Original
ISSN 2413-0516

A Novel Study of Protein Kinase C Beta Gene Polymorphism (rs3760106) and Protein Kinase C Activity Levels as a Predictor of Nephropathy Complications in Iraqi Diabetic Patients

Fadhil Jawad Al-Tu'ma*, Zahraa Abdul Adheem Al-Maiyaly

Department of Chemistry and Biochemistry, College of Medicine, University of Kerbala, Kerbala, Iraq.
*Correspondence to: Fadhil Jawad Al-Tu'ma (E-mail: fadhil.jawad@uokerbala.edu.iq)
(Submitted: 13 January 2022 – Revised version received: 29 January 2022 – Accepted: 18 February 2022 – Published online: 26 April 2022)

Abstract

Objectives: This study aimed to investigate the relation between the risk of (PRKCB-1504 C/T) gene polymorphisms and diabetic nephropathy pathogenesis and its association with its levels and some other biomarkers in serum of Iraqi diabetic nephropathy patients.

Methods: A cross-sectional study was performed on 130 samples obtained from Al-Hussein Teaching Hospital/Kerbala – Iraq during Feb., 2020 to March 2021. Seventy three patients of them with type 2 diabetes mellitus (T2D) and the remaining patients with type 2 diabetes mellitus with nephropathy complications (DN). Phenotypic analysis included determination of protein kinase C (PKC) activity levels, HbA1c%, serum insulin levels and renal function tests. Five ml of whole blood was obtained from each patients, 4.0 ml was centrifuged for serum separation used for biomarkers determinations and the remaining 1.0 ml was used for genomic DNA extraction for molecular analysis and polymorphism of PRKCB (rs3760106) by allele specific-amplification refractory mutational system-polymerase chain reaction (allele specific ARMS-PCR), followed by electrophoresis on 1% agarose gel. Various statistical analyses were applied to analyze the obtained data.

Results: The amplification of the PRKCB gene gives one genotypes as indicated by (200 bp) bands for those with homozygous wild type (CC), homozygous mutant (TT) genotypes and two genotypes bands (200 bp) for those with heterozygous (CT). Genotype frequencies of rs3760106 polymorphism were found to be consistent with Hardy–Weinberg equilibrium. Allele frequencies (23.3%, 26.7%, 50%) of CC, CT, TT in cases of DN group. While the frequencies in the non-DN group were (30%, 45%, 25%) and for healthy control group were (50%, 33.4%, 16.6%) for wild, heterozygous, and homozygous in an order. In healthy control group, the risk of diabetic nephropathy was significantly higher among carriers of T allele under codominant TT (OR = 6.42, 95% CI = (1.66–24.85), $P = 0.007$), dominant CT + TT (OR = 3.2, 95% CI = (1.08–9.95), $P = 0.03$) and recessive model (OR = 5, 95% CI = (1.51–16.56), $P = 0.008$) while in T2DM without nephropathy the risk of diabetic nephropathy was significantly higher among carrier of T allele under recessive model (OR = 3, 95% CI = (1.09–28.25), $P = 0.003$). Serum level of PKC-B1 activity was significantly higher among DN group than T2DM and control groups (43.35 ± 18.69, 30.35 ± 11.96, 27.42 ± 10.31, $P = 0.001$), also PKC-B1 activity in DN group was significantly correlated with fasting blood glucose, HOMA-IR and renal function tests such as GFR, urea and creatinine.

Conclusion: This study indicates that the PRKCB (C/T-1504) rs3760106 single nucleotide polymorphism was significantly associated with increased diabetic nephropathy susceptibility of Iraqi patients with T2DM and serum level of PKCB activity was associated with increase the risk of diabetic nephropathy complications.

Keywords: Type 2 diabetic nephropathy, PRKCB gene polymorphism, pathogenesis, protein kinase C

Introduction

Diabetes mellitus (DM) is consider as the most common and chronic metabolic disorder worldwide. The prevalence of this disorder keep increasing and it is expected to impact 439 million individuals by 2030.[1] It is the major cause of severe complications such as "retinopathy, nephropathy, and neuropathy" identified in T2DM by resistance to insulin action in adipose tissues, muscle, liver, and elsewhere.[2-4] Insulin is an important hormone released from the pancreas. It has a critical function in which enhance the transporting of glucose from the bloodstream into the body's cells where the glucose is converted into energy. Insulin deficiency or the cell's failure to respond to insulin result in elevated level of blood glucose, or hyperglycemia, which is the hallmark of diabetes. Different organs especially the eyes, kidneys, nerves, and also blood vessels effected and damaged with chronic hyperglycemia of DM.[5,6] Multiple genetic and environmental factors greatly influence it, for this reason there is extensive attempts made to recognize the disease-affecting genes in order to better understanding the pathogenesis of the disease, develop new clinical therapy targets and improved prediction of the disease.[7]

