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The Study of the Correlation between Cannabinoid Receptor-1 Gene Polymorphisms and Risk of Diabetic Nephropathy in Iraqi People

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Abstract

Background: Cannabinoid receptor-1 (CNR1) gene polymorphism is reportedly associated with diabetic nephropathy (DN) in patients with type 2 diabetes mellitus (T2DM). It has been confirmed that CNR1 plays an important role in the peripheral organs, including the kidneys. Alterations of cannabinoid receptor expression and activity have been discovered in various renal diseases such as diabetic nephropathy. The goal is to find out if the CNR1 genetic variations rs750464422 T/C and rs1776965150 T/A affect how likely T2DM Iraqi patients are to get DN. Methods: In this cross-sectional study, 100 patients were included who had T2DM. We classified the participants into two groups: the first group was comprised of 50 patients with DN (the case group), and the second group was comprised of 50 patients without DN (the control group). Cannabinoid receptor-1 rs750464422, and rs1776965150 polymorphisms were genotyped in DN patients and controls by polymerase chain reaction (PCR). Results: Data analysis revealed that there was a significant difference in the frequencies of the TC genotypes of CNR1 rs750464422 between patients with DN and controls (p=0.001), while there were non-significant differences in the distribution of CC genotypes (p=0.6). Moreover, the C allele of this polymorphism was associated with an increased risk of DN compared to the T allele (p=0.001, OR=3.29 and 95% CI= 1.58-7.07). We predicted that patients with the rs1776965150 genetic polymorphism have a significant difference in the TA genotype (p=0.05, OR= 0.95, and 95% CI= 0.1-1.10), while the A allele frequency shows a non-significant difference (p=0.2). Conclusion: Cannabinoid receptor-1 genetic variations, rs750464422 polymorphisms were correlated with the development of DN, while rs1776965150 polymorphisms were not associated with DN.

Keywords: Cannabinoid receptor-1, Diabetes mellitus, Diabetic nephropathy, Gene polymorphism

Introduction

Diabetes nephropathy is the most significant microvascular consequence of diabetes [(1)]. It is a significant public health issue characterized by increased urine albumin excretion, decreased glomerular filtration rate, or both. The frequency of diabetic nephropathy is expected to rise further in the future, offering a significant burden to the healthcare system and resulting in increasing morbidity and death [(2-3-4)]. Endocannabinoids (ECs) are endogenous, bioactive lipid mediators' molecules that primarily act through two G protein-coupled receptors, the cannabinoid-1 (CB1) and cannabinoid-2 (CB2) receptors. These receptors' signaling is complex, and depending on the cell type, it may involve inhibition (and in some cases activation) of adenyl-cyclase activity, as well as activation of various mitogen-activated protein kinases (MAPKs) [(5)]. It was previously considered that the CB1 receptor was primarily expressed in the central nervous system. However, throughout the last two decades, numerous investigations have shown its presence and importance in peripheral organs, including the kidneys. Both glomeruli and tubular epithelial cells express the CB1 receptor (CNR1). The CB1 receptor is found in the endothelium of intrarenal arteries. The ECs system has several functions in kidney function. The EC system can maintain renal homeostasis under normal settings by modulating renal hemodynamics, tubular salt reabsorption, and urine protein excretion [(5)]. These effects are predominantly caused by CNR1 activation. Cannabinoid receptor expression and activity have been reported to be altered in a variety of renal disorders, including diabetic nephropathy [(6)]. Diabetes produces the most serious renal consequences, such as progressive kidney disease and diabetic nephropathy [(7)], by increasing ROS generation, which eventually leads to diabetic nephropathy (through many routes) [(8-9)]. The effects of cannabinoid receptors in the kidney on oxidative stress are inconsistent. The CB1 receptor promotes inflammation, oxidative/nitrative damage, and cell death by activating p38-MAPK [(10)]. Despite the fact that the pathophysiology of DKD is very complex and there is rising evidence that genetic variables

