Association of CD40 *rs1883832* Polymorphism with Susceptibility of Diabetic Nephropathy and Neuropathy in Egyptian Population

Dalia Ghareeb[1], Mohamed Fawzy[2], Ahmed I. Maaty[3]

[1]Clinical Pathology Department, Faculty of Medicine, Suez University, Suez, Egypt, [2]Internal Medicine Department, Nephrology Unit, Faculty of Medicine, Suez University, Suez, Egypt and [3]Physical Medicine, Rheumatology & Rehabilitation, Faculty of Medicine, Suez Canal University, Ismailia, Egypt.

Diabetic nephropathy (DN) and peripheral neuropathy (DPN) are unpredictable diabetic complications with narrow management alternatives. CD40-CD40 ligand system may be an essential pathway for diabetic microvascular complications. No previous studies had evaluated the relationship between CD40 *rs1883832* polymorphism and DN/DPN. This study aimed to investigate the association between CD40 *rs1883832* polymorphism and the risk of nephropathy and neuropathy in Egyptian patients with type 2 diabetes mellitus. A total of 106 diabetic patients (53 with nephropathy and 53 with neuropathy) and 53 healthy controls (without DM or other overt chronic conditions) were recruited from Suez Canal University Hospitals. Genotyping of CD40 gene polymorphisms was carried out using the polymerase chain reaction-restriction fragment length polymorphism assay. Patients with TT genotype and T allele carry a higher risk of developing DN (odds ratio (OR)=5.40, *P*=0.0026) and OR=2.56, *P*=0.0009, respectively). Likewise, the risk of DPN was significantly higher in patients carrying TT genotype (OR=2.91, *P*=0.045) and T allele (OR=1.84, *P*=0.028), respectively. In conclusions, the T allele is significantly associated with DN and DPN. Further studies with larger sample sizes are necessary to confirm our observations.

The prevalence of nephropathy among Egyptian patients with diabetes mellitus (DM) is ranging from 42% to 78% [1,2]. In the Menoufia governorate, Egypt; the prevalence of micro- and macro-albuminuria is 34.2% and 12.8%, respectively [3]. Diabetic nephropathy (DN) is the principal cause of the end-stage renal disease (ESRD). The incidence of ESRD due to DM is approximately 44% in some Arab countries like Jordon, Qatar, and Kuwait. About 50% of those patients need renal replacement measures [4].

Diabetic peripheral neuropathy (DPN) is one of the most frequent chronic complications of DM (40%). Distal symmetric polyneuropathy (DSPN) is the most common type of chronic DPN (75%) [5]. The early prediction and treatment of DPN are required for many reasons. First, several management choices are existing for symptomatic DPN. Second, DPN may be asymptomatic in 50% of patients. Third, severe complications of DPN, like recurrent feet injuries, ulcers and Charcot arthropathies which may ultimately end by amputation, can be prevented by early diagnosis and suitable management. Proper interventions can decrease foot ulcers by 60% and amputations by 85% [6]. The DPN has a very high economic burden with enormous exhaustion of the financial resources. After the DPN diagnosis, the annual direct cost/case is increased by 46% during the last decade [7].

Cumulative evidence advocates that the tumor necrosis factor (TNF) receptor (R) (TNFR) family member CD40 and its ligand

(CD40L) participate in the DM-associated inflammation, immune response and consequent development of microvascular complications (e.g. DN and DPN). CD40 and CD40L are expressed on pancreatic cells (e.g. islet β- and ductal cells) and this expression is increased upon exposure to pro-inflammatory cytokines (e.g. TNF-α) [8–10]. In addition to conventional modifiable risk factors (e.g. hypertension, smoking, and hyperlipidemia), genetic components are also considered to be significant risk factors for DN and DPN. Therefore, detecting the genetic factors can be very useful in the prediction and prevention of DN and DPN. No studies, to our knowledge, had been performed to explore the association between CD40 gene *rs1883832* single-nucleotide polymorphism (SNP) and the risk of developing DN and DPN in the Egyptian patients with type 2 DM (T2DM).