One of the most frequent and common complications of diabetes and the direct cause of end-stage renal disease (ESRD) is diabetic nephropathy, 40% of all individuals with type 2 diabetes develop diabetic nephropathy.[3] Clinically, diabetic nephropathy is characterized by an increase in the excretion of albumin in urine and a decline in GFR which result in decline in the function of the kidney. The classification of diabetic nephropathy is based on "estimated glomerular filtration rate (eGFR)" and the level of proteinuria.[2]

It is obvious that both hemodynamic and metabolic pathways contribute to trigger the progression of DKD.[8] Patients with DN manifest at the beginning hyper filtration that mean high or normal level of GFR with the occurrence of micro albuminuria, then the patient manifest a gradual decrease of the GFR and occurrence of macro albuminuria that comes before mild and subsequently moderate proteinuria. The final stage is identify by severe proteinuria and sever decline in GFR < 15% with chronic renal insufficiency that result in ESRD and dialysis. These abnormalities result from structural

modifications at the cellular level, which encompass of vascular permeability, podocytes apoptosis, accumulation of extracellular matrix (ECM) in the mesangium (mesangium expansion) and thickening of glomerular basement membrane. Then, the formation of mesangial nodules which also called Kimmelsteil-Wilson nodules (glomerulosclerosis) and ultimately tubule-interstitial fibrosis.[9]

Protein kinase C (PKC) is a family of serine- and threonine-specific protein kinases that can be activated by calcium and second messenger diacylglycerol. PKC family members phosphorylate a wide variety of protein targets and are known to be involved in diverse cellular signaling pathways. PKC family members also serve as major receptors for phorbol esters, a class of tumor promoters.[10,11] The protein kinase family consists of over 15 subgroups with more than 500 kinases, each of which is involved in the regulation of gene expression; thereby, the down-regulation or up-regulation of these kinases induces severe consequences in the progression of disorders including neurodegenerative diseases[12,13] and involve in numerous signaling processes such as modification of gene expression, cell division, migration, proliferation, differentiation, cell survival and apoptosis.[14] Chronic hyperglycemia which is one of the hallmark of DM result in increases the second messenger diacylglycerol "DAG" production, that is one of the important protein kinase C (PKC) activating factors with calcium ion, so that PKC may have a critical role in diabetes mellitus and its vascular complications.[15] The role of PKCB isoform in the pathogenesis of DN has been the most intensively investigated among all the PKC isoenzyme. It has been shown that in diabetic glomeruli PKCB was raised or activated and has led to glomerular hypertrophy and expansion of the extracellular matrix. Recent studies have shown that LY333531 (a PKCβ-selective inhibitor) selectively inhibits PKCB over other PKC isoforms, can help improve glomerular hyperfiltration, albuminuria and mesangial expansion. Such findings strongly indicate that PKCB may have a significant role in the development of diabetic nephropathy.[16]

In addition to environmental factors, the genetic variations may have an significant effect on the progression of DN and ESRD. It is well known that gene susceptibility to DN have a critical role in individuals, even with the same environmental exposure.[3] Protein kinas C beta gene (PRKCB) is a new and strong candidate gene for DN, is located on chromosome 16p11.2.[17] However, the relationship between PRKCB gene polymorphisms and DN was validated in a Chinese as well as Indians population[17,18] confirmed that variant (PRKCB -1504 C/T rs3670106) was effect the expression and activity of PKCB isoform and associated with DN.

The aim of the current study was to assay the association between the risk of PRKCB (-1504 C/T) rs3760106 gene polymorphism with DN susceptibility, and the serum level of PKCB isoform and some biomarkers in the Iraqi patients.