play a role in susceptibility, there is accumulating evidence that genetic factors contribute to DKD risk. The role of genetic markers in disease etiology has received much attention, but no such research has been undertaken in Iraq. Genetic research may reveal the pathobiology of DKD and potential therapeutic targets. Numerous single nucleotide polymorphisms in numerous genes have been found [(11)], resulting in a plethora of gene variants that play a substantial role in the genetic vulnerability to diabetic nephropathy [(13)]. The discovery of genetic variations at the biomarker level has the potential to improve diabetic nephropathy diagnosis, treatment, and early prevention. This is because it allows for the identification of people who are more likely to get the condition [(14)]. CNR1 is a polypeptide with 473 amino acids that is encoded by CNR1 genes. This gene belongs to the inhibitory G protein-coupled receptors family and is located on chromosome 6q14-q15 [(12)]. Several polymorphisms in the CNR1 gene have been identified in various groups. Recent research suggests a link between the CNR1 gene polymorphism and DN, as well as several clinical situations such as metabolic syndrome [(13)]. This syndrome is distinguished by a decrease in high-density lipoprotein cholesterol, higher triglyceride and insulin levels, and obesity, all of which are known risk factors for type 2 diabetes and coronary artery disease in T2DM patients [(13)]. To the best of our knowledge, no previous research has been undertaken in Iraq to investigate the role of SNPs within the CNR-1 gene in the pathogenesis of DKD. Thus, the current study sought to determine if the presence of SNPs in CNR1 variants (rs750464422 and rs1776965150) influenced the progression of DN in Iraqi T2DM patient.

Materials and Methods

Study subjects

This cross-sectional study included one hundred male and female participants with age over 18 years all patients with diabetic mellitus type 2, divided into two groups of fifty patients each recruited from the Specialized Center for Endocrinology and Diabetes in Al-Rusafa, Baghdad. The first group included T2DM patients with DN as a case group and T2DM patients without DN as a control group. The American Diabetes Association (ADA) guideline was used to make the diagnosis of T2DM with duration range from 5 to 10 years of diagnosis. Diabetic patients with nephropathy were chosen by a professional consultant nephrologist and diagnosed based on questionnaires, clinical features, laboratory data, and the urinary albumin creatinine ratio (ACR> 30 mg/g). Patients with type 1 diabetes, hypertension, urinary tract infections, urethral calculi tumors (any type of malignancy), gestational diabetes, severe cardiac, liver, and renal function failure, renal disease, and other diseases that cause proteinuria; obese patients (who had BMI ranges ≥ 30 Kg /m2); patients on nephrotoxic drugs; anti-inflammatory drugs; and lipid-lowering drugs were excluded from the study. A questionnaire was utilized to collect data on age, gender, duration of T2DM, blood pressure, smoking and drinking status, and medication history. The participants' height, weight, waist circumference, and body mass index (BMI=weight/height2, kg/m2) were all measured. S. creatinine, eGFR, fasting blood glucose, and HbA1c were all measured using standard biochemical procedures. A random spot urine sample was taken in a suitable urine container and promptly measured for urinary albumin and urinary creatinine in order to calculate the albumin-to-creatinine ratio (ACR).

Genotyping

For DNA extraction, two ml of the obtained venous blood specimen were kept at -20°C until DNA extraction and gene genotyping for the determination of CNR-1 gene variations by polymerase chain reaction (PCR). The Genomic DNA was extracted and purified from human whole blood samples (frozen EDTA blood samples) using the Easy Pure® Blood Genomic DNA Kit (Catalog No.: EE121) in specialized laboratory of genetic study by the author with the assistance of lab staff. The protocol that used for extraction was done by addition of 100 µl of lysis to the 250 µl of blood. Mixed thoroughly by vortexing or pipetting, then 20 µl of Proteinase K was added to the lysate and incubated at room temperature for 2 min. 20 µl of RNase A was added to the lysate (to obtain RNA-free genomic DNA). Mixed well by vortexing, then incubated at room temperature for 2 minutes. All mixtures were incubated at 65 ºC for 15 minutes with mixing. 500 µl of binding buffer2 was added and immediately mixed by vortexing for 5 seconds. Then incubated at room temperature for 10 minutes. All the lysate was transferred to a spin column, centrifuged at 12,000 rpm for 30 seconds. Removed the flow through. Followed by addition of 500 µl of clean buffer 2 and centrifuged at 12,000 rpm for 30 seconds. Removed the flow through. Another 500 µl of wash buffer 2 was added and centrifuged at 12,000 rpm for 30 seconds. Then the flow through was discarded. The last step repeated once. the column centrifuged at 12,000 rpm for 2 minutes, to remove the remaining wash buffer 2. The spin column placed in a sterile 1.5 mL microcentrifuge tube, then 100 µl of Elution Buffer was added (preheated to 65 ºC) to the column matrix. Then incubated the mixture for1minute at room temperature. Centrifuged at 12,000 rpm for 1 minute to elute the extracted DNA**.** The extracted genomic DNA was stored at − 20°C. DNA amplification of a single nucleotide polymorphism (SNP) was performed with primers (forward 5′-CCCTCTGCTTGCAATCATGG, reverse 5′-

