	GG	57.44±3.15c	170.31±3.19d
	GA	86.32±2.59c	172.77±1.27d
P. Value		0.0000***	0.0000***

rational strategy to prevent and reverse diabetic nephropathy. Increased CB1R signaling in podocytes contributes to the development of diabetic nephropathy and represents a common pathway through which both hyperglycemia and increased RAS activity exert their deleterious effects, highlighting the therapeutic potential of peripheral CB1R blockade [25].

Neutrophil gelatinase-associated lipocalin is one of the most promising tubular markers for diagnosing acute and chronic kidney diseases [26]. NGAL has been documented to promote nephrogenesis and to facilitate the transformation of mesenchymal cells into renal epithelial cells. NGAL synthesis in kidney tubules increases in response to several harmful stimuli, including ischemia-reperfusion injury, indicating that this protein plays a crucial role in tubular regeneration and repair [15].

Neutrophil gelatinase-associated lipocalin (NGAL) is a lipoprotein involved in inflammation, the transport of small hydrophobic molecules, and the regulation and preservation of iron. NGAL levels in blood and urine increase within two hours following acute kidney injury (AKI). It serves as a more sensitive biomarker than traditional renal indicators such as serum creatinine and blood urea nitrogen, allowing for earlier detection of kidney damage. Additionally, treatment with diuretics does not affect NGAL levels in either urine or blood [27].

Study by Dawood et al (2023) [28] examined at T2DM affects circulating levels of increase in NGAL in diabetic patients Starting 2-4 hours after a kidney injury, elevated NGAL levels can be seen in plasma and urine, due to changes in glomerular filtration, tubular reabsorption, and also via increased secretion in tubular epithelial cells. When the proximal tubule is injured by tubular necrosis, filtered NGAL can leave tubular reabsorption and be secreted in urine. High serum NGAL and high filtered saturated tubular capacity lead to increased urinary and serum NGAL. Kidney damage detected by serum creatinine, but is delayed in response to renal injury is usually late and the degree of injury may be underestimated. creatinine is a marker of glomerular filtration not specific to injury in other areas of the nephron. Kidney damage (KIM-1) is a substantial increase in cells of the proximal tubules. KIM-1 is lost in the lumen. Some studies have revealed that shed KIM-1 can be used as a blood biomarker for kidney damage [29].

The immunoglobulin superfamily protein known as kidney injury molecule (KIM)-1, or T cell immunoglobulin mucin domains (TIM)-1, is significantly elevated in the proximal tubule of an injured or diseased kidney; KIM-1 expression appears early in the course of the disease, and blood KIM-1 levels predict the progression of DKD to end-stage kidney disease (ESKD) independent of the urinary albumin-to-creatinine ratio (ACR), hemoglobin A1C, and estimated glomerular filtration rate (eGFR) [30]. Numerous studies have improved them as markers and indicators of injury in chronic renal disease [4].

This study highlights a significant association between specific polymorphisms in the CNR1 gene and the prevalence of chronic kidney disease among patients with type 2 diabetes mellitus. The identified genetic variations, particularly those correlated with altered levels of serum biomarkers NGAL and KIM-1, underscore the potential role of the endocannabinoid system in the progression of diabetic nephropathy. These findings contribute valuable insights into the genetic factors influencing kidney injury and may pave the way for improved risk stratification and personalized therapeutic approaches in diabetic kidney disease. Further research with larger populations is warranted to validate these associations and explore their clinical applications.

Conflict of interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethical clearance

Ethical clearance. This study has been approved by the ethics commission for, University of Middle Technical, Baghdad, Iraq. dated: 02/05/2025.

Availability of data and material

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request**.**

Authors' contributions

Both authors contributed equally to the development of the research plan, the statistical analysis of the results, and the writing of the manuscript.

