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Salma Alaa Eldin Imbaby[1], Mai Badrah[2], Yousra Hisham Abdel Fattah[3]
[1]Lecturer of Clinical Pathology, Faculty of Medicine, Alexandria University, Alexandria, Egypt
[2]Lecturer of Internal Medicine and Diabetology, Faculty of Medicine, Alexandria University, Alexandria, Egypt
[3]Lecturer of Physical Medicine, Rheumatology and Rehabilitation, Faculty of Medicine, Alexandria University, Alexandria, Egypt

The relation between IL-10 gene (-1082G/A) and VEGF gene 936 C/T polymorphism and diabetic polyneuropathy in a cohort of Egyptian patients with type 2 diabetes

ABSTRACT

Background. Polymorphisms have been described to be correlated with type 2 diabetes mellitus (T2DM) and its complications including diabetic polyneuropathy (DPN). The aim of this study was to investigate the relation between interleukin (IL)-10-1082 G/A (rs1800896) and vascular endothelial growth factor (VEGF)-936 C/T polymorphisms and DPN in T2DM patients.

Methods. This cross-sectional study included 50 T2DM patients and 40 controls. Clinical and electrophysiological assessments for DPN, fasting blood glucose level (FBS) and glycosylated hemoglobin (HbA1C) were recorded. VEGF-936 C/T polymorphism was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Genotyping for IL-10 promotor gene (-1082 G/A) (rs1800896) polymorphism was performed using Real-time PCR.

Results. Sixty percent of patients had confirmed DPN, while none of them were considered normal. IL-10 (genotype -1082G/G) was statistically higher among controls compared to T2DM patients (P = 0.008). VEGF-936-CT genotype was statistically higher among patients compared to controls (P = 0.033), but there was no significant relation between IL-10-1082 G/A or VEGF-936 C/T polymorphism genotypes and DPN. Patients with IL-10 (genotype-1082A/G) had higher HbA1c level (P = 0.041) and lower albumin/creatinine ratio, while those with IL-10 (genotype-1082G/G) had higher albumin/creatinine ratio (P = 0.024). Thus, DPN has significant correlation with the duration of diabetes, FBS and HbA1c.

Conclusion: The VEGF-936 C/T genotype may be associated with T2DM in contrast to IL-10 (genotype-1082G/G) which may be less likely to be associated with it. However, there is no association between VEGF-936 or IL-10-1082 genotypes and DPN. (Clin Diabetol 2021; 10; 5: 420–427)

Key words: type 2 diabetes mellitus, polymorphism, interleukin-10, vascular endothelial growth factor, diabetic polyneuropathy, total neuropathy score

Introduction

Type 2 diabetes mellitus (T2DM) is an immune-mediated disease that leads to defective insulin signaling and selective destruction of insulin producing beta cells in which cytokines play an important role in this destruction [1]. Diagnosis is usually delayed for many years, making the patients at higher risk for developing macro- and micro-vascular complications [2, 3].

Diabetic neuropathies (DNs) are among the most common long-term complications of diabetes which affect up to 50% of patients [4]. There are wide varieties of DNs; the commonest one is the distal symmetric diabetic polyneuropathy (DPN) [5, 6].

Hyper-secretion of pro-inflammatory cytokines in overweight and obese individuals can cause insulin

Address for correspondence:
Yousra Hisham Abdel-Fatah
Medan Khartoum, Sultan Hussein St.,
Alexandria, Egypt
e-mail: yousrahisham@gmail.com
yousra.moustafa@alexmed.edu.eg
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resistance in peripheral tissues [7] and reduction of the mass and function of beta cells [8], contributing to T2DM development. Therefore, an imbalance in the activity of these cytokines may play an important role in T2DM pathogenesis.

The production of cytokines is controlled genetically, and polymorphisms have been identified within a large number of these genes [9]. On the basis of genotype, there are 'high' and 'low' cytokine producers. By recognizing specific polymorphisms in each cytokine gene, it is possible to identify these high and low cytokine producers by genotyping. Therefore, a genetic predisposition for a hyper-reactive immune response may be responsible for T2DM development. Moreover, the development of DPN and other complications may be associated with polymorphisms in cytokine genes [10, 11].

Interleukin (IL)-10 is an anti-inflammatory and immunosuppressive substance which plays a role in the control of immune responses [12]. IL-10 gene rs1800896 polymorphism (IL-10-1082G/A polymorphism) in the promoter region could influence IL-10 gene expression [13]. This polymorphism has been described to be correlated with T2DM [14].

The vascular endothelial growth factor (VEGF) gene, located at chromosome 6p21.3, is involved in angiogenesis modulation under physiologic and pathologic conditions. It has a role in proliferation, differentiation, migration and survival of cells, as well as nitric oxide production and release of other growth factors [15]. VEGF has been identified as the main mediator in the pathogenesis of cardiovascular complications in T2DM [16].