TGTGTAGCCAAAGGTTTCCC).These primers were designed by Alpha DNA Canda company by a specialized laboratory in genetic study as lyophilized product of various picomoles concentration. The primers were presented in a freeze-dried format. They were reconstituted by dissolving them in water that was free of nucleases, to be used as a concentrated solution (stock solution) to yield a final concentration of 100 pmol/ µl. The working solution of these primers was generated by mixing10µl of primer stock solution (preserved at -20Co) to 90 µl of nuclease-free water to yield a 10 pmol/ µl working primer solution.The PCR conditions were set as follows: initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s; annealing at 60 °C for 40 s; extension-1 at 72°C for 45 s; and final extension at 72°C for 5 min. The resulting fragments were electrophoresed on a 2% agarose gel stained with ethidium bromide in order to determine the genotypes of the patients and controls for both polymorphic sites. The PCR products and primers used in the current study were sent to Macrogen, South Korea (dna.macrogen.com), for Sanger sequencing analysis to find any polymorphisms. The results were received by email and then analyzed using the Geneous prime program and the Bioedit program.

Statistical analysis

IBM SPSS Statistics 26 program was used to detect the effect of different factors on study parameters. One-way ANOVA was used to significantly compare between means. Means followed by different letters are significantly different according to Duncan's multiple range comparisons (DMRTs), Means followed by the same letter are not significantly different. Chi-square test was used to significantly compare between percentage (0.05 and 0.01 probability). Estimate of Odd ratio and CI in this study. GraphPad Prism 9 program was used to draw the Figures in this study. WINPEPI and SPSS program was used to detect the genotyping [15,16].

Results and Discussion

The demographic, anthropometric, clinical, and biochemical characteristics of all individuals enrolled in this study are summarized in Table 1. There were no significant differences in age, gender, duration of disease, smoking, or drinking habits between the study groups ($p>0.05$). On the other hand, a significant difference was found in the serum levels of Creatinine, ACR, and eGFR between the control group and the patient groups ($p<0.05$). The ACR and creatinine levels were higher in the DN patient group than in the control group, while eGFR showed the lowest levels in the DN group. Serum levels of HbA1c, FSG, and Body mass index (BMI) when compared between both groups revealed a non-significant difference ($p>0.05$). The distribution of DN patients according to the KIDGO eGFR stages was considerably different between the groups, with the majority of control patients at stage 1 and only 10 at stage 2. While the distribution of DN patients was uneven, most patients were at stage 1, as illustrated in Table (1).

Table 1. Sociodemographic parameters of the study groups

Parameters		**Group 1 patient With DN n=50**	**Group2 control Without DN n= 50**	***p*-value**
Age (year)		50.92 ±9.23	52.62±10.32	0.3
Gender	female	22 (44%)	26 (42%)	0.4
	male	28 (56%)	24 (48%)	
BMI (kg/m^2)		26.76±3.07	26.52±3.16	0.7
Smoking	smoker	11(22%)	13(26%)	0.6
	Nonsmoker	39(78%)	37(74%)	
Alcohol consumption	drinker	5 (10%)	3(6%)	0.2
	Non-drinker	45 (90%)	47(94%)	
T2DM duration (year)		8.7±4.33	8.08±4.3	0.4
HBA1c %		8.89 ±2.32	8.13±2.30	0.1
FSG mmol/L		9.84 ± 2.44	8.95 ± 2.12	0.3
ACR 3-30mg/g		85.33 ±66.80	13.12±6.92	0.0001**
S. Creatinine mmol/L		73.48 ± 26.89	58.04±16.95	0.001**
eGFR		98.16±31.49	123.92±49.90	0.003**

CKD stage (eGFR ml/min/1.73m^2)			
Stage 1 (≥90)	31	40	0.01**
Stage 2 (60-90)	12	10	
Stage 3 (30-60)	7	0	
Stage 4 (15-29)	0	0	
Stage 5 (<15)	0	0	

Group 1: T2DM patient with DN, Group 2: control group T2DM patient. BMI: body mass index, T2DM: type 2 diabetes mellitus, HbA1c: glycated hemoglobin, FBS: fasting blood sugar, eGFR: estimated glomerular filtration rate, ACR: urinary albumin-creatinine ratio, CKD: chronic kidney disease) Continuous variable expressed as mean ±SD; *: significant difference between the groups (p<0.05), **: highly significant difference between the groups (p<0.01),Chi-square test was used to significantly compare between the percentages and ANOVA to compare between the means.