Materials and Methods

Participants

One-hundred and six patients diagnosed with T2DM for at least 5 years, according to the American Diabetes Association Classification and Diagnosis of Diabetes [11], were recruited from the Internal Medicine department and Nephrology Unit, Suez Canal University Hospitals, Ismailia, Egypt. The diabetic group was subdivided into two groups: 53 diabetic patients with DN whose albumin/creatinine ratio (ACR) was >30 mg/g creatinine, and 53 diabetic patients with DPN whose ACR was <30 mg/g creatinine. DSPN associated with T2DM was diagnosed according to American Association of Neuromuscular & Electrodiagnostic Medicine (AANEM) diagnostic criteria [12] and its update [13]. DPN is defined as a combination of neuropathic a symptom(s) or sign(s) confirm DSPN and abnormal electrodiagnostic studies. Patients with preexisting autoimmune inflammatory disease, chronic liver, or kidney disease were excluded from the study. Patients' results were compared to those from 53 age- and gender-matched healthy controls without DM or other overt medical diseases.

Clinical Evaluations

Anthropometric measures of weight and height were performed to calculate body mass index (BMI) according to the equation: BMI = weight (kg)/ height (m^2). Weight was measured to the nearest 0.1 kg, while height was measured to the nearest 0.5 cm according to a standardized protocol. Neuromuscular examinations of the participants were completed. The reported neuropathic symptoms were alterations of sensation (numbness, burning, prickling, paresthesia, dysesthesia, and allodynia) involving the feet or hands. We described neuropathic signs as abnormalities of sensory (pain, touch, vibration or proprioception), motor (weakness or atrophy), or tendon reflexes (diminished or absence).

Laboratory Investigations

All biochemical and immunochemical assays were performed by a fully automated analyzer (Cobas® 6000 Auto-analyzer, Roche Diagnostics, Mannheim, Germany) using kits provided by the manufacturer. Turbidimetric Inhibition Immunoassay was used for HbA1c evaluation (Tina-quant® HBA1C assay, Roche Diagnostics, Mannheim, Germany) and albumin in urine, while serum and urine creatinine was measured by a kinetic colorimetric assay based on the Jaffe method. Measurement of serum triglycerides (TG) was based on the enzymatic determination of glycerol using the enzyme glycerol phosphate oxidase after hydrolysis by lipoprotein lipase. Total cholesterol (TC) and high-density lipoprotein-cholesterol (HDL-C) were evaluated by enzymatic colorimetric method, according to the manufacturer's standards. Low-density lipoprotein-cholesterol (LDL-C) was estimated by the Friedewald's formula: LDL-C = TC – HDL-C – (TG/5), if the TG was less than 400 mg/dL and it was measured by enzymatic colorimetric method, if the TG was more than or equal 400 mg/dL. ACR was measured by dividing urinary albumin concentration (mg) by creatinine concentration (g) in spot urine collection. Microalbuminuria was defined as ACR 30-300 mg/g and values above 300 mg/g were considered as macroalbuminuria. Estimated Glomerular filtration rate (eGFR) was calculated from serum creatinine (S_{cr}) using the Modification of Diet in Renal Disease formula as follows: GFR (mL/min/1.73 m^2) = $175 \times (S_{cr})^{-1.154} \times (Age)^{-0.203} \times$ (0.742 if female).

Electrophysiological Assessments

Electrodiagnostic studies of sural sensory, ulnar sensory, and median sensory nerves, and peroneal, tibial, median, and ulnar motor nerves with F waves were performed. If the response is absent for any of the studied nerves (sensory or motor), nerve conduction study (NCS) of the contralateral nerve was performed. "The minimum case definition criterion for electrodiagnostic confirmation of DSPN is an abnormality of any attribute of NCS in two separate nerves, one of which must be the sural nerve".