Therefore, the aim of the current study was to investigate the relation between IL-10 promoter gene -1082 G/A (rs1800896) and VEGF-936 C/T polymorphisms and DPN in a cohort of T2DM Egyptian patients.

Methods

Subjects

This cross-sectional study was conducted on 50 patients with T2DM, who were attending the Endocrine and Physical Medicine, Rheumatology and Rehabilitation outpatient clinics in Alexandria University Hospitals, Alexandria, Egypt. These patients were diagnosed according to the guidelines of the American Diabetes Association [17]. Forty age- and sex-matched healthy individuals were included as a control group. The study was conducted over a period of 1 year.

Patients with T2DM were excluded if they were on thiazolidinedione or received it in the previous three months. Patients with a history of the following conditions were also excluded from the study: type I diabetes, recent acute infection, autoimmune diseases, endocrine disorders with secondary diabetes, as well as history of acute pancreatitis, acute or chronic hepatitis B or C, human immunodeficiency virus and malignant disease within the past five years. Patients on long-term treatment with immunosuppressive, immunomodulatory and anti-inflammatory drugs as well as certain anti-depressants and drugs with possible pro-diabetic effects as glucocorticoids were also excluded.

In the control group, T2DM was excluded based on the fasting blood glucose (FBS) (7 mmol/L) and glycosylated hemoglobin (HbA1c) (< 6.5%).

Clinical and laboratory evaluation: All participants were subjected to full history taking and physical examination. Detailed medical history (age at diabetes diagnosis, duration of T2DM, medication history and history of complications) was recorded and body mass index (BMI) was calculated. Diabetic retinopathy was evaluated by direct fundoscopy under mydriasis. FBS, HbA1c and albumin/creatinine ratio were recorded.

Diabetic polyneuropathy assessment

Diabetic polyneuropathy was evaluated by neurological examination [18] and nerve conduction study (NCS) of both lower limbs [19]. The total neuropathy score-reduced (TNS-r), a combined clinical and electrophysiological score, was used to calculate DPN severity [20].

NCS was considered abnormal: if the abnormality was ≥ 1 in 2 separate nerves [21].

Categorization of patients according to DPN was as follows: patients with no clinical or electrophysiological evidence of neuropathy were categorized as normal, those with clinical signs or symptoms of neuropathy were categorized as possible clinical DPN, those with a combination of clinical signs and symptoms of neuropathy were categorized as probable clinical DPN, those with neither clinical signs nor symptoms of neuropathy but with abnormal NCS were categorized as subclinical DPN and those with a combination of clinical signs and symptoms of neuropathy plus abnormal NCS were categorized as confirmed DPN [22, 23].

Blood sample collection: Peripheral blood samples were obtained by venipuncture using a sterile aseptic technique using EDTA vacutainer tubes. About 5 milliliters of venous blood were withdrawn.

Genotyping: Total genomic deoxyribonucleic acid (DNA) was purified according to the manufacturer protocol using the QIAamp DNA Blood Mini Kit, (QIAGEN, Germany). DNA quantity and purity were assayed using a Nanodrop 2000 spectrophotometer (Thermo Scientific, USA). The A260:A230 ratio greater than 1.6 and A260:280 ratio greater than 1.8 were considered as indicators for highly pure DNA.

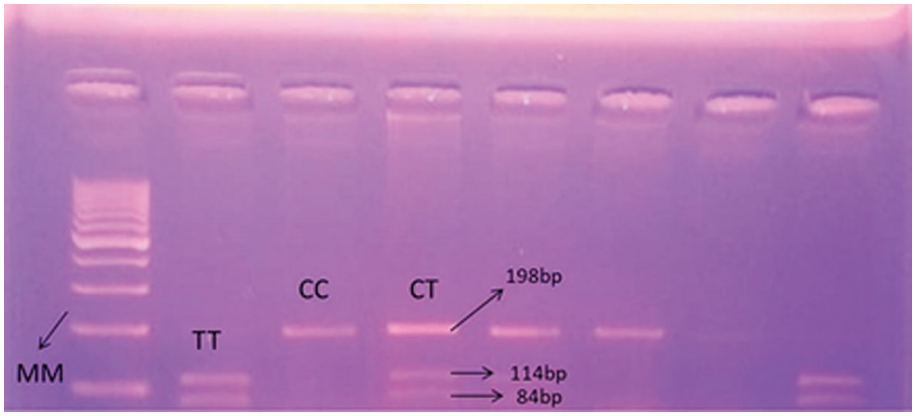

Figure 1. Electrophoresis of Digested PCR Product. CC genotype consists of 1 band (198 bp), CT genotype consists of 3 bands (198 bp, 114 bp, and 84 bp) and TT genotype consists of 2 bands (114 bp and 84 bp). Marker 100 bp on lane 1

VEGF-936 C/T single nucleotide polymorphism (SNP) genotyping was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. DNA amplification using PCR technique was first performed using Thermo Scientific DreamTaq Green master mix with a final volume of 20 μl. The PCR reaction was carried out on the Simpli-Amp Thermal Cycler (Applied Biosystems, USA). Two sequence-specific primers were used for the targeted fragment of VEGF gene. The PCR primers were: [24]

Forward: 5'-AAG GAA GAG GAG ACT CTG CGC3.'