The products of DNA extraction were then fractionated on a 1.5% agarose gel, stained with ethidium bromide, and visualized using a gel imaging system, as shown in Figure (1).

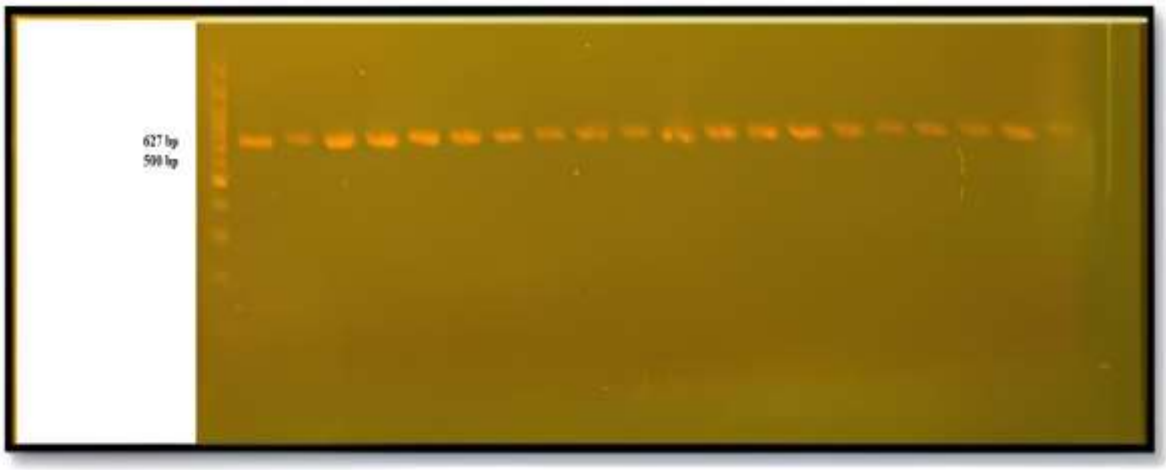

Figure 1. Electrophoresis of a 627 bp fragment of human CNR1 gene as electrophoresed for 1 hour on ethidium bromide-stained agarose (1.5%) gel at 45 volt/cm2 and 1× TBE buffer.

All DNA samples of patients and controls were undergone sequencing for CNR1 gene. The results of the CNR1 gene sequencing revealed absence for G1359A variant (rs1049353) but other SNPs were detected, rs750464422, rs1776965150 were undergone analysis.

Results of the analysis of the rs750464422 SNP of the CNR1 gene using Sanger sequencing are shown in Figure (2). A single "C" peak is indicative of a C homozygous genotype. A single "T" peak is indicative of a T homozygous genotype. The presence of the "C" and "T" peaks is indicative of a T/C heterozygous genotype.

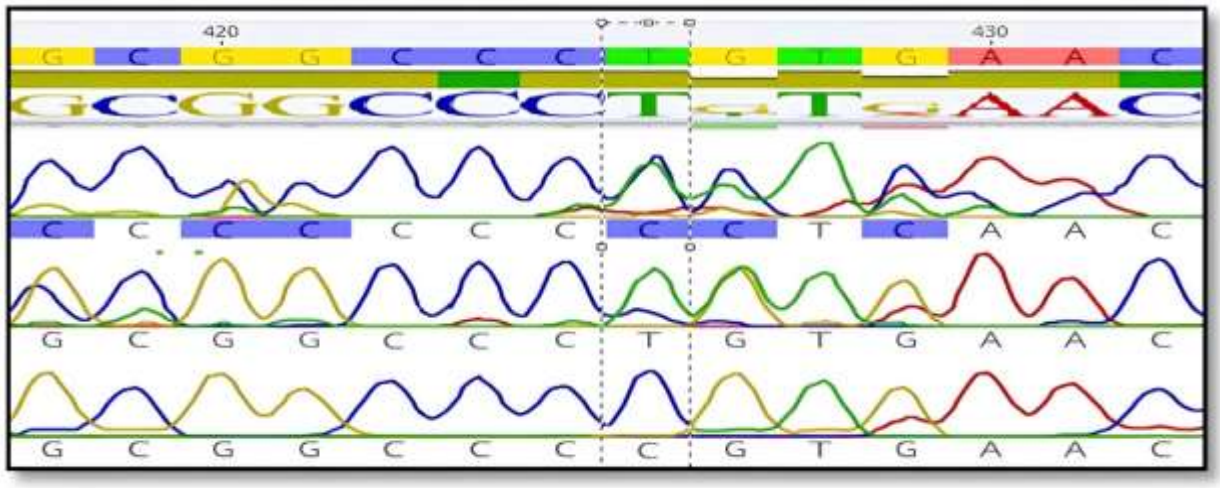

Figure 2. Analysis of Rs 750464422 SNP of CNR1 gene.