Materials and Methods

This cross-sectional study was conducted on 100 subjects obtained from Al-Hassan Center of Endocrinology, Al-Hussein Teaching Hospital, Al-Hussein Medical City, Kerbala Health Directorate/Kerbala - Iraq during Nov., 2019 to Sep., 2020. The subjects were classified into three groups: 30 type 2 diabetic patients with nephropathy complication with age ranged between 35–74 years, of whom 63% were males and 37% females, 40 type 2 diabetic patients without nephropathy complication having the similar age range, and including 53% males and 47% females and 30 subjects as apparently healthy control group with the same age range, 66% were male and 34% were female. The exclusion criterion was T2DM patients with no DN or no dialysis. Subjects with eGFR< 60 (ml/min, 1.73 m^2 body surface area) and urinary albumin excretion "UAE" >300 mg/day were considered as the DN group. While subjects with eGFR > 60 ml/min 1.73 m^2 and UAE < 30 mg/day consider as T2DM without nephropathy and control group.

Blood sample (5 ml) was collected from each group; 2 ml was added to EDTA tube for molecular studies, the remaining sample, 3 ml was collected in a gel tube used for serum separation after centrifugation at 3000 × *g* and utilized for various biochemical investigations, including lipid profile, blood glucose, renal function tests and PKC-B level. The PKC-B enzyme activity level was measured by ELISA, the other biomarkers were assessed, using the Roche COBAS c311, and HOMA-IR and eGFR by equations (REF).

The DNA was extracted from frozen whole blood of each group, using the Geneaid kit and stored at −20°C. The PCR reaction is performed by using allele specific amplification refractory mutation system polymerase chain reaction (ARMS-PCR) which is a rapid and simple technique to detect a SNP.[19,20] A tri primer ARMS-PCR was used for the detection of −1504 C/T polymorphism of *PRKCB* (rs3760106). Sequences of primers are presented in Table 1.

The PCR products were analyzed by agarose gel electrophoresis, using 1% agarose gel, and visualized by staining with ethidium bromide (Promega, USA). PCR cycling conditions were as follows (Table 2).

The data were expressed as mean ± SD, the one-way ANOVA was used for calculating the probability (*P* value). The PAST version 3.09 used for calculating the probability value (*P* value), chi-square (χ^2), odds ratio (OR) and confidence interval 95% (CI 95%), and to express a significance between the study groups (polymorphisms and biochemical

Table 1. **Primer sequence for alleles of PRKCB gene**

Primers	Sequence
Mutant forward	**5'ACTACAAAGCATCCTGGTGACAAATGA 3'**
Normal forward	**5' ACTACAAAGCATCCTGGTGACAAATGG 3'**
Common Reverse	**5' AGGAGAGGGACATTCTATTATTGCAGCC 3'**

Table 2. **PCR reaction program**

Type of cycle	Temperature °C	Time	No. of cycle
Initial denaturation	95	5 min	1 cycle
Denaturation	95	30 sec	35 cycle
Annealing	62	30 sec	35 cycle
Extension	72	60 sec	35 cycle
Final Extension	72	5 min	1 cycle
Total Time: 1 hour and 35 min			

parameters). In all statistical analysis, the $P = 0.05$ was considered as a significant and $P < 0.01$ as highly significant.

Results

The characteristics of patient such as age, gender, and biochemical parameters such as HDL-C had no significant difference among DN and non-DN groups (Table 3, $P > 0.05$). While the PKCB isoform level, other characteristics were significantly different among the study groups (Table 3, $P < 0.05$) eGFR was very low in DN group as compared to DM and control group while serum urea and creatinine was very high in DN group.

Genotype frequencies of the *PRKCB* gene were not consistent with the Hardy–Weinberg equilibrium (HWE) in DN group. While, with the non-DN groups were consistent with HWE. Allele frequencies were (23.3%, 26.7%, 50%) of CC, CT, TT in cases of DN group, while the frequencies in the T2DM without nephropathy group were (30%, 45%, 25%) and for healthy control group were (50%, 33.4%, 16.6%) for wild, heterozygous, and homozygous in an order (Table 4).

In healthy control group, the risk of diabetic nephropathy was significantly higher among carriers of T allele under codominant TT (OR = 6.42, 95% CI = (1.66–24.85), $P = 0.007$), dominant CT + TT (OR = 3.2, 95% CI = (1.08–9.95), $P = 0.03$) and recessive model (OR = 5, 95% CI = (1.51–16.56), $P = 0.008$) while in T2DM without nephropathy the risk of diabetic nephropathy was significantly higher among carrier of T allele under recessive model (OR = 3, 95% CI = (1.09–28.25), $P = 0.003$), (Table 5).