Reverse: 5'- TAT GTG GGT GGG TGT GTC TAC AGG-3.'

The PCR cycling conditions was as follows: initial denaturation for 1 cycle at 95°C for 2 minutes, followed by 35 cycles at 95°C for 30 seconds, 62°C for 30 seconds, 72°C for 60 seconds and final extension for 1 cycle at 72°C for 10 minutes. A PCR product of 198 base pairs (bp) was generated. The amplified PCR products were digested with NlaIII enzyme with buffer G 10X (Thermo Scientific) at 37°C for 120 minutes followed by 65°C for 20 mins. Digested product and marker were analyzed in 2% agarose gel electrophoresis. After the electrophoresis process (110 V for 30 minutes), the result was photographed and analyzed. Digested products were (Fig. 1):

1) CC genotype: 1 band with product length of 198 bp
2) CT genotype: 3 bands with product length of 198 bp, 114 bp, and 84 bp
3) TT genotype: 2 bands with product length of 114 bp and 84 bp.

Genotyping for IL-10 promotor gene 1082 G/A polymorphism (rs1800896) was performed using real time PCR (TaqMan genotyping assay). The PCR reaction mix contained 10 ul TaqMan Universal PCR Master Mix (Applied Biosystems, USA), 0.5ul of TaqMan SNP Genotyping Assay 20X (Assay ID C___1747360_10), 20 ng DNA/reaction, and DNase free water to final volume of 20 ul. Thermal cycling was done using Stratagene as follows: initial AmpliTaq enzyme activation at 95°C for 10 minutes, 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 1 minute.

Ethical consideration

This research was conducted on human participants in accordance with the ethical standards of local ethical committee on human experimentation and Helsinki Declaration of 1964 and later versions. The study was approved by the Ethics Committee of Alexandria University, Egypt (serial number: 0304298-18/04/2019). A written informed consent was obtained from all participants enrolled in this study.

Statistical analysis

Data were analyzed using IBM SPSS software package version 20.0. Comparison between different groups regarding categorical variables was tested using chi square (χ^2) test. The distributions of quantitative variables were tested for normality using Kolmogorov–Smirnov test. Normally distributed data were expressed using mean ± standard deviation and compared using independent t test (between two groups) or one-way ANOVA (three or more groups). Abnormally distributed data were described using minimum, maximum, and median and compared using Mann-Whitney test (between two groups) or Kruskal-Wallis H test (three or more groups). Statistical significance was considered at $P < 0.05$. Genotype frequencies were compared with Hardy-Weinberg equilibrium (HWE) using χ^2 test. If $P > 0.05$, this means that the observed frequencies do not significantly differ from those expected and thus, the population is in HWE. Odds ratio was used

Table 1. Comparison between the two studied groups according to demographic data

	Patients (n = 50)	Control (n = 40)	Test of sig.	P
Age (years)				
Mean ± SD	52.96 ± 10.75	49.83 ± 9.44	t = 1.450	0.151
Sex				
Male	16 (32%)	18 (45%)	$\chi^2 = 1.598$	0.206
Female	34 (68%)	22 (55%)		
Occupation				
Housewife	26 (52%)	23 (57.5%)	$\chi^2 = 6.580$	$^{MC}P = 0.085$
Worker	9 (18%)	13 (32.5%)		
Desk job	8 (16%)	2 (5%)		
Retired	7 (14%)	2 (5%)		
BMI (kg/m^2)				
Mean ± SD	33.24 ± 5.97	34.40 ± 5.45	t = 0.953	0.343

χ^2: Chi square test; MC: Monte Carlo test; t: Student t-test; P: P value for comparing between the two groups; *: Statistically significant at P ≤ 0.05; SD: slandered deviation; BMI: body mass index

to quantify the degree of relation between exposure (genotype/allele) and outcome (patient status).

Power calculation

A sample size of 90 achieves 85% power to detect a medium effect size (W) of 0.35 in VGEF genotype between patients with DPN and controls using 2 degrees of freedom Chi-Square test with a significance level (alpha) of 0.05, according to Barus *et al.* in 2018 [25]. The sample size was calculated using NCSS 2004/PASS 2000 software.

Results

This cross-sectional study was conducted on 50 patients with T2DM collected over a period of 1 year and 40 healthy age- and sex-matched individuals. Most participants in both groups were middle-aged, obese and housewife females. There was no statistically significant difference between the 2 groups regarding the demographic data (Table 1).