The result of the analysis of the rs1776965150 SNP of the CNR1 gene using Sanger sequencing is illustrated in Figure (3). A single "A" peak is indicative of an "A" homozygous genotype. A single "T" peak is indicative of a T homozygous genotype. The presence of the "C" and "A" peaks is indicative of a T/A heterozygous genotype.

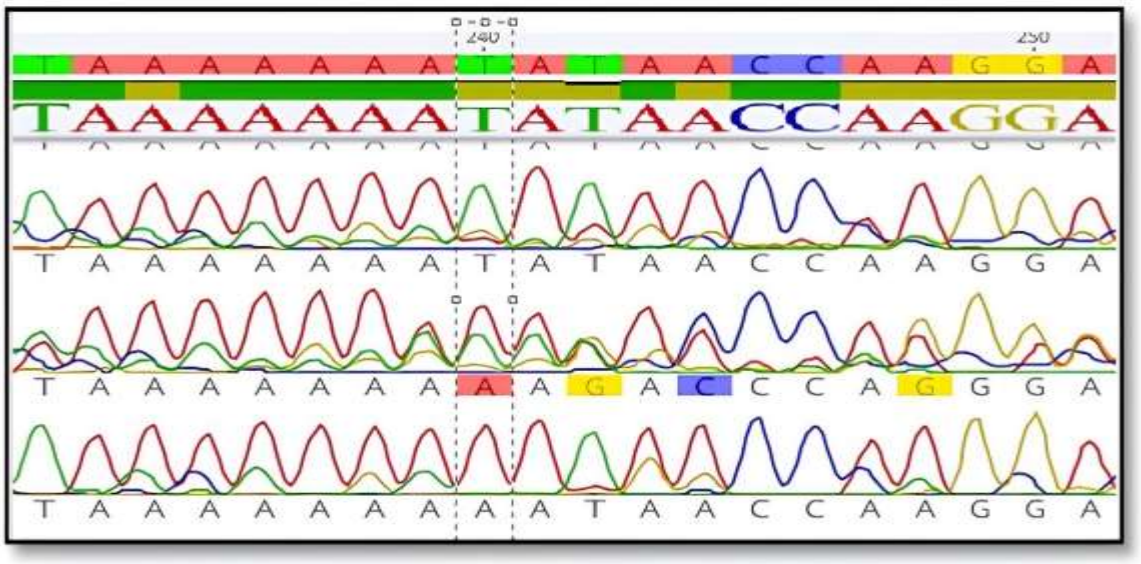

Figure 3. Analysis of rs1776965150 T/A SNP of CNR1 gene

The association between CNR1 gene polymorphisms and risk of DN in different genetic models of Iraqi patients with T2DM is shown in Table (2). The analysis of this study expressed that there was a significant difference in the frequencies of the TC genotypes of CNR1 rs750464422 between patients with diabetic nephropathy and control subjects (p=0.001), while there were non-significant differences in the distribution of CC genotypes (p=0.6). Moreover, the C allele of this polymorphism was associated with an increased risk of diabetic nephropathy compared to the T allele (p=0.001) with an OR of 3.29 and 95%CI = 1.58-7.07. Furthermore, patients with rs1776965150 genetic polymorphism have a significant difference in the TA genotype (p<0.05) which is a borderline for significance and OR = 0.95; 95% CI = 0.1–1.10; while the AA genotype and A allele frequency show a non-significant difference (p=0.6 and 0.2, respectively).

Table 2. Genotypes and allele frequency of rs750464422 and rs1776965150 in patients with diabetic nephropathy and patients without diabetic nephropathy groups.