CD40 gene *rs1883832* polymorphism

The DNA was extracted from the study subjects' peripheral blood leucocytes using a commercially available Spin-column technique kit for DNA extraction (QIAamp®DNA Blood Mini Kit) (Avenue, Stanford, Valencia, CA, US). Genotyping of CD40 gene *rs1883832* polymorphism was carried out using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay using the following primer pairs forward F: 5′-CCC CGA TAG GTG GAC CGC GAT TG-3′ and reverse R: 5′-CCC GCC CTC TGA ACC CCC TAC CA-3′

PCR mixture included 5µl genomic DNA, 12.5µl Maxima Hot Start green master mix, 5.5µl RNase free water, and 1µl of each primer with the final volume of 25µl. Amplification was carried out using Thermo-cycler (Mastercycle personal Eppendorf, China) and it consisted of initial denaturation at 95°C for 4 min., followed by denaturation at 95°C for 30 sec., annealing at 60°C for 30sec and extension at 72°C for 1 min, followed by final extension at 72°C for 5 min. The PCR product was digested using restriction enzyme (*NcoI*) FastDigest by adding 25µl of PCR product, 3µl restriction enzyme Buffer and 0.5µl of restriction enzyme (*NcoI*) FastDigest, and then mixed by pipetting. The Eppendorf were incubated for 1 hour at 37°C.

The PCR products were analyzed by electrophoresis on 3% (w/v) agarose gel in which 6 µl of ethidium bromide was added to the gel and the DNA bands were visualized under ultraviolet (UV) light and photographed using gel documentation system (Syngene, UK). The DNA bps were compared using DNA ladder; homozygous CC was represented by the presence of a single band of 503 bp, homozygous TT was represented by the presence of two bands of 373 bp and 130 bp, and heterozygous CT was represented by the presence of three bands of size 503, 373 and 130 bp [14].

We follow in strict accordance with the instructions of DNA extraction kit to extract the genomic DNA. The concentration and purity of DNA sample must meet the PCR requirements. PCR laboratory was divided into (1) reagent preparation area, (2) sample preparation area, (3) PCR area, and (4) product analysis area. The laboratory was always sterilized to avoid cross contamination. Manual Pipettes and PCR instruments were periodically calibrated to ensure the accuracy. The positive control and negative control were used in PCR–RFLP progress. Ten percent of the DNA samples (n=16) were randomly selected to be re-amplified and re-genotyped by PCR to ensure that our laboratory work is precise and produce the same results for the replicated samples. PCR and enzyme-digested products were confirmed by electrophoresis. In PCR progress, one of the above DNA samples of successful PCR was used to be positive control, and water as negative control. The wild and mutant homozygous genotypes of DNA samples were acted as the quality control samples in each enzyme digestion reaction.

Statistical Analysis

Quantitative variables were represented as means ±standard deviation (SD) or as medians and interquartile range (IQR) according to the data distribution. Qualitative variables were presented as frequencies and percentages. For a comparison of two groups, unpaired t, Mann-Whitney U or chi-square (χ^2) tests were used according to the type of data. One-way analysis of variance (ANOVA) was used to compare the three groups. Bonferroni's method was used for pairwise comparisons. Pearson's χ^2 goodness-of-fit test was used to assess Hardy-Weinberg equilibrium and a p-value >0.05 is considered to specify equilibrium. The association between variables was evaluated by the odds ratio (OR) and 95% confidence interval (CI). The ORs and 95% CIs were calculated with multiple logistic regression analysis. The statistical analyses were performed using IBM SPSS statistics version 23.0. *P*-values <0.05 were considered statistically significant.