The mean duration of T2DM was 9.58 ± 6.88 years. Thirty five patients (70%) were on oral hypoglycemic drugs, 14 patients (28%) were on combined oral hypoglycemic drugs and insulin therapy and only 1 patient was not medicated, he just was on diet control and exercise. Fourteen patients (28%) had no associated conditions while 30 patients (60%) had associated conditions. The most common associated conditions were musculoskeletal complains in 17 patients (34%) in the form of muscle cramps, knee osteoarthritis and shoulder periarthritis, followed by hypertension in 12 patients (24%) and gout in 8 patients (16%). The least common associated conditions were hypothyroidism in 2 patients (4%) and fatty liver with elevated liver enzymes in 1 patient (2%). Only 3 patients (6%) had serious associated medical conditions in the form of muscle weakness in both lower limbs with difficulty in walking, stroke with consequent hemiplegia and myocardial infarction. Non-proliferative diabetic retinopathy was found in 14 patients (28%).

Distribution of patients according to diabetic polyneuropathy

Neurological symptoms, signs of DPN or a combination of both were found in 48 patients (96%), while 33 patients (66%) had abnormal NCS and the mean TNS-r was 9.44 ± 5.90 ranging from 1–22. Most patients (n = 30) (60%) had confirmed DPN, 10 patients (20%) had probable clinical DPN, 8 patients (16%) had possible clinical DPN and only 2 patients (4%) had subclinical DPN. None of the patients was considered normal.

Distribution of patients according to genetic polymorphisms:

Regarding the studied genetic polymorphisms, patients with T2DM showed a higher frequency of IL-10 (genotype -1082A/G) genotype in comparison to controls (60% vs. 42.5% P = 0.099); however, this was not statistically significant. The GG genotype was statistically higher among controls compared to T2DM patients (P = 0.008). The frequency of IL-10–1082 A Allele was higher among patients compared to controls (60% vs. 46.3%, P = 0.066), but this was not statistically significant (Table 2).

Moreover, T2DM patients showed a statically significant higher frequency of VEGF-936 C/T genotype compared to controls (44% vs. 22.5%, P = 0.033).

Table 2. Comparison between the two studied groups according to IL-10 and VEGF genotypes

	Patients	Control	c2	p	OR	95% CI (LL – UL)
IL-10-1082 gene	(n = 50)	(n = 40)				
GG	5 (10%)	13 (32.5%)	7.031*	0.008*	0.231	0.074–0.719
AG	30 (60%)	17 (42.5%)	2.728	0.099	2.029	0.872–4.723
AA	15 (30%)	10 (25%)	0.277	0.599	1.286	0.504–3.282
Allele frequency	(n = 100)	(n = 80)				
A	60 (60%)	37 (46.3%)	3.382	0.066	1.743	0.962–3.16
G	40 (40%)	43 (53.8%)			0.574	0.317–1.039
P for Hardy	0.077	0.358				
VEGF gene	(n = 50)	(n = 40)				
CC	26 (52%)	28 (70%)	3.0	0.083	0.464	0.194–1.114
CT	22 (44%)	9 (22.5%)	4.549*	0.033*	2.706	1.069–6.851
TT	2 (4%)	3 (7.5%)	0.519	FEP = 0.652	0.514	0.082–3.235
Allele frequency	(n = 100)	(n = 80)				
C	74 (74%)	65 (81.3%)	1.328	0.249	0.657	0.321–1.346
T	26 (26%)	15 (18.8%)			1.523	0.743–3.120
P for Hardy	0.310	0.098				

IL: interleukin, VEGF: vascular endothelial growth factor, χ^2: Chi square test, FE: Fisher Exact
P: P value for comparing between the two groups; *: Statistically significant at $p \leq 0.05$
LL: Lower limit; UL: Upper limit; CI: Confidence interval. If $P < 0.05$ — not consistent with HWE (Hardy-Weinberg)

Also, they showed lower CC genotype frequency (52% vs. 70%, P = 0.083), but this was not statistically significant. The frequency of T allele was higher among patients than controls, but this was not statistically significant (26% vs 18.8%, P = 0.652) (Table 2).

The genotype distribution of IL-10-1082 polymorphism conformed to HWE in both groups (P = 0.07 for patients and P = 0.358 for controls). Also, the genotype distribution of VEGF-936 C/T genotype conformed to HWE in both groups (P = 0.310 for patients and P = 0.098 for controls) (Table 2).

Relation between diabetic polyneuropathy and genetic polymorphisms

In order to investigate if the described relation between either IL-10-1082 gene genotype (rs1800896) or VEGF-936 genotype and DPN was a consequence of other risk factors (phenotype characteristics), an analysis of the studied groups with different genotypes was performed in relation to sex, age and anthropometric characteristics. There was no statistically significant difference between subjects with different genotypes for the IL-10-1082 G/A (rs1800896) and VEGF-936 C/T polymorphisms in terms of age, sex, or BMI.