Genotype/allele	rs750464422			
	Control group (n=50) *n*(%)	**Patients group (n=50) *n*(%)**	***p*-value**	**OR and 95% CI**
TT	40(80)	22(44)	-------	1.00 reference
TC	8(16)	25(50)	0.001*	5.25(2.05-13.89)
CC	2(4)	3(6)	0.6	1.53(0.22-13.32)
T allele	88(88)	69(69)	------	1.00 reference
C allele	12(12)	31(31)	0.001*	3.29(1.58-7.07)
	rs1776965150			
TT	34(68)	41(82)	-------	1.00 reference
TA	14(28)	6(12)	0.05	0. ٩٥(0.10-1.10)
AA	2(4)	3(6)	0.6	1.53(0.22-13.32)
T allele	82(82)	88(88)	------	1.00 reference
A allele	18(18)	12(12)	0.2	0.62(0.28-1.37)

* Significant difference between groups; rs:- reference SNP, Qi-square test was used to compare between the percentages & calculation of odd ratio.

Fasting serum glucose and glycated hemoglobin were calculated in both groups, and one-way ANOVA analysis revealed no significant effect of the variant on the glycemic index, as illustrated in Table (3).

Table 3. Association between RS 750464422 and HbA1c, FSG

Groups	RS 750464422	HbA1c	FSG
Patient	TT	9.25±2.17 a	10.44±4.36 a
	TC	8.68±2.52 a	9.696±4.53 a
	CC	7.75±0.35 a	5.30±2.40 a
	p-value	0.5	0.2
Control	TT	7.99±2.22 a	8.72±5.25 a
	TC	8.24±2.17a	9.80±4.32 a
	CC	10.40±4.81 a	10.40±8.49 a
	p-value	0.3	0.8

Data are presented as Mean±SD. FSG: fasting serum glucose; HbA1c glycated hemoglobin, one-way anova test was used to compare between the means, Means followed by the same letter are not significantly different.

There were no significant differences in S. creatinine, eGFR, or ACR between genotypes in the control group, while there is a significant difference only in S. creatinine level and eGFR between the wild and non-wild genotypes in the patient group, as shown in Table (4).

Table 4. association between rs750464422 on serum creatinine, estimated glomerular filtration rate, albumin creatinine ratio

Groups	rs750464422	S. creatinine (mg/dL)	eGFR	ACR
Patient	TT	83.09±32.04 a	86.45±31.96 b	80.37±58.27 a
	TC	63.58±18.15 b	109.27±28.33 a	91.17±75.67 a
	CC	96.50±2.12 a	82.65±15.16 b	64.03±.19.59 a
	p-value	0.01**	0.03*	0.7
Control	TT	59.43±17.30 a	120.60±48.33 a	13.09±6.73 a
	TC	54.50±16.34 a	136.34± 63.24 a	11.34±7.47 a
	CC	44.50±2.12 a	140.80±26.16 a	21.04±6.39 a
	p-value	0.3	0.6	0.2

Data are presented as mean±SD, Means followed by different letters are significantly different according to Duncan's multiple range comparisons (DMRTs), Means followed by the same letter are not significantly different,* Significant difference between groups, ** highly significant difference between groups, one-way ANOVA test was used to compare between the means.

One-way ANOVA analysis shows no significant effect of the variant on the development of the glycemic index, as illustrated in Table (5). As shown in table (6), there is no significant effect of the variant seen on S. creatinine, eGFR, or ACR between wild and non-wild genotypes in the control group, while there is a significant difference in serum creatinine and eGFR between the genotypes in the patients' groups (p=0.02 and 0.03, respectively).

Table 5. association between rs1776965150 and HbA1c, FSG

Groups	rs1776965150	HbA1c	FSG
Patient	TT	8.83±2.49 a	9.77±4.64 a
	TA	9.42±1.05 a	11.25±3.27 a
	AA	8.75±0.49 a	7.25±2.76 a
	p-value	0.8	0.5
Control	TT	8.05±2.39 a	8.85±5.20 a
	TA	8.69±2.14 a	9.90±5.12 a
	AA	6.35±0.35 a	4.65±2.05 a
	p-value	0.3	0.4

Data are presented as mean±SD. Means followed by the same letter are not significantly different, FSG; fasting serum glucose; HbA1c: glycated hemoglobin, one-way ANOVA test was used to compare between the means.

Table 6. association between rs1776965150 on serum creatinine, estimated glomerular filtration rate, and albumin creatinine ratio

Groups	rs1776965150	S. creatinine	eGFR	ACR
Patient	TT	70.24±24.99 b	100.76±30.10 b	89.72±69.99 a
	TA	100.33±30.79 a	70.83±31.75 b	68.68±48.43 a
	AA	61.00±1.414 b	125.55±11.67 a	43.23±9.28 a
	p-value	0.02*	0.03*	0.5
Control	TT	58.40±18.74 a	126.90±55.77 a	13.13±6.90 a
	TA	57.85±13.19 a	119.29± 35.02 a	13.91±7.20 a
	AA	53.00±1.41 a	102.10±2.12 a	8.01±6.59 a
	p-value	0.9	0.7	0.5

Data are presented as mean±SD, Means followed by different letters are significantly different according to Duncan's multiple range comparisons (DMRTs), Means followed by the same letter are not significantly different, one-way ANOVA test was used to compare between the means.