Results

As shown in Table 1, there were insignificant differences between patients and controls regarding age (*P*=0.69), gender

(*P*=0.59) and BMI (*P*=0.57). Descriptive analyses also showed that patients with DN and DPN had a significantly higher mean total cholesterol (*P*=0.013), TG (*P*<0.0001) and LDL-cholesterol (*P*=0.048) in comparison to healthy control. Concerning serum creatinine and ACR, DN patients had higher values than DPN and controls (*P*<0.0001), meanwhile, there were insignificant differences between DPN patients and controls (*P*>0.05). Besides, eGFR was significantly higher among DPN and healthy controls than DN patients (*P*<0.0001). According to pairwise comparison, the frequencies of TT genotype were significantly higher among DN (49.1%) and DPN (41.5%) patients compared to control subjects (20.8%). Likewise, the differences were statistically significant between DN/DPN patients versus controls regarding T allele (67.9%/60.4% *vs* 45.3%, respectively). In contrast, there were insignificant differences between DN and DPN patients regarding the TT genotype and T allele (*P*>0.05).

Table 1. Characteristics of the studied populations.

Variables	DN (n=53)	DPN (n=53)	Control (n=53)	*P* value
Age-mean years ±SD	56.3±8.8	57.7±13.9	56.1±7.4	NS
Female gender-n. (%)	32 (60.4%)	37 (69.8%)	35 (66.0%)	NS
BMI (kg/m^2)-mean ±SD	35.1±5.6	34.4±16.4	32.9±7.2	NS
HbA1c (%)-mean ±SD	8.5±2.4	8.1±4.1	4.9±0.4	<0.0001*
Lipid profile				
Total cholesterol (mg/dl)-mean ±SD	195.3±46.5	207.7±50.8	182.7±29.3	0.013*
TG (mg/dl)-mean ±SD	152.8±51.4	133.1±47.6	103.2±12.6	<0.0001*
HDL (mg/dl)-mean ±SD	38.8±17.5	45.8±19.4	46.5±16.6	NS
LDL (mg/dl)-mean ±SD	128.2±45.2	129.9±59.1	108.8±38.7	0.048*
Kidney functions				
Serum creatinine (mg/dl)-median (IQR)	1.8 (0.70) [a]*	0.63 (0.42) [b]	0.65 (0.17) [c]*	<0.0001*
ACR (mg/g)-median (IQR)	137.3 (49.7) [a]*	21.2 (8.3) [b]	23.3 (15.5) [c]*	<0.0001*
eGFR (mL/min/1.73 m2)-median (IQR)	76.1 (19.4) [a]*	96.8 (33.2) [b]	105.8 (21.6) [c]*	<0.0001*
Genotype frequencies				
CC-n. (%)	7 (13.2%)	11 (20.8%)	16 (30.1%)	
CT-n. (%)	20 (37.7%)	20 (37.7%)	26 (49.1%)	NS
TT-n. (%)	26 (49.1%) [a]	22 (41.5%) [b]*	11 (20.8%) [c]*	0.008*
Allele frequencies				
C-n. (%)	34 (32.1%)	42 (39.6%)	58 (54.7%)	
T-n. (%)	72 (67.9%) [a]	64 (60.4%) [b]*	48 (45.3%) [c]*	0.003*

DN, diabetic nephropathy; DPN, diabetic peripheral neuropathy; BMI, body mass index; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate; ACR, albumin/creatinine ratio; HbA1c, hemoglobin A1c. **P*-value at >0.05 is not significant (NS), [a] DN *vs.* DPN, [b] DPN *vs.* control, [c] DN *vs.* control.

Table 2 shows that DN patients who carry TT genotype were significantly more obese than those who carry other genotypes (*P*<0.0001). The TT genotype of the CD40 *rs1883832* gene was also significantly associated with higher levels of total

cholesterol (*P*=0.039) and LDL-cholesterol (*P*=0.015), and lower levels of HDL-cholesterol (*P*=0.023). Significantly heavier proteinuria (*P*<0.0001) and lower eGFR (*P*<0.0001) were existing in patients with TT genotype compared to CC and CT genotypes.

In DPN patients, however, only total cholesterol showed significantly higher levels in individuals with TT genotype than CT and CC genotypes (*P*<0.05). Also, the TT genotype was associated with higher HBA1c% in comparison to CT and CC genotypes (11.2±5.2% *vs* 7.6±2.6% *vs* 7.1±3.8%, respectively).

