ORIGINAL ARTICLE

Risk factors associated with diabetic neuropathy in Mexican patients

Factores de riesgo asociados a neuropatía diabética en pacientes mexicanos

*Katy Sánchez-Pozos[1], Jazmín Monroy-Escutia[1], Joel Jaimes-Santoyo[1], Mª de los Ángeles Granados-Silvestre[2], Marta Menjivar[2], and Mª Guadalupe Ortiz-López[1]**

[1]Laboratory of Molecular Endocrinology, Hospital Juárez de México; [2]Faculty of Chemistry, Universidad Nacional Autónoma de México, Mexico City, Mexico

Abstract

Introduction: *Diabetic neuropathy (DN) is one of the most common complications of type 2 diabetes (T2D) and is a leading cause of lower limb amputation. The aim of the present study was to evaluate the risk factors contributing to DN in Mexican patients through the comparison of T2D patients with and without DN.* **Materials and methods:** *This cross-sectional study consisted of 509 subjects from Mexico who were classified as with DN and without DN. DN was assessed according to Douleur Neuropathique 4 questionnaire. Logistic regression analysis was performed to analyze risk factors contributing to DN.* **Results:** *The prevalence of DN in the studied population was 28.3%. The risk factors associated with DN were T2D duration (odds ratio [OR]: 2.51; 95% confidence interval [CI] 1.36-4.65), glycemic exposure index (OR: 1.82; 95% CI 1.01-3.64), low- and high-density lipoprotein levels (OR: 1.53; 95% CI 1.02-2.31), metformin treatment (OR: 2.08; 95% CI 1.11-3.91), diabetic retinopathy (OR: 1.65; 95% CI 1.07-2.54), and smoking (OR: 1.51; 95% CI 1.00-2.26).* **Conclusions:** *Therefore, the early identification of risk factors for DN development in Mexican population would allow implementing personalized strategies to improve the overall T2D patients' quality of life and reduce healthcare costs in our country.*

Key words: *Diabetes complications. Diabetic neuropathy. Glycemic index. Insulin resistance. Type 2 diabetes.*

Resumen

Antecedentes: *La neuropatía diabética (ND) es una de las complicaciones más comunes de la diabetes tipo 2 (DT2).* **Objetivo:** *Investigar los factores de riesgo que contribuyen al desarrollo de ND en población mexicana con DT2, comparando pacientes diabéticos con y sin ND.* **Método:** *Estudio transversal con 509 pacientes mexicanos con DT2, clasificados en dos grupos, con y sin ND. La ND fue diagnosticada con el cuestionario DN4. La identificación de los factores de riesgo de ND se realizó mediante un análisis de regresión logística.* **Resultados:** *La prevalencia de ND en la población de estudio fue del 28.3%. Los factores de riesgo asociados a ND fueron la duración de la DT2 (odds ratio [OR]: 2.51; intervalo de confianza del 95% [IC 95%]: 1.36-4.65), el índice glucémico (OR: 1.82; IC 95%: 1.01-3.64), las concentraciones bajas de colesterol unido a lipoproteínas de alta densidad (OR: 1.53; IC 95%: 1.02-2.31), el tratamiento con metformina (OR: 2.08; IC 95%: 1.11-3.91), la retinopatía diabética (OR: 1.65; IC 95%: 1.07-2.54) y el tabaquismo (OR: 1.51; IC 95%: 1.00-2.26).* **Conclusiones:** *La identificación temprana de los factores de riesgo para el desarrollo de ND en población*

Correspondence:
*Mª Guadalupe Ortiz-López
Avda. Instituto Politécnico Nacional, 5160
Magdalena de Las Salinas
C.P. 07760, Mexico City, Mexico
E-mail: gortizl@prodigy.net.mx

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mexicana permitirá implementar estrategias personalizadas para mejorar la calidad de vida de los pacientes con DT2 y disminuir el gasto del sector salud originado por esta complicación.

Palabras clave: *Complicaciones de diabetes. Neuropatía diabética. Índice glucémico. Resistencia a la insulina. Diabetes tipo 2.*

Introduction

Diabetes currently affects over 425 million people worldwide, and it is predicted to rise to be 642 million people in 2040, which represents a 50% more than the actual records; as a consequence, complications derived from diabetes will increase too[1]. In Mexico, approximately 1 out of 10 adults are affected with type 2 diabetes (T2D), which represents a health burden for the country and is among the leading causes of death[2]. Moreover, the T2D prevalence in younger age groups has increased; at present, there are more cases of children and adolescents affected (25% of diabetes cases in Mexico occur in young adults under 43 years of age)[3]. Hence, T2D is a complex disease to manage not only because of the lack of timely detection and the multiple complications it generates but also because addressing its causes and reducing its risk factors goes beyond the traditional limits of the health system.