Discussion

Diabetic nephropathy is a primary cause of end-stage renal disease; it is a common and deadly complication of diabetes that shortens life expectancy and is still not fully treated (17,18). Hyperglycemia has been identified as a main cause of glomerulopathy. The prevalence of progressive kidney disease in diabetes individuals with reasonably good glucose control (albeit greater than in type 2 diabetic patients) supports the inclusion of other pathogenic variables (20). As a result, it is critical to find other factors that may influence the progression of DN, such as the possible role of the cannabinoid type 1 receptor gene polymorphism in type 2 diabetes with nephropathy. A previous study (14), in this regard, confirmed that DN is caused by a mix of genetic and environmental variables. Previous research has shown that overactivity of the endocannabinoid/cannabinoid1 receptor (CB1R) system in podocytes contributes to the development of diabetes and its complications (5,19), and that it does so through two main pathogenic factors (hyperglycemia and increased renin angiotensin system (RAS) activity) (19). These findings supported Buraczynska's (2014) assertion that there is a relationship between CB1R and diabetic nephropathy, as suggested by a documented association between a polymorphism in the CNR1 gene and nephropathy in T2DM patients (21). As a result, CNR1 gene polymorphisms may influence CB1R signaling in podocytes, either indirectly (via metabolic consequences) or directly by increasing diabetes-associated inflammation and ROS generation, promoting tissue injury, and the development of diabetic complications. The genetic connection of the rs750464422 CNR1 polymorphism with diabetic nephropathy susceptibility has investigated in the Iraqi population in this study. The findings demonstrated a statistically significant variation in the distribution of rs750464422 genotypes and alleles between the patients as illustrated in Table (2). According to the findings of this investigation, persons with the CNR1 rs750464422 polymorphism carrying TC genotypes had a higher risk of developing DN. Furthermore, this studied SNP had been associated with increased chance of disease which is ensure by significant differences in the glomerular filtration rate accompanied with higher serum creatinine level & ACR levels in subject carrying non wild genotype in compared to wild genotype in patients' group as showed in Table (4).Also, when compared to the T allele, the mutant homozygote C allele of this polymorphism was significantly related with an increased risk of diabetic nephropathy, This suggests that this SNP may have similar effect as another common SNP in CB1R rs1049353 which is associated with reductions in parameters of glucose metabolism, probably caused by upregulation of gluconeogenic transcription factors as the result of increased CB1 receptor activity(13,22)Concerning the rs1776965150 polymorphism, we discovered that patients with the TA genotype are on the borderline of significance, despite having a higher frequency in controls (28% vs. 12% in patients) and an odd ratio (0.95) that is nearly one; we couldn't consider this genetic variant as protective; because all renal parameters indicated decline in renal function as illustrated in Table (6). If it has a protective role, the deterioration in creatinine & eGFR may be less. perhaps if we have a larger sample size, it will appear as a protective SNP, furthermore, this variant has a significant effect on creatinine and eGFR levels in patients with DN, but not in patients without DN. This could be due to the variant interacting with other unique genetic and environmental factors associated with DN, which may involve pathways or mechanisms specifically involved in the development and progression of DN. also, the mutant homozygote (AA) was not significant because of its low frequency variant besides the small sample size. Furthermore, the A allele of this polymorphism was not linked to an elevated risk of diabetic nephropathy; thus, we deem this genetic variant unrelated to the condition. Besides, negative interactions exist between the

CNR1 rs1776965150 and some parameters that increased risk of DN, including HBA1c, FSG and ACR. As a result, the current study represents a novel discovery in a Middle Eastern/Iraqi community in Baghdad area. Surprisingly, these SNPs have never been investigated before. Several genome-wide association studies (GWASs) have recently been done to study the relationship between other SNPs in T2DM and its association with nephropathy (13,21), however no previous study on the same SNPs was found and link between the CNR1 variants rs750464422 and rs1776965150 and the presence of diabetic nephropathy. Despite the success of Genome-Wide Association Studies (GWAS) as a potent tool in the analysis of complex diseases, identifying all genetic risk factors connected with the disease is a difficult undertaking due to its complexity. The mechanism through which the CNR1 gene polymorphism leads to diabetic nephropathy susceptibility remains unknown. Because CNR1 may influence a variety of metabolic activities, the potential impact of CNR1 polymorphisms on the risk of developing diabetic nephropathy may be influenced by differences in an individual's metabolic profile, such as glycemic control, dietary habits, response to treatment, obesity, blood pressure, and other related factors.