Patients showing IL-10 (genotype -1082A/A) or VEGF (genotype -936 T/T) showed a higher TNS-r score, but this was statistically insignificant (H = 3.423, P = 0.181, H = 0.289, P = 0.868, respectively) (Tables 3, 4).

Relation between other diabetic traits and genetic polymorphisms:

There were significantly lower HbA1c and albumin/creatinine ratio among patients showing IL-10 (genotype-1082A/G) compared to the other genotypes (P = 0.041 and P = 0.024 respectively), while the homozygous GG genotype had a significantly higher albumin/creatinine ratio (Table 3). On the other hand, no significant relations were found between the different diabetic traits and different VEGF-936 genotypes (Table 4).

Correlation between diabetic polyneuropathy and other diabetic factors

Patients with non-proliferative diabetic retinopathy had statistically higher TNS compared to patients with normal vessels and disc on fundus examination [12 (7–22) and 9 (1–22), respectively], with a statistically significant difference between the two groups (U = 155.5, P = 0.036). Also, there was a very high statistically significant positive correlation between TNS and the duration of diabetes (r_s = 0.721, P < 0.001), FBS (r_s = 0.548, P < 0.001) and HbA1c levels (r_s = 0.581, P < 0.001).

Discussion

The strong influence of ethnic differences on genetic predisposition of multifactorial diseases highlights the importance of regional studies that present a chan-

Table 3. Relations between IL-10 gene and other parameters for diabetic patients (n = 50)

	IL10 AA (n = 15)	AG (n = 30)	GG (n = 5)	Test of sig.	P
TNS-r					
Mean ± SD.	11.67 ± 5.56	8.38 ± 5.43	9.2 ± 8.76	H = 3.423	0.181
Median (Min.–Max.)	12 (2-22)	9 (1–20)	10 (1–22)		
HbA1c					
Mean ± SD. (%)	9.13 ± 1.85	7.61[a] ± 1.80	7.84 ± 2.16	F = 3.422*	0.041*
Median (Min.–Max.) (%)	9 (6–12.7)	7.1 (5.4–11.9)	9 (5.1–10.1)		
Median (Min.–Max.) (mmol/mol)	75 (42–115)	54 (36–107)	75 (32–87)		
Albumin / Creatinine ratio					
Mean ± SD	102.79 ± 111.8	67.71[a] ± 168.90	171.82[b] ± 296.08	H = 7.424*	0.024*
Median (Min.–Max.)	55 (2.2–400)	5.65 (2–700)	50 (2–700)		

F: F for ANOVA test; H: H for Kruskal Wallis test; P: P value for comparing between the studied categories; *: Statistically significant at P ≤ 0.05; IL: interleukin; HbA1c: glycosylated hemoglobin; TNS-r: total neuropathy score-reduced

Table 4. Relations between VEGF-936 genotype and other parameters for diabetic patients (n = 50)

	VEGF CC (n = 26)	CT (n = 22)	TT (n = 2)	Test of sig.	P
TNS					
Mean ± SD.	9.5 ± 5.13	9.14 ± 6.29	12 ± 14.14	H = 0.289	0.868
Median (Min.–Max.)	10 (1–21)	9.5 (1–22)	12 (2–22)		
HbA1C					
Mean ± SD. (%)	8 ± 1.93	8.16 ± 1.93	8.45 ± 3.46	F = 0.074	0.929
Median (Min.–Max.) (%)	7.75 (5.1–11.9)	8.1 (5.6–12.7)	8.45 (6–10.9)		
Median (Min.–Max.) (mmol/mol)	61.2 (32–107)	65 (37.7–115)	68.9 (42.1–95.6)		
Albumin/Creatinine ratio					
Mean ± SD.	74.14 ± 148.52	106.92 ± 198.58	76.10 ± 104.51	H = 0.063	0.969
Median (Min.–Max.)	10.1 (2–700)	12.5 (2–700)	76.1 (2.2–150)		

F: F for ANOVA test; H: H for Kruskal Wallis test; P: P value for comparing between the studied categories; *: Statistically significant at P ≤ 0.05; VEGF: vascular endothelial growth factor; HbA1c: glycosylated hemoglobin; TNS-r: total neuropathy score-reduced

nel for a greater understanding of the pathogenesis and improvement of prevention and treatment of these diseases.

In this cross-sectional study, we analyzed the association between IL-10 gene (-1082G/A) rs1800896 and VEGF-936 C/T polymorphisms and DPN in a group of Egyptian patients with T2DM. Although the sample size used in this study is smaller than that usually used for gene polymorphism studies, the power calculation showed a medium effect size supporting the validity of the study results.