It is estimated that half of patients with diabetes are unaware of their disease and they are thus more prone to developing diabetic complications[4]. An observational study of 28 countries in Asia, Africa, South America, and Europe reported that half of patients with T2D showed microvascular complications and 27% had macrovascular complications. Specifically, neuropathy was the highest microvascular complication reported in all regions, ranging from 25% in South Asia to 83% in Russia[5]. The global prevalence of diabetic neuropathy (DN) is approximately 30% in hospitalized diabetic patients and 20-30% in community-based diabetic patients[6]. Diabetic neuropathies are a heterogeneous group of disorders with diverse clinical manifestations, with the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after exclusion of other causes[7]. In Mexico, diabetes complications account for the 24.5-34.9% and DN prevalence varies from 17.1% to 81.1%; however, the studies in this field are scarce[8,9]. Furthermore, despite that 41.2% of the population in Mexico presented burning, pain or loss of sensitivity in the feet as reported by the ENSANUT MC 2016 (Encuesta Nacional de Salud Medio Camino 2016), only 20.9% of T2D patients reported a feet annual revision[10].

The typical symptoms of DN can be found in up to 50% of T2D patients[11]. Nevertheless, due to patients do not experimented symptoms in the course of mild neuropathy it is hard to identify subclinical DN, coupled with the fact that it starts to develop earlier than considered, as early as prediabetes[12]. Results from several studies suggest that risk factors for DN include diabetes duration, levels of hemoglobin A1C (HbA1c), dyslipidemias, retinopathy, smoking, high body mass index (BMI), and hypertension, among others[13-15]. Consequently, the identification of risk factors could be helpful in the prevention and early diagnosis of DN, especially in Mexico, where there is a lack of information in this area to establish measures to avoid the progress of diabetes. At present, there is no drug that can halt or reverse the progression of the disease. In fact, most of the therapies aim at providing only symptomatic relief; therefore, the identification of new modifiable risk factors is essential[16]. According to some authors, it is necessary to explore treatments that consider modifiable risk factors, such as metabolic syndrome components and inflammatory process[17,18]. Hence, if the risk factors are modifiable, it is possible to propose lifestyle changes that could improve the condition of the affected patients, delaying the DN development. Thus, the present study aimed to evaluate the risk factors contributing to DN in Mexican patients through the comparison of T2D patients with and without DN, incorporating two new indexes triglyceride–glucose (TyG) and glycemic exposure (GE) as risk factors of DN.

Materials and methods

Study population

The present was a descriptive and cross-sectional study. The study included adults > 18 years with T2D attending to Internal Medicine and Endocrinology service at Hospital Juárez de México from July 2013 to December 2017 (50% of which are from Estado de Mexico and 25% from Gustavo A Madero Alcaldia, the rest of the participants were from central and south

Mexico). The method for collect the samples was for convenience sampling, in this sense, all patients were invited to participate but only those with complete data were included in the study. Diagnosis of diabetes (according to American Diabetes Association, ADA)[19] was determined as follows: previous self-reported or diagnosed diabetes defined as doctor-diagnosed diabetes (in the interview and/or medical prescription for T2D or recent diagnosis of diabetes if the participant showed in the recruitment interview: (1) a fasting plasma glucose of ≥ 7 mmol/L; and/or (2) a Hb1Ac value ≥ 6.5%[20]. Exclusion criteria were: severe somatic diseases, pregnancy, and patients receiving corticosteroids (at doses equal to or greater than 5 mg/day of prednisone or equivalent doses of other corticosteroids), with terminal illnesses, patients with anemia (hemoglobin < 13 g/dL in men and < 12 g/dL in women), type 1 diabetes, renal failure, recent infection history, and autoimmune diseases and incomplete clinical history. Furthermore, patients were excluded if they had known non-diabetic causes of neuropathy (vitamin deficiencies, uremia, thyroid disease, lumbar, or cervical radiculopathy or inflammatory neuropathy).

The medical diagnosis of DN was performed by a certified physician according to symptoms and signs using the Douleur Neuropathique 4 (DN4) questionnaire[21,22]. The DN4 includes 10 questions (7 for symptoms and 3 for signs); a score ≥ 4 identifies neuropathic pain with high sensitivity (80%-84%) and specificity (90%)[23,24].

The Research and Ethics Committees of the Hospital Juárez de México approved the study, and each participant provided written informed consent (HJM0227/16-1).

Clinical variables

Age, sex, time of disease progression, and medical treatment status for T2D were recorded in the interview. Weight and height measurements of the participants were taken wearing light clothing and without shoes. The same evaluator took the measures. The BMI was calculated with the formula: weight in Kg divided by the square of height in meters.

Biochemical measurements

During interview a sample of blood was collected after a 9-12 h overnight fast. Plasma was obtained for determinations of glucose, total cholesterol, triglycerides, low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C), creatinine, urea, uric acid, and transaminases (AST/ALT) performed at the Hospital Juárez de México by commercially available standardized methods according to manufacturer's instructions using the ADVIA 1800 Chemistry System Siemens AutoAnalyzer. The HbA1c was measured in total blood on ADVIA 2120i Hematology System Siemens Analyzer. Analytical quality determinations were monitored by means of an internal quality control system and the participation of an external quality assurance program (Programa de Aseguramiento de la Calidad, PACAL).