Study Limitation

Our study, like most association studies, has some limitations. The small sample size and concentration on a single facility in a single city (Baghdad) limited the scope of this study. As a result, before generalizing the findings of this study to the entire country, extreme caution should be exercised. Diabetes and its complications are also affected by the combination of multiple risk alleles, environmental variables, medication adherence, and dietary habits. The combined influence of these various factors might lead to an overestimation or underestimating of the importance of an investigated polymorphism in defining the phenotype.

Conclusion

Based on the findings of the present study, it can be concluded that there is a predictable association between cannabinoid receptor-1 genetic polymorphism rs750464422 and susceptibility to diabetic nephropathy, while rs1776965150 were not associated to the disease.

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Conflicts of Interest:

The authors declare no conflicts of interest.

Funding:

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Ethics Statements

The study was approved by the Ethical and Scientific Committee of the College of Pharmacy at the University of Baghdad, Iraq with Ethical approval with the number (REAFUBCP-632022A).

Author Contribution

The authors confirm contribution to the paper as follows: study conception and design: Eman saadi; data collection: raghda hisham ; analysis and interpretation of results: eman saadi saleh,raghda hisham khalil ; draft manuscript preparation: eman saadi,raghda hisham. All authors reviewed the results and approved the final version of the manuscript.

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العلاقة بين تعدد الأشكال الجيني لمستقبلات القنب -١ وخطر الإصابة باعتلال الكلية السكري في الشعب العراقي

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الخلاصة

تشير الدراسات الحديثه الى العلاقه بين تعدد الأشكال الجيني لمستقبلات القنب -١ ومرض الاعتلال السكري في مرضى السكري من النوع الثاني.مستقبلات القنب من النوع الاول تلعب دورا مهما في الاعضاء المحيطية بما فيها الكلى.تم اكتشاف تأثير التغييرات في تعبير مستقبلات القنب ونشاطها في امراض الكلى مثل اعتلال الكلية السكري.

هومعرفة ما اذا كانت الاختلافات الجينيه لمستقبلات الكانابينويد ١ تؤثرعلى زيادة احتمالية اصابة مرضى داء السكري من النوع الثاني على الاعتلال الكلوي ولتحقيق هذا الهدف تم اشراك مئة فرد عراقي من مرضى داء السكري من كلا الجنسين، تتراوح أعمارهم من 18 عامًا وما فوق، تم تقسيمهم إلى مجموعتين تحتوي كل منهما على خمسين مريضًا. المجموعة الأولى (مجموعة التحكم) تألفت من أشخاص مصابين بداء السكري من النوع الثاني بدون اعتلال كلوي، بينما المجموعة الثانية تضمنت مرضى داء السكري من النوع الثاني الذين يعانون من الاعتلال الكلوي.

بعد تضخيم الحمض النووي المستخرج باستخدام تفاعل البلمرة المتسلسل، تم إجراء تسلسل باستخدام طريقة سانغر لتحديد تعدد الانماط الجينية لمستقبل الكانابينويد-١ . النتائج: أظهر تحليل البيانات بالنسبة لمجموعة مرضى داء السكري الذين يعانون من الاعتلال الكلوي.إن النمط الجيني المتغاير TC ل rs750464422 ذات القيمة (P = 0.001) يمثل قيمة ذات دلاله احصائية عالية بينما النمط الجيني المتغاير TA لـ rs1776965150.فأن قيمة ال (P = 0.05)على حافة الاهتمام للدلاله الاحصائية.

أظهرت النتائج أن تعدد الأشكال الجيني للطفرة -(rs750464422) قد يكون مرتبط بتطور مرض الاعتلال الكلوي في مرضى داء السكري من النوع الثاني بينما الطفره ,(rs1776965150) غير مرتبطه بتطور الاعتلال الكلوي لدى مرضى داء السكري من النوع الثاني

الكلمات الأساسية: مستقبلات القنب -١ ، داء السكري ، اعتلال الكلية السكري ، تعدد الأشكال الجيني .