The amplification of the PRKCB-1504 C/T gene gives one genotypes as indicated by (200 bp) bands for those with homozygous wild type (CC), homozygous mutant (TT) genotypes and two genotypes bands (200 bp) for those with heterozygous (CT), (Figure 1).

The analysis of data showed that DN patients, harboring TT genotype showed a higher significant PKCB activity level, blood glucose, urea, creatinine and eGFR as compared to those with the CT and CC genotype ($P < 0.05$), while in T2DM without nephropathy group expect blood glucose and HbA1c% there is no significant difference in other biological parameters in TT and CT genotype ($P > 0.05$). In healthy control group there is no significant difference in all biological parameters in CT, TT and CC genotype ($P > 0.05$) (Table 6).

Discussion

Various biomarker and genetic investigations in type 2 diabetic Iraqi patients with and without nephropathy

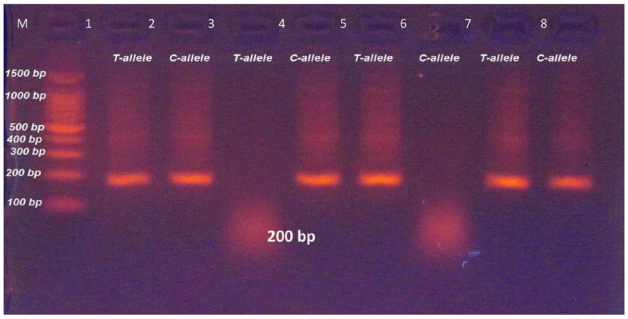

Fig. 1 **The amplification of the PRKCB1 -1504 C/T gene indicated by (200 bp) bands for those with homozygous wild type (CC), homozygous mutant (TT) genotypes and two genotypes bands (200 bp) for those with heterozygous (CT).**

Table 3. **Anthropometric and biochemical characteristics of study subjects**

Parameters	Control Mean ± SD N = 30	T2DM Mean ± SD N = 40	DN Mean ± SD N = 30	*P*-value
Age (y)	56.62 ± 6.55	57.72 ± 7.13	59.29 ± 7.55	0.1
BMI (kg/m^2)	28.3 ± 3.3	29.4 ± 5.4	28.3 ± 3.5	0.5
Duration of DM (y)	–	10.7 ± 3.7	16.36 ± 5.02	0.001*
FBS (mg/dl)	96.15 ± 10.4	168.4 ± 30.6	173.2 ± 22.02	<0.001*
HbA1c %	5.2 ± 0.9	8.93 ± 2.7	6.8 ± 1.32	<0.001*
Insulin (μu/ml)	4.54 ± 1.24	30.6 ± 11.49	41.82 ± 14.3	<0.001*
HOMA-IR	1.21 ± 0.49	9.91± 3.07	22.6 ± 11.2	<0.001*
Urea (mg/dl)	27.86 ± 6.83	30.4 ± 13.9	136.25 ± 14.6	<0.001*
Creatinine, (mg/dl)	0.6 ± 0.16	0.8 ± 0.2	7.6 ± 1.8	<0.001*
GFR (ml/min.1.732 m^2)	114.3 ± 19.2	106.16 ± 24.5	7.5 ± 2.7	<0.001*
T. Cholesterol, (mg/dl)	143.62 ± 28.24	168.4 ± 40.8	163.1 ± 34.5	0.03*
TG (mg/dl)	136.18 ± 40.3	188.3 ± 39.2	173.76 ± 21.6	0.01*
HDL-C (mg/dl)	40.73 ± 7.6	36.26 ± 7.77	38.39 ± 10.5	0.14
LDL-C (mg/dl)	78.02 ± 19.2	118.134.5	97.8 ± 11.3	<0.001
PKC-B (IU/min)	27.42 ± 10.31	30.35 ± 11.96	43.35 ± 18.69	<0.001*

BMI, body mass index; FBS, fasting blood sugar (glucose); eGFR, estimated glomerular filtration rate; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoproteins-cholesterol; LDL-C, low-density lipoproteins-cholesterol; VLDL-C, very low density lipoproteins-cholesterol; DN, diabetic nephropathy; DM, diabetes mellitus; HC, healthy control. Data were expressed as mean ± SD. *P value < 0.05 is significant, by one way ANOVA test.