The TyG index was calculated using the published formula, TyG = Ln[TG(mg/dL)xFPG(mg/dL)/2], where TG is fasting triglycerides and FPG is fasting plasma glucose[25]. TyG index was used as a reference of insulin resistance.

The glycemic exposure (GE) index was calculated using the following equation[26]:

GE index = $(HbA1c)^{1/2} \times (\text{duration of T2D in years})^{1/8}$.

Statistical methods

Quantitative variables are described by means and 95% confidence interval (CI), and median and interquartile ranges (IQR), and qualitative variables by frequency distribution. Kolmogorov test was applied to test normality. The continuous variables were log-transformed when it was necessary, applying the corresponding tests. Student's t-test or Mann–Whitney U test were used to compare continuous variables to test the hypothesis that there is a difference between the groups without and with ND. Qualitative variables were compared by Chi-square. Logistic regression models adjusted for BMI, age, and sex were considered as confounder variables to explore DN risk factors. The risk factors to be analyzed were: Clinical (gender, onset age, diabetes duration, smoking, and BMI); biochemical (HbA1c, GE index, total cholesterol, HDL-C, triglycerides, and TyG index); treatment (insulin and metformin therapy); and complications (diabetic retinopathy). Dichotomic variables were analyzed by logistic regression analysis performed in all patients with "presence of DN" as the dependent variable (0 = DN absent; 1 = DN present) and gender (0 = male; 1 = female), onset age (0 ≤ 40 years (early), 1 ≥ 40 years old (late)), BMI (0 < 25.0 kg/m^2, 1 ≥ 25.0 kg/m^2), diabetes duration (0 < 10 years, 1 ≥ 10 years), glycemic control (0 = HbA1c < 7%, 1 = HbA1c ≥ 7%), GE index (0 < p75, 1 ≥ p75), total

cholesterol (0 < 200 mg/dL, 1 ≥ 200 mg/dL), HDL-C (0 < 45 mg/dL, 1 ≥ 45 mg/dL), triglyceride levels (0 < 150 mg/dL, 1 ≥ 150 mg/dL), TyG index (0 < p75, 1 ≥ p75), treatment with insulin (0 = not treated, 1 = treated), metformin treatment (0 = not treated, 1 = treated), diabetic retinopathy (0 = absent, 1 = present), and current smoking (0 = no, 1 = yes) as the independent variables. The T2D population with DN was stratified by IQR TyG and GE index, differences among groups were analyzed by ANOVA or Kruskal–Wallis tests according to normality of data. SPSS software for Mac version 21.0 was used for data analysis. A $p < 0.05$ was considered significant.

Results

Clinical and biochemical characteristics of T2D patients

The study involved 509 adults > 18 years with T2D. The patients were classified into two groups: with and without DN. The 100% of diabetic patients with DN have hypoesthesia, and none in the group without DN, also 95.1% displayed numbness and 3.6% in diabetic patients with and without DN respectively. Out of the 509 participants with T2D, 144 patients presented DN (a score ≥ 4 for DN4 questionnaire), corresponding to the 28.3% of the studied population with T2D (Table 1).

Table 1 shows the main differences in biochemical parameters between groups that included disease evolution, GE index, urea, and transaminases. DN group presented significantly higher disease evolution, GE index, and urea than group without DN, while DN group had significantly lower plasma transaminases values compared with group without DN.

Diabetes complications were similar in both groups. However, there were differences regarding “diabetic foot” between patients with (36.8%) and without DN (0%) ($p < 0.05$).

In general, both groups presented high percentage of hypertension (61.5%), dyslipidemias (45.8%), and overweight and obesity (28.4%).

Clinical and biochemical characteristics of patients with DN according to TyG index

To elucidate if insulin resistance is an independent risk factor related with DN, we used TyG index to dissect its effect in patients with DN. In table 2, patients with DN were classified according to IQR TyG index. Patients in third quartile (> Q3) presented significantly higher plasmatic glucose, HbA1c levels, GE index, and triglycerides compared with patients in first and second quartiles (< Q1 and Q1-Q3) ($p < 0.05$).

Clinical and biochemical characteristics of patients with DN according to GE index

To elucidate the role of GE index as a risk factor for DN development, we classified the patients with DN according to IQR GE index. As it shown in table 3, patients in third quartile (> Q3) showed higher disease evolution, plasmatic glucose, HbA1c levels, and TyG index, than patients in first and second quartiles (< Q1 and Q2-Q3) ($p < 0.05$). Moreover, these patients have the highest disease evolution compared with the remaining groups.

Risk factors for DN development

According to regression analysis, risk factors associated with DN were diabetes duration, GE index, low levels of HDL-C, metformin treatment, and smoking. Furthermore, diabetic retinopathy was associated with DN ($p < 0.05$) (Table 4).

Discussion

T2D is a leading cause of morbidity and mortality in Mexico and worldwide. Even though 87.8% of the affected population receives pharmacological treatment, only 5.3% are currently under adequate control, while 38% and 56% have poor and very poor control, respectively[10,27]. This is consistent with the prediction that in 20 years, 65% of the population with T2D will have chronic complications of the disease[28].