Table 2. Characteristics of the patients with diabetic nephropathy (DN)/peripheral neuropathy (DPN) according to the genotypes of CD40 *rs1883832* gene.

Variables	DN (n=53) genotypes				DPN (n=53) genotypes			
	CC (n=7)	CT (n=20)	TT (n=26)	*P* value	CC (n=11)	CT (n=20)	TT (n=22)	*P* value
Age (years)	57.1±5.2	56.6±9.3	58.9±8.8	NS	56.9±11.3	54.7±15.7	60.2±16.8	NS
Female gender (%)	4 (57.1%)	10 (50.0%)	18 (69.2%)	NS	8 (72.7%)	17 (85.0%)	12 (54.5%)	NS
BMI (kg/m^2)	35.0±7.1	31.1±2.9	39.4±6.3	<0.0001*	34.8±15.9	32.7±18.7	35.4±12.5	NS
HbA1c (%)	8.5±3.5	8.7±4.1	8.3±5.5	NS	7.1±3.8	7.6±2.6	11.2±5.2	0.007*
Cholesterol (mg/dl)	173.4±37.4	187.6±52.1	221.2±56.7	0.039*	183.1±42.8	198.7±49.6	229.9±61.3	0.044*
TG (mg/dl)	151.2±48.7	161.4±57.3	149.9±60.1	NS	121.4±58.6	130.6±42.1	148.7±72.3	NS
HDL (mg/dl)	46.9±20.1	43.1±18.6	30.8±15.3	0.023*	45.7±18.3	46.1±23.2	44.4±17.5	NS
LDL (mg/dl)	101.2±27.6	132.7±34.5	159.6±60.2	0.015*	128.2±67.3	119.3±53.7	134.5±74.1	NS
Creatinine (mg/dl)	1.81 (0.7)	1.78 (0.5)	1.75 (0.8)	NS	0.59 (0.31)	0.63 (0.22)	0.71 (0.18)	NS
ACR (mg/g)	25.8 (19.5)	142.4 (66)	179.6 (59.9)	<0.0001*	18.9 (5.5)	19.3 (7.2)	24.8 (10.4)	NS
eGFR (mL/min/1.73 m2)	102.3 (20)	78.6 (18.5)	75.2 (13.3)	<0.0001*	121.9(35.4)	112.2(45.2)	92.9 (21.5)	NS

BMI, body mass index; TG, triglycerides; HDL, high density lipoprotein; LDL, low density lipoprotein; eGFR, estimated glomerular filtration rate; ACR, albumin/creatinine ratio; HbA1c, hemoglobin A1c. **P*-value at >0.05 is not significant (NS),

Our results revealed that HDL- and LDL-cholesterol were significantly associated with DN (OR=7.09 and OR=3.15, respectively), whereas, total cholesterol was significantly associated with DPN (OR=2.97) (Table 3). We confirmed the association between DN/DPN and CD40 polymorphism using multiple logistic regression analyses. Approximately 5-folds risk of DN was observed in patients carrying TT genotype (OR=5.40) and the risk was about 3-folds for T allele (OR=2.56). Similarly, around 3- and 2-folds risk of DPN were detected in patients carrying TT genotype (OR=2.91) and T allele (OR=1.84), respectively, as demonstrated in Table 3.

Table 3. Multiple logistic regression models to predict diabetic nephropathy (DN)/ peripheral neuropathy (DPN).