Regarding IL-10 gene (-1082G/A) polymorphism (rs1800896), our current study has shown higher frequency of the AG genotype among patients (60%) compared to controls (42.5%) but with no statistical significance. The IL-10 GG genotype was statistically higher among the included controls compared to T2DM patients. In a review and meta-analysis by Hua et al. in 2013 [26], a significant association between IL-10-1082 G/A polymorphism and the risk of T2DM was observed under heterozygote comparison (GA vs. AA: OR = 1.21, 95% CI = 1.03–1.14) and dominant genetic model (GA/GG vs. AA: OR = 1.22, 95% CI = 1.05–1.41). In the stratified analysis by ethnicity, IL-10 gene (-1082G/A) polymorphism (rs1800896) was associated with a significantly increased risk of T2DM in Asian descendants under dominant genetic model (GA/GG vs. AA: OR = 1.69, 95% CI = 1.21–2.38). However, no significant association was found in European or African descendants [26]. This controversy may be attributed to the racial and ethnic differences. This is consistent

with another study which evaluated the role of this polymorphism with diabetic retinopathy [27]. The direct association of the IL-10 gene (-1082G/A) polymorphism (rs1800896) with T2DM remains elusive. The results of a meta-analysis published in 2013, which included 10 studies, demonstrated that the IL-10 gene (-1082G/A) polymorphism (rs1800896) was clearly associated with T2DM, but only in the Asian population, not in the European or African populations [26].

In a study performed by Kolla et al. in 2009 [10], the development of DPN was associated with high production of IL-10 (genotype -1082G/G) which is contrary to our study that showed no significant association between this polymorphism and DPN among cases with T2DM. However, our study showed a significant association between IL-10-1082 polymorphism (rs1800896) and the albumin/creatinine ratio, suggesting a possible association between this polymorphism and diabetic nephropathy. Nonetheless, further analysis on a wider scale with more specific and accurate investigations is needed to evaluate the role of IL-10-1082 polymorphism(rs1800896) in diabetic nephropathy. Other studies evaluated the role of this polymorphism in relation to other micro-vascular complications such as retinopathy, showing that IL-10 gene rs1800896 polymorphism is associated with a decreased risk of proliferative diabetic retinopathy [27].

Regarding the VEGF gene 936 C/T polymorphism, the frequency of CC genotype among patients was 52% compared to 70% among controls. In addition, the CT genotype was significantly higher among patients (44%) compared to controls (22.5%). This finding disagrees with the study of Ghisleni et al. in 2015 [28] in which no statistical significance was found between the two groups. Hardy-Weinberg analysis showed a p value of 0.098 meaning that the genotype frequency fulfilled the HWE.

The studies focusing on the expression of VEGF in diabetes patients with neuropathic complications exhibit contradictory findings [29-32]. Our study didn't find any statistical association between VEGF-936 C/T polymorphism and DPN. Another study performed by Kim et al. in 2009 [31] also demonstrated that there was no significant relationship between VEGF gene 936 C/T polymorphism and DPN. However, a study by Tavakkoly-Bazzaz et al. in 2010 [32] suggested that allele C was considered probably related to increased risk of developing DPN, while allele T might be protective [32]. The exact role of VEGF gene 936 C/T polymorphism in DPN remains controversial. Nonetheless, other studies evaluated the role of this SNP with other microvascular complications in patients with T2DM and their results showed that the TT genotype was more frequent among T2DM patients with retinopathy [31].

The significant correlation between TNS-r and other important diagnostic and therapeutic traits in diabetes as the duration of diabetes, FBS and HbA1c along with the fact that all T2DM patients included in this study had a grade of DPN and none was considered normal supports the importance of tight glycemic control to protect against this complication and delay its onset and severity [33]. Also, the presence of a grade of DPN in patients with short duration of diabetes (less than 3 years of diagnosis), even if considered subclinical, supports the reports demonstrating that the diagnosis of T2DM is usually delayed for many years due to the gradual onset of symptoms [2].

Conclusion

The VEGF 936 C/T genotype may be associated with T2DM, while the IL-10-GG genotype may be less likely to be associated with it; however, there is no association between VEGF-936 or IL-10 genotypes and DPN. The different traits showed different importance in T2DM-related traits, while DPN is correlated with the most important prognostic and disease monitoring traits of diabetes. When considering the results of other studies, a substantial heterogeneity in the findings is observed, demonstrating a complex link between T2DM risk factors and genetic predisposition. Such complexity can be explained largely by the variability in ethnicity of the studied populations and the influence of environmental factors on genetic expression.

Study limitations

Further studies regarding other genetic variants related to glycemic and metabolic function, ethnicity, especially the ones presumably related to nerve structure and function are needed to detect the strongest genetic factors associated with DPN. Also, population-based studies are recommended to help elucidating the role of these polymorphisms in T2DM complications. In addition, since this is a cross-sectional study, the disease outcome and prognosis in relation to a specific genotype is still unknown.