The prevalence of DN in the present study was 28.3%, which is different from early reports in Mexico (17.1-81.1%); hence, these marked differences could be related to the different diagnosis criteria and to an under-diagnosis[6,8,9].

We observed differences between patients with ND and without ND in the evolution of the disease, GE index, urea concentration, and transaminase activity ($p < 0.05$) (Table 1). Several reports have demonstrated that duration of diabetes is a risk factor for DN independent of patient´s age[29]. In the present study, patients with DN have longer disease evolution than patients without DN, as reported previously[12]. On the

Table 1. Clinical and biochemical characteristics of T2D patients

N (F/M)		Without DN				With DN				Total	
		365 (213/152)				144 (79/65)				509 (292/217)	
		Median	Q1, Q3	Mean	95% CI	Median	Q1, Q3	Mean	95% CI	Median (Q1, Q3)	Mean (95% CI)
Disease evolution		15	8, 20	15.6	14.6-16.5	17	11, 23*	18.5	17.0-19.9	16 (9, 21)	16 (16-17)
BMI (kg/m^2)		27.9	25.5, 31.2	28.4	27.7-29.0	27.3	24.7, 32.0	28.3	26.9-29.6	27.7 (25.4, 31.3)	28.4 (27.7-29.0)
Glucose (mg/dL)		137	105, 193	169	158-180	151	120, 215	178	160-196	141 (107, 200)	171 (162-181)
HbA1c (%)		7.9	6.5, 10.0	8.5	8.2-8.7	8.0	7.0, 10.0	8.6	8.2-9.0	8.0 (6.6, 10.0)	8.5 (8.3-8.7)
GE index		3.89	3.47, 4.49	4.0	3.9-4.1	4.08	3.60, 4.58*	4.1	4.0-4.2	3.92 (3.48, 4.52)	4.0 (4.0-4.1)
Total cholesterol (mg/dL)		175	146, 199	178	171-185	180	155, 205	185	175-194	179 (148, 202)	180 (174-185)
LDL-C (mg/dL)		100	85, 120	104	100-108	109	93, 127	110	102-118	105 (86, 121)	105 (102-109)
HDL-C (mg/dL)	F	46.9	40.6, 56.6	50.3	47.0-53.6	51.6	42.4, 58.5	52.9	47.8-58.1	47.5 (41.2, 57.3)	51.0 (48.2-53.8)
	M	44.7	35.1, 47.5	41.3	39.2-43.4	47.5	39.6, 51.0	45.6	40.5-50.7	45.9 (36.3, 47.5)	42.4 (40.4-44.5)
Triglycerides (mg/dL)		146	114, 198	190	173-207	135	112, 182	161	144-178	165 (113, 185)	182 (169-195)
TyG index		9.30	8.94, 9.83	9.39	9.31-9.46	9.33	8.93, 9.74	9.36	9.26-9.47	9.31 (8.93, 9.83)	9.38 (9.32-9.44)
Creatinine (mg/dL)	F	0.8	0.6, 1.0	1.1	1.0-1.3	0.8	0.6, 1.1	1.1	0.9-1.4	0.8 (0.6, 1.0)	1.1 (1.0-1.2)
	M	1.0	0.8, 1.5	1.5	1.2-1.7	1.0	0.8, 1.8	2.1	1.5-2.7	1.0 (0.8, 1.5)	1.7 (1.4-1.9)
Urea (mg/dL)		34	25, 47	46	42-50	40	28, 55*	52.9	46-60	36 (26, 49)	48 (44-51)
Uric acid (mg/dL)		5.6	4.7, 6.6	5.7	5.4-5.9	5.6	4.9, 6.8	5.6	5.3-6.0	5.6 (4.7, 6.3)	5.7 (5.5-5.9)
AST (UI/L)		27	21, 32	31	29-33	25	20, 30*	28	24-31	27 (21, 31)	30 (28, 32)
ALT (UI/L)		28	18, 33	31	29-33	25	16, 30*	26	24-29	26 (17, 31)	30 (28, 32)
Diabetes complications (%)											
Acute complications (hypoglycemia, DKA, HONK)				29.6				22.9			27.7
Cardiovascular disease				2.7				4.2			3.1
Cerebrovascular				1.6				0.7			1.4
Diabetic foot				0.0				36.8§			10.4
Diabetic retinopathy				21.1				30.6			23.8
Diabetic nephropathy				21.6				29.2			23.8
Acute kidney injury				6.3				3.5			5.5
Comorbidities (%)											
Hypertension				60.0				65.3			61.5
Dyslipidemias				44.9				47.9			45.8
Hypothyroidism				9.3				8.3			9.0
Hyperuricemia				4.7				6.9			5.3
Overweight and obesity				28.5				28.4			28.4
Smoking				28.5				37.5			31.0
Treatment (%)											
Oral hypoglycemic agents				81.9				72.2			79.2
Insulin				52.1				56.9			53.4