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References

1. Banday MZ, Sameer AS, Nissar S (2020) Pathophysiology of diabetes: an overview. Avicenna J Med 10(4): 174–188 doi: 10.4103/ajm.ajm_73_20
2. Ahmed MH, Haddad NI, Nori E (2022) Correlation between albuminuria levels and chitinase 3 like 1 protein in Iraqi patients with type 2 diabetes mellitus. Iraqi J Sci 63: 21–32
3. Darraji M, Saqban L, Mutar T, Rasheed M, Hussein A (2022) Association of candidate genes polymorphisms in Iraqi patients with chronic kidney disease. J Adv Biotechnol Exp Ther 6(1): 687
4. Quang TH, Nguyet MP, Thao DP, Thi MH, Phuong Thi Dam L, Thi HH, et al. (2020) Evaluation of urinary neutrophil gelatinase associated lipocalin and kidney injury molecule-1 as diagnos- tic markers for early nephropathy in patients with type 2 dia- betes mellitus. Diabetes Metab Syndr Obes 13: 2199–2207 doi: 10.2147/DMSO.S273142
5. Yahya AA, Kadhim DJ, Abdalhadi NA (2023) Kidney injury molecule-1 and cystatin C as early biomarkers for renal dysfunction in Iraqi type 2 diabetes mellitus patients. J Adv Biotechnol Exp Ther 6(3): 60
6. Ivanac-Janković R, Lovčić V, Magaš S, Šklebar D, Kes P (2015) The novella about diabetic nephropathy. Acta Clin Croat 54(1): 83–90
7. Colhoun HM, Marcovecchio ML (2018) Biomarkers of diabetic kidney disease. Diabetologia 61(5): 996–1011 doi: 10.1007/s00125-018-4545-6
8. Said SM, Nasr SH (2016) Silent diabetic nephropathy. Kidney Int 90(1) doi: 10.1016/j.kint.2016.02.002
9. Sauriasari R, Safitri DD, Azmi NU (2021) Current updates on protein as biomarkers for diabetic kidney disease: a systematic

review. Ther Adv Endocrinol Metab 12: 20420188211049612 doi: 10.1177/20420188211049612