Table 4. **Result of polymorphic allele frequency for PRKCB gene polymorphism at (rs3760106) locus in healthy control, T2DM and DN groups**

Group	Genotype frequency	Expected	Observed	χ^2	*P*-value
DN (*N* = 30)	CC (wild)	4.0	7 (23.3%)	5.44	0.01*
	CT (hetero)	13.9	8 (26.7%)		
	TT (mutant)	12.0	15 (50%)		
	C allele Freq.	0.37 (37%)			
	T allele Freq.	0.63 (63%)			
T2DM (*N* = 40)	CC (wild)	11	12 (30%)	0.38	0.5
	CT (hetero)	20	18 (45%)		
	TT (mutant)	9	10 (25%)		
	C allele Freq.	0.52 (52%)			
	T allele Freq.	0.48 (48%)			
Healthy control (*N* = 30)	CC (wild)	13.3	15 (50%)		
	CT (hetero)	13.3	10 (33.4%)	1.87	0.6
	TT (mutant)	3.3	5 (16.6%)		
	C allele Freq.	0.67 (67%)			
	T allele Freq.	0.33 (33%)			

Table 5. **Genotype and allele frequency of PRKCB gene SNP at (rs3760106) locus between T2DM and DN**

SNP rs3760106 C/T	T2DM *N* = 40	DN *N* = 30	OR (95%CI)	*P*-value
Codominant				
CC (Reference)	12	7		
CT	18	8	0.6 (0.2–2.6)	0.6
TT	10	15	2.5 (0.7–8.7)	0.1
Dominant				
CT + TT	28	23	1.4 (0.4–4.16)	0.5
Recessive				
CT + CC (Reference)	30	15		
TT	10	15	3 (1.09–28.25)	0.003*
Allele Frequency				
C (wild allele)	0.52	0.37		
T (mutant allele)	0.48	0.63	1.8 (1.4–3.2)	0.03*

SNP, single nucleotide polymorphisms; T2DM, Type 2 Diabetes Mellitus; DN, diabetic nephropathy; OR, observed risk; CI, confidence interval. *P* value < 0.05 is significant by risk odd ratio.

complications and acute kidney injury were performed as reported by Al-Tu'ma, et al. in 2017 and 2018.[21,22] In the present study, the genetic association of the *PRKCB rs3760106 polymorphism* with diabetic nephropathy susceptibility was examined in the Iraqi population. The results of this study indicated that subjects who carrying TT and CT genotypes had a greater chance to getting DN. In addition, the activity of PKCB isoform is higher in DN group than in non-DN subjects. The observed data indicated that there is a significant association between PRKCB rs3760106 polymorphism and the etiology of DN, genetic susceptibility. In addition to hyperglycemia, is identified as an significant factor affecting the development of diabetic nephropathy. In this study, we obtained fundamental evidence supporting the theory that polymorphism in *PRKCB gene*, which encodes PKCB-I and PKCB-II was significantly associated with diabetic nephropathy in Iraqi subjects. The results of the present study is compatible with the previous studies in Indian subjects[17] as well as Chinese subjects[18] which found that SNP (-1504 C/T, rs 3760106) of PRKCB1 gene have a significant association with ESRD in diabetic patients. This SNP are found in the regulatory region of PRKCB gene "the promoter" and consequently could affect the transcription of *PRKCB* gene. the functional roles of the -1504 C/T SNP are unclear but many studies show that the SNP may locate in the binding position for transcription factors (Sp1) so that affect the expression of the gene, also diabetes mellitus and hyperglycemia may stimulate glycosylation of Sp1 and consequently may induce the pathogenesis of diabetic complications.[17]

Table 6. **Biochemical characteristics of DN in relevance to the genotypes of PRKCB gene polymorphism analyzed under co-dominant model (*N* = 30)**