Data are presented as median (Q1, Q3), mean with 95% CI and percentages. Mann–Whitney U test was performed for comparison of groups. ALT: alanine transaminase; AST, aspartate transaminase; ˚F, female; GE index, glycemic exposure index; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein; M, male; SD, standard deviation; TyG index, triglyceride–glucose index. $p < 0.05$. § $p < 0.05$, Chi-square was applied.

other hand, GE index is an algorithm that combines Hb1Ac, duration of diabetes, and age at onset of diabetes, which correlates significantly with complications and predicts late complications better than single components[30]. Patients with DN presented higher GE index values than patients without DN;

Table 2. Clinical and biochemical characteristics of patients with DN classified by IQR TyG index

		TyG index		
		< Q1	Q1-Q3	> Q3
Characteristics				
N (F/M)		32 (20/12)	76 (35/41)	36 (24/12)
Disease evolution		16 (11-23)	17 (11, 23)	20 (14, 25)
BMI (kg/m^2)		27.1 (23.4, 31.5)	27.8 (25.4, 32.9)	26.5 (24.9, 28.0)
Glucose (mg/dL)		106 (82, 137)[b,c]	145 (127, 183)[a,c]	231 (178, 312)[a,b]
HbA1c (%)		8.0 (6.8, 11.1)[c]	7.5 (6.5, 8.7)[c]	9.5 (8.3, 11.8)[a,b]
GE index		3.98 (3.50, 4.62)	3.90 (3.53, 4.32)[c]	4.53 (3.82, 4.83)[b]
Total cholesterol (mg/dL)		165 (144, 204)	179 (151, 200)	179 (168, 219)
LDL-C (mg/dL)		105 (85, 122)	105 (95, 126)	118 (102, 128)
HDL-C (mg/dL)	F	49 (45, 61)	48 (42, 59)	48 (41, 52)
	M	48 (40, 51)	47 (40, 48)	48 (39, 52)
Triglycerides (mg/dL)		91 (73-116)[b,c]	160 (115, 182)[a,c]	209 (181, 261)[a,b]
Creatinine (mg/dL)	F	0.70 (0.60, 1.10)	0.80 (0.60, 1.10)	0.90 (0.70, 1.65)
	M	1.10 (0.75, 1.37)	1.00 (0.80, 2.00)	0.95 (0.75, 1.67)
Urea (mg/dL)		36 (28, 58)	43 (30, 53)	36 (27, 59)
Uric acid (mg/dL)		4.9 (4.2, 5.9)	5.6 (5.1, 6.5)	5.6 (5.0, 6.3)
AST (UI/L)		24 (18, 31)	25 (21, 30)	26 (20, 30)
ALT (UI/L)		26 (16, 29)	25 (16, 30)	24 (20, 30)

Data are presented as medians (Q1, Q3) and percentages. ANOVA or Kruskal–Wallis test were performed for comparison of groups. ALT: alanine transaminase; AST: aspartate transaminase; GE index: glycemic exposure index; HDL-C: high-density lipoprotein; LDL-C: low-density lipoprotein; TyG index: triglyceride–glucose index; [a]p < 0.05 versus < Q1; [b]p < 0.05 versus Q1-Q3; [c]p < 0.05 versus > Q3.

Table 3. Clinical and biochemical characteristics of patients with DN according to GE index

		Glycemic exposure index		
		< Q1	Q1-Q3	> Q3
Characteristics				
N (F/M)		35 (13/12)	73 (46/30)	36 (20/16)
Disease evolution		11 (7, 16)[b,c]	17 (14, 23)[a]	23 (18, 27)[a]
BMI (kg/m^2)		27.8 (25.6, 32.9)	27.9 (25.8, 32.4)	25.0 (22.0, 27.3)
Glucose (mg/dL)		121 (95, 153)[b,c]	159 (130, 207)[a]	189 (127, 254)[a]
HbA1c (%)		6.0 (5.8, 6.7)[b,c]	8.0 (7.2, 9.0)[a,c]	11.6 (11.0, 13.0)[a,b]
Total cholesterol (mg/dL)		179 (153, 198)	180 (165, 207)	179 (143, 198)
LDL-C (mg/dL)		105 (101, 134)	105 (90, 126)	107 (85, 120)
HDL-C (mg/dL)	F	46 (39, 53)	48 (44, 58)	48 (43, 60)
	M	48 (40, 48)	48 (41, 48)	43 (38, 48)
Triglycerides (mg/dL)		132 (114, 182)	175 (115, 182)	163 (105, 195)
TyG Index		9.12 (8.92, 9.35)[c]	9.38 (9.11, 9.62)	9.70 (8.92, 10.06)[a]
Creatinine (mg/dL)	F	0.60 (0.60, 0.90)	0.85 (0.60, 1.20)	0.95 (0.70, 1.50)
	M	1.25 (1.00, 4.70)	1.00 (0.75, 1.65)	0.89 (0.80, 1.10)
Urea (mg/dL)		35 (28, 63)	41 (28, 56)	36 (30, 50)
Uric acid (mg/dL)		5.6 (5.6, 6.9)	5.5 (4.9, 5.8)	4.8 (4.2, 6.3)
AST (UI/L)		28 (21, 31)	27 (20, 30)	23 (21, 28)
ALT (UI/L)		21 (16, 30)	29 (16, 32)	22 (17, 29)