Variables	DN (n=53)		DPN (n=53)	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age (years)	1.00 (0.86-1.91)	NS	0.99 (0.78-1.22)	NS
Female gender	0.78 (0.36-1.68)	NS	1.21 (0.53-2.72)	NS
BMI (kg/m^2)	0.99 (0.92-1.43)	NS	1.01 (0.36-1.89)	NS
Cholesterol (mg/dl)	2.38 (0.94-6.03)	NS	2.97 (1.21-7.28)	0.016*
Triglyceride (mg/dl)	2.59 (0.62-10.79)	NS	1.00 (0.79-1.80)	NS
HDL (mg/dl)	7.09 (2.48-20.27)	<0.0001*	1.39 (0.88-1.92)	NS
LDL (mg/dl)	3.15 (1.18-8.38)	0.019*	1.21 (0.48-1.78)	NS
Presence of CT genotype	1.76 (0.61-5.11)	NS	1.10 (0.43-2.90)	NS
Presence of TT genotype	5.40 (1.74-16.8)	0.0026*	2.91 (1.01-8.42)	0.045*
Presence of T allele	2.56 (1.46-4.48)	0.0009*	1.84 (1.07-3.21)	0.028*

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OR, odds ratio; CI, confidence interval. **P*-value at >0.05 is not significant (NS).

Discussion

We demonstrated for the first time in Egypt, that there is an association between CD40 *rs1883832* polymorphism and the risk of nephropathy and neuropathy in adult Egyptian patients with T2DM. During the last decade, several investigational and clinical studies have recognized the connection of CD40 molecule in the pathogenesis, progression, prognosis, and management of DM [15–18]. However, there is lack of data regarding the association between CD40 *rs1883832* polymorphism and T2DM [19].

Our study reported that the genotype of CD40 C/T polymorphism was significantly higher among the patients with T2DM than the healthy subjects. These results were similar to the study done by Jinchuan *et al.* [20], who found a significantly over-expression of CD40-CD40L in diabetic patients compared with nondiabetic controls ($P<0.05$). Some studies had investigated the role of CD40 molecule in DM. In humans, both pancreatic duct cells and islet beta cells and have been shown to express CD40. Upon ligation with CD40L, these cells produce pro-inflammatory cytokines, that may contribute to the pathogenesis of DM upon encounter with activated T cells. Patients with DM have also been shown to have significantly higher levels of CD40L as well as surface CD40L expression, suggesting other sources of ligation for these CD40-expressing pancreatic cells [15,21,22].

There are no studies, to our knowledge, have assessed the association between *rs1883832* C/T polymorphism and DN, however, some studies had measured plasma levels of CD40 and CD40L in diabetic patients with or without DN. For example, an observational study performed by Lajer

and colleagues [10] stated that subjects with DN had significantly higher levels of CD40L than controls. These remarks were also noticed in an Egyptian study [23] that aimed to evaluate CD40L levels in adolescents with T1DM and its relation to microvascular complications (microalbuminuria and peripheral neuropathy). They reported high serum CD40L levels in patients with microvascular complications, particularly those with microalbuminuria. This also coincides with Chiarelli *et al.* [24] who found that patients with high CD40 had a greater risk of persistent microalbuminuria. These data were further supported by another study [25], where the authors found that the T allele frequency of CD40 C/T polymorphism was associated with a higher risk of the acute coronary syndrome in Chinese patients with DM. Despite using different methods by these studies, it may give a general idea about the relationship between the CD40-CD40L system and DM.

The important role of CD40 in renal injury has been extensively studied. CD40 is expressed and up-regulated on the renal parenchymal cells including proximal tubule via transforming growth factor-beta (TGF-β) mediated pathways. CD40-mediated signaling is associated with inflammation, atherosclerosis, and thrombosis which increase renal fibrosis and damage. Moreover, elevated levels of CD40 may account for ESRD associated immunodeficiency [22,26].

In this study, the frequencies of the TT genotype/ T allele of the CD40 gene were significantly higher among DPN patients compared to controls. A prediction of incident DPN by biomarkers of subclinical inflammation such as CD40 has been studied previously [8]. The hypothesis for such an association between CD40 and DPN is that the CD40L binds to its receptor CD40 and induces tissue factor expression on macrophages and endothelial cells. CD40-CD40L system induces microangiopathy in diabetic nerves which consequently causes tissue ischemia. This nerve ischemia activates a series of signaling mechanisms implicated in the pathogenesis of DPN (e.g. oxidative stress, lipid metabolism, glycation) [9].