Clinical Characteristics	TT (*N* = 15) Mean ± SD	CT (*N* = 8) Mean ± SD	CC (*N* = 7) Mean ± SD	*P*-value
BMI (kg/m^2)	28.31 ± 3.98	28.13 ± 3.62	28.05 ± 3.89	0.9
Age (in year)	62.4 ± 9.91	59.13 ± 7.8	54.5 ± 8.42	0.3
FBS (mg/dl)	214.9 ± 26.10	170.12 ± 30.9	121.4 ± 2.77	0.01*
HbA1c%	7.41 ± 1.34	6.3± 1.20	6.41 ± 0.99	0.1
Insulin	39.5 ± 14.2	42.7 ± 12.5	50.3 ± 26.9	0.6
HOMA-IR	23.7 ± 11.6	21.6 ± 12.7	21.5 ± 6.7	0.9
Urea (mg/dl)	140.19 ± 19.9	136.04 ± 17.4	114.12 ± 4.9	0.05*
Creatinine, (mg/dl)	8.49 ± 1.47	7.61 ± 1.37	6.77 ± 1.54	0.04*
eGFR	6.40 ± 1.34	8.03 ± 1.85	8.66 ± 2.4	0.02*
T. Cholesterol, mg/dl	140.5 ± 26.7	146.6 ± 33.7	139.2 ± 23.5	0.5
Triglyceride, (mg/d)	94.41 ± 24.7	169.6 ± 36.4	179.3 ± 37.33	0.2
LDL-C (mg/dl)	82.7 ± 22.8	75.8 ± 18.6	71.13 ± 11.69	0.4
HDL-C (mg/dl)	40.51 ± 6.69	34.8 ± 10.5	40.97 ± 11.5	0.5
PKC-B activity (IU/min)	44.71 ± 4.9	36.51 ± 4.32	33.16 ± 7.2	0.0008*

BMI, body mass index; FBS, fasting blood sugar (glucose); eGFR, estimated glomerular filtration rate; TC, total cholesterol; TG, triglycerides; HDL-C, high density lipoproteins cholesterol; LDL-C, low-density lipoproteins cholesterol; VLDL-C, very low density lipoproteins cholesterol; DN, diabetic nephropathy. Data were expressed as mean ± SD. *P* value < 0.05 is significant, by one way ANOVA test.

The result of the present study indicated that the minor allele frequency (T allele frequency) was significantly higher in DN group than T2DM and control subjects (0.63, 0.48, 0.33 respectively) as well as homozygous carriers of the minor allele (TT) were significantly more frequent among DN patients than among T2DM and control subjects (50%, 25%, 16.6% respectively) this pattern is consistent with a recessive mode of inheritance. Also genotype distributions were significantly different among DN patients as compared with T2DM and control subjects. Individuals carrying the T allele of the rs3760106 SNP increased the risk of diabetic nephropathy as compared with non-carriers (odds ratio, 1.8, 3.4). This result is compatible with the results of (Araki, et al., 2015)[17] which found that T allele of rs 3760106 was significantly associated with increase susceptibility of DN and (Ma, et al., 2010) in which 18 SNPs throughout PRKCB1 gene were genotyped in cohort study and the patients were followed for 8 years.[18] This study indicated that two PRKCB SNPs, rs3760106 and rs2575390, were significantly associated with renal failure in type 2 diabetes mellitus.

The activity of PKCB is significantly higher in diabetic nephropathy (43.35 ± 18.69) as compared with T2DM without nephropathy and apparently control. The results of the present study agree with the results of (Langham, et al., 2008; Yang and Zhang, 2015) which found that the expression and activity of PKCB and other clinical parameters such as "FBS, HbA1c%, GFR and serum creatinine" was significantly higher in type 2 diabetic patients with nephropathy as compared with "diabetic patients without nephropathy" and also agree with (Toyoda, et al., 2004) which shown that in glomeruli of DN patients the activity of PKC-MAPK (Mitogen Activated Protein Kinase) pathway and the expression of TGFB-1 is significantly higher than glomeruli of normal subjects, with this results they proved that activation of protein kinas C (PKC) is a major signaling pathway for transforming growth factor (TGF)−B stimulate extracellular matrix (ECM) production in diabetic nephropathy (DN) and activation of PKCB have an critical role in the progression of glomerular damage in DN.[23-25]

The PKC-β has 2 subtypes (PKC-βI and PKC-βII) produced by the same gene PRKCB by "alternative splicing". High glucose level regulate the expression and activity of PKB-II while PKCB-I not effected.[26,27] In diabetic nephropathy group there is a significant correlation between PKC-B level with creatinine and eGFR respectively (r = 0.7, P = 0.03, r = –0.6, P = 0.05). Estimation of GFR considers the most useful general index for the evaluation the severity of kidney damage. Losing of 75% of renal tissue result a decline in GFR of 50% (less than 60 ml/min. 1.73 m^2). As glomerular function deteriorates, compounds that are normally cleared by the kidneys, such as urea and creatinine, accumulate in plasma so that with a decline in GFR as in DN (due to the structural abnormalities in glomerular such as mesangial expansion and thickening of GBM), plasma urea concentration and creatinine tend to rise. These data were in agreement with another study performed by Noor et al., in 2020.[28]