Data are presented as medians (Q1, Q3) and percentages. ANOVA or Kruskal–Wallis test were performed for comparison of groups. ALT: alanine transaminase; AST: aspartate transaminase; GE index: glycemic exposure index; HDL-C: high-density lipoprotein; LDL-C: low-density lipoprotein; TyG index: triglyceride–glucose index. [a]p < 0.05 versus <Q1; [b]p < 0.05 versus Q1-Q3; [c]p < 0.05 versus > Q3

Table 4. Regression analysis of risk factors for neuropathy diabetic development

Risk factors	OR (95% CI)	p	OR (95% CI) Adjusted by sex, age, and BMI	p	OR (95% CI) Adjusted by HbA1c and duration of T2D	p
Gender						
Male						
Female	1.153 (0.782-1.701)	0.473	1.008 (0.575-1.766)	0.978	1.113 (0.743-1.668)	0.604
Onset age (years)						
Late						
Early	1.165 (0.773-1.756)	0.465	2.222 (0.956-5.165)	0.069	1.052 (0.680-1.626)	0.821
BMI (kg/m^2)						
< 25						
≥ 25	1.510 (0.812-2.808)	0.193	1.482 (0.794-2.767)	0.217	1.329 (0.694-2.547)	0.391
Diabetes duration (years)						
< 10						
≥ 10	2.216 (1.365-3.598)	0.001*	2.513 (1.357-4.654)	0.003*	2.209 (1.356-3.599)	0.001*
HbA1c						
< 7%						
≥ 7%	1.488 (0.876-2.525)	0.141	1.375 (0.721-2.622)	0.334	1.377 (0.786-2.413)	0.264
GE index						
< Q3						
≥ Q3	1.356 (0.859-2.142)	0.191	1.820 (1.011-3.637)	0.049*	1.259 (0.566-2.798)	0.572
Total cholesterol (mg/dL)						
< 200						
≥ 200	1.282 (0.835-1.968)	0.256	1.333 (0.751-2.369)	0.326	1.325 (0.849-2.067)	0.215
HDL-C (mg/dL)						
≥ 45						
< 45	1.533 (1.020-2.306)	0.049*	1.081 (0.606-1.928)	0.791	1.492 (0.976-2.280)	0.069
Triglycerides (mg/dL)						
< 150						
≥ 150	1.067 (0.723-1.575)	0.743	1.119 (0.653-1.919)	0.683	1.096 (0.732-1.642)	0.657
TyG index						
≤ Q3						
> Q3	1.208 (0.783-1.862)	0.393	1.400 (0.889-2.204)	0.146	1.276 (0.796-2.045)	0.311
Insulin treatment						
No						
Yes	1.281 (0.863-1.904)	0.219	1.286 (0.735-2.253)	0.378	1.038 (0.681-1.582)	0.862
Metformin treatment						
No						
Yes	1.658 (1.101-2.497)	0.016*	2.083 (1.109-3.910)	0.022*	1.606 (1.052-2.452)	0.028*
Diabetic retinopathy						
No						
Yes	1.646 (1.066-2.542)	0.025*	1.707 (0.934-3.120)	0.082	1.588 (1.006-2.506)	0.047*
Smoking						
No	1.506 (1.003-2.262)					
Yes		0.049*	1.050 (0.562-1.964)	0.878	1.401 (0.916-2.144)	0.120

ORs were calculated by a logistic regression analysis. *($p < 0.05$). GE index: glycemic exposure index; HDL-C: high-density lipoprotein; TyG index: triglyceride–glucose index.

therefore, patients with DN have a higher GE compared with patients without DN; this is important to note, because higher GE index values correlates and predicts microvessel complications[30]. A recent study in Indian population has suggested that high urea levels correlates with poor glycemic control, indicated by GE index[31]. Ata et al. have suggested moderate elevations of serum ALT with poor glycemic control,

suggesting that patients with DN might have more time exposed to chronic hyperglycemia as implied by GE index, which impacts in levels of urea, AST, and ALT[32].

The frequency of complications associated with T2D was not different between patients without DN or with DN, as we would have expected. However, the phenomenon that calls our attention (Table 1) is the high prevalence of hyperuricemia (5.3% in the total studied population) in T2D patients. In Mexico, the epidemiological data in this matter are scarce; the most recent report informed a prevalence of 2.4-4.7% in general population[33], which is inferior compared with the frequency in T2D patients in this study. It is known that patients with gout have a high frequency of cardiovascular and renal type comorbidities; moreover, hyperuricemia in patients with metabolic disorders affect patient prognosis and treatment[34]. Interestingly, the frequency of hypothyroidism in T2D patients was similar to the prevalence reported in the general Mexican population, which suggest a underestimation in our country, though further studies are needed (9.0% vs. 5.7%, respectively)[35].