Also, our results highlighted that DN patients with homozygous TT genotypes had significantly higher BMI, while, there was an insignificant difference between T and C alleles regarding BMI in DPN patients. Some of our results and conclusions seem different and contradictory, likely because microvascular and neuropathic complications in DM are not necessarily a direct result of obesity, but a consequence of a multiplicity of other risk factors. According to previous studies in the Arabic region, the prevalence of nephropathy was clearly and significantly related to BMI. In one Egyptian study, Elnajjar *et al.* [2] described that the risk of DN was 4.49 times higher in the obese group than in the nonobese one. One Yemeni study also supports a correlation between obesity and diabetic microvascular complications in patients with T2DM [27].

Previous studies emphasized that dyslipidemia is commonly complicated with DM and DN and it is an essential risk factor in the progression of DN [28]. In similar prospect, our patients with DN and DPN had higher mean total cholesterol, TG and LDL-cholesterol in comparison to controls. HDL- and LDL-cholesterol were significant predictors of DN and the TT genotype of CD40 were significantly associated with hypercholesteremia and lower HDL-cholesterol. In our DPN patients, total cholesterol displayed higher levels in

patients with TT genotype than CT/CC genotypes. Similar results were observed by El-Asrar *et al.* [23] who found that diabetic patients with and without microvascular complications had significantly higher total cholesterol, LDL-cholesterol, and TG, while serum HDL cholesterol was lower than controls. Patients with microalbuminuria and peripheral neuropathy had significantly higher lipids compared with non-complicated cases. In the same view, Clipollone et al. [29] demonstrated a significant association between CD40L and hypercholesterolemia. In contrast to these findings, the study achieved by Davì *et al.* [30] reported that CD40L levels were not affected by total cholesterol levels.

Our data presented that there were significantly higher levels of albuminuria and lower values of eGFR in subjects with DN who expressed TT genotype compared to CC/CT genotypes. In Xie *et al.* study [26], DM was the main cause of chronic kidney disease and their findings show that the levels of CD40 had prognostic value in the prediction of decreases in eGFR in diabetic patients. Lajer et al. [10] found that CD40L levels did not predict the progression of type 1 DN to ESRD or the reduction in GFR. The contradictory conclusions of the previous researches and our research may be due to the differences in study design, methods and population characteristics.

A significantly higher risk of DPN and DN was associated with the presence of TT genotype and T allele. This association may be explained by the upregulation of CD40 leading to hypoxia-inducible factor-1α expression in endothelium and nerve fibers, which induce microangiopathy, thrombosis, and inflammatory infiltrates with degeneration in diabetic nerves [9] and renal tissue hypoxia [31].

Few studies have evaluated the effects of genetic markers on diabetic control and prognosis. In this prospect, our study demonstrated that patients carrying T allele, in the DPN group, had a higher risk of poor glycemic control as evidenced by high HBA1c in these patients. Also, the study confirmed that DN subjects with T allele had poorer kidney functions as demonstrated by higher albuminuria and lower eGFR.

In conclusions, the foremost findings may suggest that over-expression of the CD40 system contributes to the progress of microvascular complications in DM. Thus, the presence of the T allele of the CD40 gene in diabetic patients may be one of the predictors and risk factors for persistent microalbuminuria and peripheral neuropathy. These conclusions stimulate further assessment of the CD40-CD40L system as a long-term marker for DM and its complications.

Compliance with Ethical Standards

This research has been reviewed and approved by the Clinical Research Ethics Review Committee of Suez Canal University from the viewpoint of scientific, ethical and medical validity, and it is being implemented with the permission of Suez Canal University Hospitals Director. The study has been conducted according to the principles expressed in the Helsinki Declaration. Before recruitment in the study, all patients signed informed consent paperwork disclose the aim, the benefits and the outcome of the study and the patients' agreement in participation with preservation of their rights to withdrawal at any time without any consequences.

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