Conclusion

This cross-sectional study indicates that the PRKCB (C/T-1504) rs3760106 SNP was significantly associated with increased DNs susceptibility in Iraqi patients with T2DM and serum level of PKCB activity was associated with increased risk of DN complication.

Conflicts of Interest

None.

References

1. Jain, A. K. C., Apoorva, H. C., Kumar, H., Kumar, K., and Rajagopalan, S. (2018). Analyzing diabetic foot ulcer through Amit Jain's classification: a descriptive study. Int J Surg Sci, 2(4), 26-32.575–569 : (1).
2. Kwak, S. H. and Park, K. S. (2015). Genetic Studies on Diabetic Microvascular Complications: Focusing on Genome-Wide Association Studies. Endocrinol Metab, 30:147–158.
3. Wei, L., Xiao, Y., Li, L., Xiong, X., Han, Y., Zhu, X. and Sun, L. (2018). The Susceptibility Genes in Diabetic Nephropathy. Kidney Diseases, 4:226–237.
4. Heindel, J. J., Blumberg, B., Cave, M., Machtinger, R., Mantovani, A., Mendez, M. A. and Vandenberg, L. N. (2017). Metabolism disrupting chemicals and metabolic disorders. Reproductive toxicology, 68: 3–33.
5. Tan, . J. H., Hong, C. C., Shen,. L. and TAY,. E. Y., 2015. Costs of Patients Admitted for Diabetic Foot Problems. Ann Acad Med Singapore, 44(12): 567–570.
6. Al-Tu'ma, A. F., Jeddoa, Z.M. and El–Yassin, H. D. (2019). Molecular Basis of Gene Encoding Human Leukocyte Antigens and its Association with Type 1 Diabetes Mellitus in Children of Kerbala Province. WJPMR, 5(1): 218–225.
7. Ahlqvist, E., Ahluwalia, T. S. and Groop, L. (2011). Genetics of type 2 diabetes. Clinical chemistry, 57: 241–254.
8. Warren, A. M., Knudsen, S. T., and Cooper, M. E. (2019). Diabetic nephropathy: an insight into molecular mechanisms and emerging therapies. Expert opinion on therapeutic targets, 23(7): 579–591.
9. Schena, F. P. and Gesualdo, L. (2005). Pathogenetic mechanisms of diabetic nephropathy. Journal of the American Society of Nephrology, 16 (3 suppl 1): S30–S33.
10. Kishimoto, A.; Takai, Y.; Mori, T.; Kikkawa, U.; Nishizuka, Y. (1980) Activation of calcium and phospholipid-dependent protein kinase by diacylglycerol, its possible relation to phosphatidylinositol turnover. J. Biol. Chem., 255: 2273–2276.
11. Kirouac, L.; Rajic, A.J.; Cribbs, D.H.; Padmanabhan, J. (2017). Activation of Ras-ERK signaling and GSK-3 by amyloid precursor protein and amyloid beta facilitates neurodegeneration in Alzheimer's disease. Eneuro, 4 (2): 0149
12. Do Van, B.; Gouel, F.; Jonneaux, A.; Timmerman, K.; Gelé, P.; Pétrault, M.; Bastide, M.; Laloux, C.; Moreau, C.; Bordet, R. (2016). Ferroptosis, a newly characterized form of cell death in Parkinson's disease that is regulated by PKC. Neurobiol. Dis., 94: 169–178.
13. Gordon, R.; Singh, N.; Lawana, V.; Ghosh, A.; Harischandra, D.S.; Jin, H.; Hogan, C.; Sarkar, S.; Rokad, D.; Panicker, N. (2016). Protein kinase Cδ up-regulation in microglia drives neuro-inflammatory responses and dopaminergic neuro-degeneration in experimental models of Parkinson's disease. Neurobiol. Dis., 93: 96–114.
14. Mérida, I., Arranz-Nicolás, J., Rodríguez-Rodríguez, C., and Ávila-Flores, A. (2019). Diacylglycerol kinase control of protein kinase C. Biochemical Journal, 476(8): 1205–1219.
15. Noh, H., and King, G. L. (2007). The role of protein kinase C activation in diabetic nephropathy. Kidney International, 72: S49–S53.