Several studies have proposed the TyG index as a surrogate for estimating insulin resistance[25,36]. TyG index results from the product of fasting triglycerides and fasting glucose levels and has been recently recommended as a simple and inexpensive index to evaluate insulin resistance, with a good correlation with gold standard hyperglycemic clamp according to studies in Brazilian population[25,36]. To evaluate the effect of insulin resistance as TyG index, we classified patients with DN in three quartiles (Table 2). The groups in second and third quartile showed higher values of HbA1c and GE index compared with group in first quartile. The accumulation of glycated hemoglobin in tissues may adversely affect vessel walls and induce endothelial cell dysfunction, which contributes to atherosclerosis[37]. Furthermore, the TyG index has been considered as a good indicator of overall glycemic control and possible risk for long-term complications[38]. Recent studies have proposed the TyG index as a predictor of cardiovascular disease (CVD) events in clinical practice, subclinical atherosclerosis, progression of coronary artery calcification, and cardiac autonomic neuropathy, suggesting that patients in second and third quartile have a greater risk of CVD events and cardiac autonomic neuropathy compared with patients in Q1[39].

It should be pointed out that patients in third quartile when grouping by GE index, presented the highest TyG index compared with the first and second quartiles, which suggest that this group is mostly exposed no only to chronic hyperglycemia but to insulin resistance, both risk important factors to the development of DN, although further studies in this matter are needed in our population (Table 3)[40].

The factors associated with DN in Mexican population were diabetes duration, GE index, and metformin treatment after adjustment by BMI, sex, and age as confounder's variables (Table 4). As mentioned above, it is well established that duration of diabetes is a risk factor for development of DN[29]. Although there was a trend ($p = 0.069$) in the increase of the risk in patients with early onset T2D vs. late onset, the significance was not achieved; however, the association of DN with duration of diabetes remained significant independently of the diagnostic methodology. It is important to mention that a stringent metabolic control can reduce the risk of DN[41]. In a 24-year follow-up of patients with type 1 diabetes, 64% of participants with poor glycemic control and none in good glycemic control developed DN[42]. Some studies have associated plasmatic levels of HbA1c with DN; however, in this study, we failed to find an association. Nevertheless, GE index (as determinant of chronic hyperglycemia exposure) was associated with DN after adjustment of sex, age, and BMI[13,43].

Despite levels of LDL-C, total cholesterol and triglycerides were not associated with DN, low levels of HDL-C were associated (Table 4). After adjustment by sex, age, and BMI, the significance was lost; however, factors like lowering lipid drugs use was not considered in the analysis. A 6-year follow-up study of 48 Korean patients revealed that reduced HDL-C (odds ratio [OR]: 5.292, $p = 0.05$) and high triglycerides (OR: 6.129, $p = 0.043$) significantly increased the risk of diabetic distal symmetrical polyneuropathy, after adjusting for age and sex[40]. Tesfaye et al. showed in a 7-year follow-up study in the European population that there is no association of DN with dyslipidemia[43]. Hence, more studies that consider covariates such as lowering lipid drugs therapy, diabetes duration, smoking, and chronic hyperglycemia exposure are needed.

Several studies have suggested a clinical association between metformin use and Vitamin B12 deficiency, and as a consequence DN development[44,45]. Nevertheless, other studies have shown a negative correlation between metformin treatment and Vitamin B12 levels and the association with DN, while others have shown that despite metformin therapy is associated with lower