Conflict of interest

None.

REFERENCES

1. Pickup JC. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes Care. 2004; 27(3): 813–823, doi: 10.2337/diacare.27.3.813, indexed in Pubmed: 14988310.
2. Okemah J, Peng J, Quiñones M. Addressing Clinical Inertia in Type 2 Diabetes Mellitus: A Review. Adv Ther. 2018; 35(11): 1735–1745, doi: 10.1007/s12325-018-0819-5, indexed in Pubmed: 30374807.

3. Cieślak M, Cieślak M. Role of purinergic signalling and proinflammatory cytokines in diabetes. Clinical Diabetology. 2017; 6(3): 90–100, doi: 10.5603/dk.2017.0015.
4. Tesfaye S, Selvarajah D. Advances in the epidemiology, pathogenesis and management of diabetic peripheral neuropathy. Diabetes Metab Res Rev. 2012; 28 Suppl 1: 8–14, doi: 10.1002/dmrr.2239, indexed in Pubmed: 22271716.
5. Juster-Switlyk K, Smith AG. Updates in diabetic peripheral neuropathy. F1000Res. 2016; 5, doi: 10.12688/f1000research.7898.1, indexed in Pubmed: 27158461.
6. Elkhamisy E, Khalel M, Elbioumy A, et al. Beta-endorphin levels in both painful and painless diabetic peripheral neuropathy and its relations to pain characters and severity. Clinical Diabetology. 2017; 6(5): 159–171, doi: 10.5603/dk.2017.0027.
7. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest. 2006; 116(7): 1793–1801, doi: 10.1172/JCI29069, indexed in Pubmed: 16823477.
8. Donath MY, Størling J, Maedler K, et al. Inflammatory mediators and islet beta-cell failure: a link between type 1 and type 2 diabetes. J Mol Med (Berl). 2003; 81(8): 455–470, doi: 10.1007/s00109-003-0450-y, indexed in Pubmed: 12879149.
9. Bidwell J, Keen L, Gallagher G, et al. Cytokine gene polymorphism in human disease: on-line databases. Genes Immun. 1999; 1(1): 3–19, doi: 10.1038/sj.gene.6363645, indexed in Pubmed: 11197303.
10. Kolla VK, Madhavi G, Pulla Reddy B, et al. Association of tumor necrosis factor alpha, interferon gamma and interleukin 10 gene polymorphisms with peripheral neuropathy in South Indian patients with type 2 diabetes. Cytokine. 2009; 47(3): 173–177, doi: 10.1016/j.cyto.2009.06.007, indexed in Pubmed: 19608431.
11. Lee JH, Lee W, Kwon OhH, et al. Cytokine profile of peripheral blood in type 2 diabetes mellitus patients with diabetic retinopathy. Ann Clin Lab Sci. 2008; 38(4): 361–367, indexed in Pubmed: 18988929.
12. Moore KW, de Waal Malefyt R, Coffman RL, et al. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol. 2001; 19: 683–765, doi: 10.1146/annurev.immunol.19.1.683, indexed in Pubmed: 11244051.
13. Lin MT, Storer B, Martin PJ, et al. Relation of an interleukin-10 promoter polymorphism to graft-versus-host disease and survival after hematopoietic-cell transplantation. N Engl J Med. 2003; 349(23): 2201–2210, doi: 10.1056/NEJMoa022060, indexed in Pubmed: 14657427.
14. Tarabay M, Elshazli R, Settin A. African vs. Caucasian and Asian difference for the association of interleukin-10 promotor polymorphisms with type 2 diabetes mellitus (a meta-analysis study). Meta Gene. 2016; 9: 10–17, doi: 10.1016/j.mgene.2016.02.006, indexed in Pubmed: 27114918.
15. Thanigaimani S, Kichenadasse G, Mangoni AA. The emerging role of vascular endothelial growth factor (VEGF) in vascular homeostasis: lessons from recent trials with anti-VEGF drugs. Curr Vasc Pharmacol. 2011; 9(3): 358–380, doi: 10.2174/157016111795495503, indexed in Pubmed: 20807189.
16. Yang B, Cross DF, Ollerenshaw M, et al. Polymorphisms of the vascular endothelial growth factor and susceptibility to diabetic microvascular complications in patients with type 1 diabetes mellitus. J Diabetes Complications. 2003; 17(1): 1–6, doi: 10.1016/s1056-8727(02)00181-2, indexed in Pubmed: 12505748.
17. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2014; 37 Suppl 1: S81–S90, doi: 10.2337/dc14-S081, indexed in Pubmed: 24357215.