16. Wang, Z. B., Zhang, S., Li, Y., Wang, R. M., Tong, L. C., Wang, Y. and Li, L. (2017). LY333531, a PKCβ inhibitor, attenuates glomerular endothelial cell apoptosis in the early stage of mouse diabetic nephropathy via down-regulating swiprosin-1. Acta Pharmacologica Sinica, 38(7): 1009–1023.
17. Araki, S. I., Ng, D. P., Krolewski, B., Wyrwicz, L.,Rogus, J. J., Canani, L. and Krolewski, A. S. (2003). Identification of a common risk haplotype for diabetic nephropathy at the protein kinase C-β1 (PRKCB1) gene locus. Journal of the American Society of Nephrology, 14(8):2015–2024.
18. Ma, R. C., Tam, C. H., Wang, Y., Luk, A. O., Hu, C., Yang, X. and Tong, P. C. (2010). Genetic variants of the protein kinase C-β 1 gene and development of end-stage renal disease in patients with Type 2 diabetes. Jama, 304(8): 881–889.
19. Hashemi, M., Hoseini, H., Yaghmaei, P., (2011). Association of polymorphisms in glutamate-cysteine ligase catalytic subunit and microsomal triglyceride. DNA Cell Biol. 30(8): 569–75.
20. Al-Koofee, D. A., and Mobarak, S. M. (2018). PRIMER1: a network service for tetra-arms PCR primer design based on well-known dbSNP. Research Journal of Pharmacy and Technology, 11(8): 3633-37.
21. Al-Tu'ma, F.J. ; Dheyauldeen, M.H. and Al-Saegh, R.M. (2017). Measurement of urinary kidney injury molecule-1 as a predictive biomarker of contrast-induced acute kidney injury. J Contemp Med Sci., 3(9): 178–181.
22. Al-Tu'ma, F.J. and Obed K. H. (2018). Association between Fat Mass and Obesity Associated (FTO) gene polymorphism (rs9939609) and lipid profile in type 2 diabetic obese Iraqi male. Iraq Med J, 2 (1): 15–19.
23. Langham, R. G., Kelly, D. J., Gow, R. M., Zhang, Y., Cox, A. J., Qi, W. and Gilbert, R. E. (2008). Increased renal gene transcription of protein kinase C-β in human diabetic nephropathy: relationship to long-term glycaemic control. Diabetologia, 51(4): 668-674.
24. Yang, J., and Zhang, J. (2015). Influence of protein kinase C (PKC) on the prognosis of diabetic nephropathy patients. International Journal of Clinical and Experimental Pathology, 8(11): 14925.
25. Toyoda, M., Suzuki, D., Honma, M., Uehara, G., Sakai, T., Umezono, T. and Sakai, H. (2004). High expression of PKC-MAPK pathway mRNAs correlates with glomerular lesions in human diabetic nephropathy. Kidney international, 66(3): 1107-1114.
26. Becker, K. P. and Hannun, Y. A. (2004). Isoenzyme-specific translocation of PKCbII and not PKCbI to a Juxtanuclear subset of recycling endosomes. Involvement of phospholipase D. Journal of Biological Chemistry, 279: 28251–56.
27. Patel, N. A., Yamamoto, M., Illingworth, P., Mancu, D., Mebert, K., Chappell, D. S. and Cooper, D. R. (2002). Phosphoinositide-3-kinase mediates protein kinase C βII mRNA destabilization in rat A10 smooth muscle cell cultures exposed to high glucose. Archives of biochemistry and biophysics, 403(1): 111–120.
28. Noor, T., Hanif, F., Kiran, Z., Rehman, R., Khan, M. T., Haque, Z., and Nankani, K. (June 2020). Relation of Copeptin with Diabetic and Renal Function Markers Among Patients with Diabetes Mellitus Progressing Towards Diabetic Nephropathy. Archives of Medical Research, 03, 51(6): 548–555
29. Stumvoll, M., Goldstein, B. J. and Van Haeften, T. W. (2005). Type 2 diabetes: principles of pathogenesis and therapy. The Lancet, 365: 1333–1346.