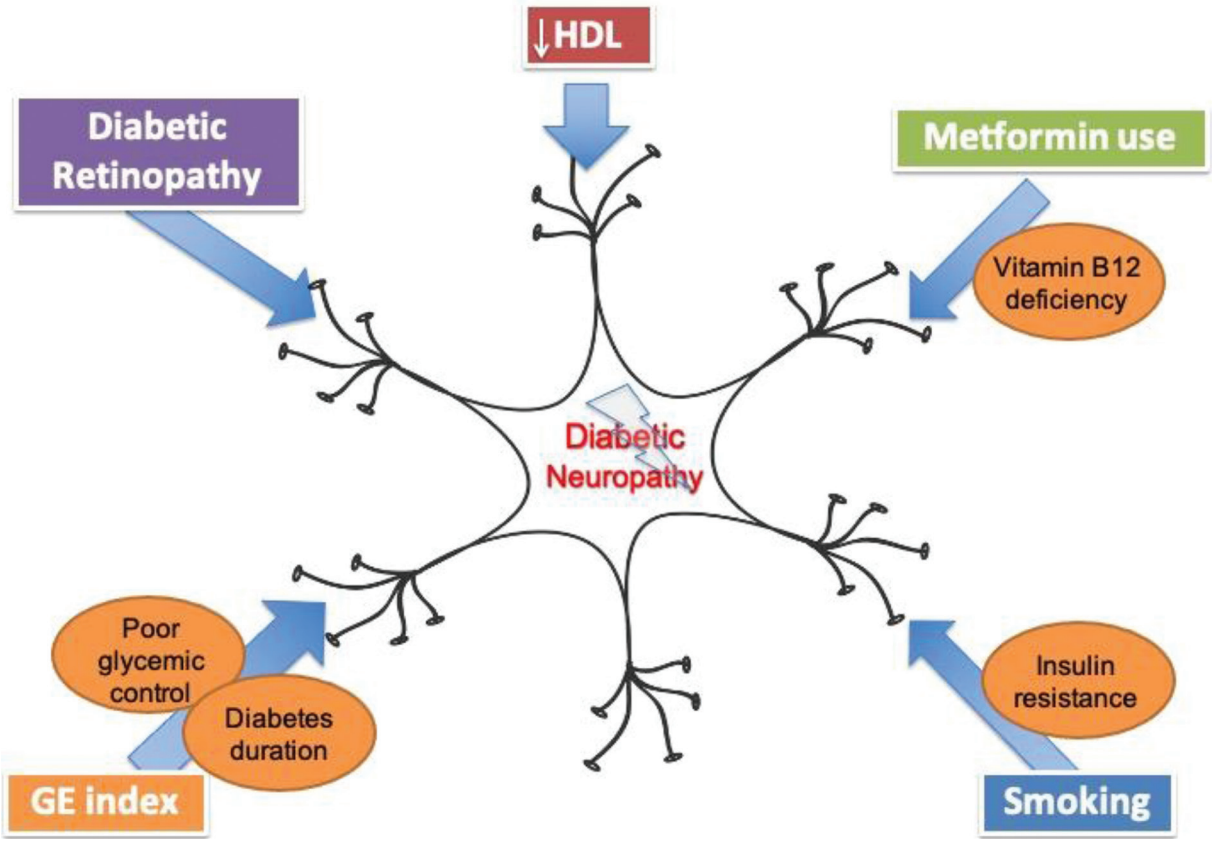

Figure 1. *Risk factors for diabetic neuropathy (DN). Risk factors associated with DN were glycemic exposure index, diabetes duration, low levels of HDL-C, metformin treatment, and smoking p < 0.05.*

Vitamin B12 status, there does not appear to be any significant effect on peripheral neuropathy in European patients receiving metformin[44-46]. In Mexico, there are no studies related with metformin therapy and DN. Indeed, this is the first study in our country that showed a significant association between the metformin treatment and DN. It is important to note that the association remained after adjustment by sex, age, and BMI, and by HbA1c and diabetes duration (OR: 2.513, p = 0.003 and OR: 2.209, p = 0.001, respectively). Furthermore, the 70% of the participants in the present study have metformin as glucose lowering treatment, which suggests double risk to develop DN as suggested by the OR obtained (Table 4). The ADA guidelines recommend periodic testing of Vitamin B12 in metformin-treated patients (especially in those with peripheral neuropathy); however, only 4.5% of Mexican T2D patients in this study have been prescribed with Vitamin B12[47]. Therefore, to prevent or delay the appearance of DN in diabetic patients, it is important to implement follow-up programs that include prescription and monitoring of Vitamin B12.

Furthermore, an association between diabetic retinopathy and DN was found (OR: 1.646, p = 0.025), nonetheless, when adjusted by sex, age, and BMI, the significance was lost. A study in T2D patients from Asia identified an association between diabetic retinopathy and cardiovascular autonomic neuropathy; in this study, the authors suggested that diabetic retinopathy is the most significant risk factor predictive of the presence of cardiovascular autonomic neuropathy in patients with T2D[48]. Further studies with a bigger sample are needed, to elucidate the relationship between DN and diabetic retinopathy development.

Smoking was associated with DN; however, when was adjusted by different covariates, the effect was lost. A robust meta-analysis including 10 prospective and 28 cross-sectional studies has found that smoking had an unadjusted OR of 1.26 for developing DN[49]. The mechanism involved is not clear, but it is well established that smoking could contribute to insulin resistance occurrence, which could impact in DN development.

Finally, in the present study, the TyG index was not a risk factor associated with DN, perhaps because the

TyG index is only an estimator of insulin resistance. It will be necessary to carry out studies to evaluate the agreement of this index with HOMA-IR in the Mexican population and to test its usefulness as a risk factor of DN. In contrast, GE index could be an important risk factor for DN development.

Limitations

Weaknesses of the study is the relatively small sample, due to the availability of the complete clinical histories and the biological samples, due to Hospital Juárez de México attend patients from various parts of the country, it is hard for them to come back. Furthermore, we use TyG index, as an estimation of insulin resistance, which is a bias for the reference of insulin resistance in this work, but this may open the way to further research that investigates the role of insulin resistance in DN development. Although there are many studies worldwide, in Mexico studies that explore risk factors associated with DN are scarce.

Conclusions

In general, the risk factors that mostly contribute to the development of DN in the Mexican population are diabetes duration, metformin therapy, GE index, and diabetic retinopathy (Figure 1). Diabetic retinopathy is associated with DN, but further studies are needed to elucidate the mechanisms involved. For 1st time, an association between metformin treatment and DN development is identified in Mexican population. Finally, it is important to recognize risk factors that contribute to the development of DN to prevent or delay diabetic complications in Mexican diabetes patients that allow us to implement personalized strategies to improve the overall T2D patients' quality of life and reduce healthcare costs in our country.

Conflicts of interest

The authors declare no conflicts of interest.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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