18. Meijer JWG, Smit AJ, Sonderen EV, et al. Symptom scoring systems to diagnose distal polyneuropathy in diabetes: the Diabetic Neuropathy Symptom score. Diabet Med. 2002; 19(11): 962–965, doi: 10.1046/j.1464-5491.2002.00819.x, indexed in Pubmed: 12421436.
19. Preston D, Shapiro B. Clinical–Electrophysiologic Correlations. Electromyography and Neuromuscular Disorders. 2013: 249–266, doi: 10.1016/b978-1-4557-2672-1.00016-7.
20. Cavaletti G, Frigeni B, Lanzani F, et al. Italian NETox Group. The Total Neuropathy Score as an assessment tool for grading the course of chemotherapy-induced peripheral neurotoxicity: comparison with the National Cancer Institute-Common Toxicity Scale. J Peripher Nerv Syst. 2007; 12(3): 210–215, doi: 10.1111/j.1529-8027.2007.00141.x, indexed in Pubmed: 17868248.
21. Dyck PJ, Carter RE, Litchy WJ. Modeling nerve conduction criteria for diagnosis of diabetic polyneuropathy. Muscle Nerve. 2011; 44(3): 340–345, doi: 10.1002/mus.22074, indexed in Pubmed: 21996793.
22. DYCK P, HUGHES R, O'BRIEN P. Quantitating Overall Neuropathic Symptoms, Impairments, and Outcomes. Peripheral Neuropathy. 2005: 1031–1051, doi: 10.1016/b978-0-7216-9491-7.50043-0.
23. Dyck PJ, Albers JW, Andersen H, et al. Toronto Expert Panel on Diabetic Neuropathy. Diabetic polyneuropathies: update on research definition, diagnostic criteria and estimation of severity. Diabetes Metab Res Rev. 2011; 27(7): 620–628, doi: 10.1002/dmrr.1226, indexed in Pubmed: 21695763.
24. Krippl P, Langsenlehner U, Renner W, et al. A common 936 C/T gene polymorphism of vascular endothelial growth factor is associated with decreased breast cancer risk. Int J Cancer. 2003; 106(4): 468–471, doi: 10.1002/ijc.11238, indexed in Pubmed: 12845639.
25. Barus J, Setyopranoto I, Sadewa AH, et al. Gene Polymorphism in Indonesian Subjects with Diabetic Polyneuropathy. Open Access Maced J Med Sci. 2018; 6(10): 1784–1789, doi: 10.3889/oamjms.2018.399, indexed in Pubmed: 30455749.
26. Hua Y, Shen J, Song Y, et al. Interleukin-10 -592C/A, -819C/T and -1082A/G Polymorphisms with Risk of Type 2 Diabetes Mellitus: A HuGE Review and Meta-analysis. PLoS One. 2013; 8(6): e66568, doi: 10.1371/journal.pone.0066568, indexed in Pubmed: 23805237.
27. Liu L, Zheng J, Xu Y, et al. Association between interleukin-10 gene rs1800896 polymorphism and diabetic retinopathy in a Chinese Han population. Biosci Rep. 2019; 39(4), doi: 10.1042/BSR20181382, indexed in Pubmed: 30890581.
28. Ghisleni MM, Biolchi V, Jordon BC, et al. Association study of C936T polymorphism of the VEGF gene and the C242T polymorphism of the p22phox gene with diabetes mellitus type 2 and distal diabetic polyneuropathy. Mol Med Rep. 2015; 12(3): 4626–1633, doi: 10.3892/mmr.2015.3988, indexed in Pubmed: 26130419.
29. Motawi TK, Rizk SM, Ibrahim IAR, et al. Alterations in circulating angiogenic and anti-angiogenic factors in type 2 diabetic patients with neuropathy. Cell Biochem Funct. 2014; 32(2): 155–163, doi: 10.1002/cbf.2987, indexed in Pubmed: 23913471.
30. Costa PZ, Soares R. Neovascularization in diabetes and its complications. Unraveling the angiogenic paradox. Life Sci. 2013; 92(22): 1037–1045, doi: 10.1016/j.lfs.2013.04.001, indexed in Pubmed: 23603139.
31. Kim HW, Ko GJ, Kang YS, et al. Role of the VEGF 936 C/T polymorphism in diabetic microvascular complications in type 2 diabetic patients. Nephrology (Carlton). 2009; 14(7): 681–688, doi: 10.1111/j.1440-1797.2009.01085.x, indexed in Pubmed: 19796028.
32. Tavakkoly-Bazzaz J, Amoli MM, Pravica V, et al. VEGF gene polymorphism association with diabetic neuropathy. Mol Biol Rep. 2010; 37(7): 3625–3630, doi: 10.1007/s11033-010-0013-6, indexed in Pubmed: 20352346.
33. Peltier A, Goutman SA, Callaghan BC. Painful diabetic neuropathy. BMJ. 2014; 348: g1799, doi: 10.1136/bmj.g1799, indexed in Pubmed: 24803311.