10. Bjornstad P, Cherney DZ, Maahs DM (2015) Update on estimation of kidney function in diabetic kidney disease. Curr Diab Rep 15: 1–12 doi: 10.1007/s11892-015-0589-x
11. Chaudhary K, Phadke G, Nistala R, Weidmeyer CE, McFarlane SI, Whaley-Connell A (2010) The emerging role of biomarkers in diabetic and hypertensive chronic kidney disease. Curr Diab Rep 10: 37–42 doi: 10.1007/s11892-009-0071-4
12. He P, Bai M, Hu J-P, Dong C, Sun S, Huang C (2020) Significance of neutrophil gelatinase-associated lipocalin as a biomarker for the diagnosis of diabetic kidney disease: a systematic review and meta-analysis. Kidney Blood Press Res 45(4): 497–509 doi: 10.1159/000508340
13. Lin C-H, Chang Y-C, Chuang L-M (2016) Early detection of diabetic kidney disease: present limitations and future perspectives. World J Diabetes 7(14): 290 doi: 10.4239/wjd.v7.i14.290
14. Singer E, Markó L, Paragas N, Barasch J, Dragun D, Müller DN, et al. (2013) Neutrophil gelatinase-associated lipocalin: pathophysiology and clinical applications. Acta Physiol (Oxf) 207(4): 663–672 doi: 10.1111/apha.12075
15. Greco M, Chiefari E, Mirabelli M, Salatino A, Tocci V, Cianfrone P, et al. (2022) Plasma or urine neutrophil gelatinase-associated lipocalin (NGAL): which is better at detecting chronic kidney damage in type 2 diabetes? Endocrines 3(2): 175–186
16. Mukhopadhyay P, Pan H, Rajesh M, Bátkai S, Patel V, Harvey-White J, et al. (2010) CB1 cannabinoid receptors promote oxidative/nitrosative stress, inflammation and cell death in a murine nephropathy model. Br J Pharmacol 160(3): 657–668 doi: 10.1111/j.1476-5381.2010.00768.x
17. Rizvi S, Raza ST, Mahdi F (2014) Association of genetic variants with diabetic nephropathy. World J Diabetes 5(6): 809 doi: 10.4239/wjd.v5.i6.809
18. Zhang X, Zhu H, Xing X, Zhang C (2020) Association between cannabinoid receptor-1 gene polymorphism and the risk of diabetic nephropathy among patients with type 2 diabetes mellitus. Pharmacogenomics Pers Med 13: 591–599 doi: 10.2147/PGPM.S258282
19. Heisig M, Łaczmański Ł, Reich A, Lwow F, Szepietowski JC (2016) Uremic pruritus is not associated with endocannabinoid receptor 1 gene polymorphisms. Biomed Res Int 2016:3567527 doi: 10.1155/2016/3567527
20. Doris JM, Millar SA, Idris I, O'Sullivan SE (2019) Genetic polymorphisms of the endocannabinoid system in obesity and diabetes. Diabetes Obes Metab 21(2): 382–387 doi: 10.1111/dom.13426
21. Matys T, Szymańska-Chabowska A, Bogunia-Kubik K, Smyk B, Kamińska M, Mazur G, et al. (2020) The relationship between selected CNR1, MC4R, LEP, FTO and VDR gene polymorphisms and several basic toxicological parameters among persons occupationally exposed to arsenic, cadmium and lead. J Clin Med 9(4): 1040 doi: 10.3390/jcm9041040
22. de Luis DA, Sagrado MG, Aller R, Izaola O, Conde R (2011) Influence of G1359A polymorphism of the cannabinoid receptor gene on anthropometric parameters and insulin resistance in women with obesity. Metabolism 60(2): 272–276 doi: 10.1016/j.metabol.2010.02.004
23. Aljorani RH, Saleh ES (2025) The study of the correlation between cannabinoid receptor-1 gene polymorphisms and risk of diabetic nephropathy in Iraqi people. Iraqi J Pharm Sci 34(1): 1–9
24. Dao M, François H (2021) Cannabinoid receptor 1 inhibition in chronic kidney disease: a new therapeutic toolbox. Front Endocrinol 12: 720734 doi: 10.3389/fendo.2021.720734
25. Jourdan T, Szanda G, Rosenberg AZ, Tam J, Earley BJ, Godlewski G, et al. (2014) Overactive cannabinoid 1 receptor in podocytes drives type 2 diabetic nephropathy. Proc Natl Acad Sci U S A 111(50): E5420–E5428 doi: 10.1073/pnas.1416112111
26. Sueud T, Hadi NR, Abdulameer R, Jamil DA, Al-Aubaidy HA (2019) Assessing urinary levels of IL-18, NGAL and albumin creatinine ratio in patients with diabetic nephropathy. Diabetes Metab Syndr Clin Res Rev 13: 564–568
27. Abed IN, Hassan HAA-H, Al Timari US (2023) Correlation between Neutrophil gelatinase-associated lipocalin and beta2-Microglobulin with serum protein electrophoresis in multiple myeloma patients. J Pak Med Assoc 73: S3–S8
28. Dawood DA-J, Rasheed EM, Hassan AS (2023) Correlation between Neutrophil Gelatinase Associated with Lipocaline and Megalin in Type 2 Diabetic patients. Adv Life Sci 10: 30–34
29. Hassan HAA-H, Jassim HS (2024) Evaluation of Serum Human Kidney Injury Molecule-1 and N-Acetyl-β-D-Glucosaminidase as Early Markers of Kidney Injury in Patients with β-Thalassemia Major in Diyala Governorate. Iraqi J Sci 65: 616–622
30. Mori Y, Ajay AK, Chang J-H, Mou S, Zhao H, Kishi S, et al. (2021) KIM-1 mediates fatty acid uptake by renal tubular cells to promote progressive diabetic kidney disease. Cell Metab 33(5): 1042–1061.e1047 doi: 10.1016/j.cmet.2021.03.012