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Peripheral blood levels of CXCL10 are a useful marker for diabetic polyneuropathy in subjects with type 2 diabetes

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Abstract

Background: Diabetic peripheral neuropathy (DPN) is a chronic complication of diabetes mellitus associated with high morbidity and mortality. Major risk factors for DPN include metabolic changes, duration of diabetes, nerve ischaemia and derangements in regeneration and nerve repair programmes. Chemokines have been previously implicated in the pathogenesis of various neuropathies and neuropathic pain processes. The aim of this pilot study was to evaluate the association between the plasma levels of chemokines (CXCL9, CXCL10 and CXCL11) in the presence of DPN in a cohort of type 2 diabetes (T2D) patients.

Materials and methods: We studied 73 patients with T2D: 36 with DPN and 37 without DPN. DPN was established through the Semmes-Weinstein test (SW). Plasma levels of circulating chemokines CXCL9, CXCL10 and CXCL11 were determined using DuoSet ELISA kits (Abingdon, UK).

Results: We found that levels of CXCL10 were significantly higher in patients with DPN than amongst patients without DPN (57.6 ± 38.3 vs 38.1 ± 33.4 pg/mL, respectively; $P = .034$). Serum levels of chemokine CXCL9 were also higher amongst patients with DPN but did not reach a statistical significance (188.1 ± 72.7 and 150.4 ± 83.6 pg/mL, respectively, $P = .06$).

Conclusions: Increased circulating levels of CXCL10 were associated with DPN in T2D patients, suggesting a role of this chemokine in the DPN. Determination of CXCL10 levels could be used as a marker for the early detection and implementation of therapeutic strategies in order to reverse and prevent the DPN.

Abbreviations: ABI, ankle-brachial index; DPN, diabetic peripheral neuropathy; HCUV, Hospital Clínico Universitario of Valencia; HPLC, high-performance liquid chromatography; MODY, maturity onset diabetes of the young; NDS, neurological disability score; NF-kB, nuclear factor kappa beta; NSS, neuropathy symptom score; SD, standard deviation; SPSS, statistical package for social sciences; SW, Semmes-Weinstein test; T2D, type 2 diabetes.

1 | INTRODUCTION

Diabetic peripheral neuropathy (DPN), as defined by the American Diabetes Association, is a diagnosis of exclusion involving the presence of signs and/or symptoms of peripheral nerve dysfunction in individuals with diabetes.[1] It is probably the most prevalent and largely underdiagnosed, chronic complication of diabetes and has high morbidity and mortality.[1,2] Major risk factors of DPN include metabolic changes associated with chronic hyperglycaemia and duration of diabetes, nerve ischaemia and derangements in regeneration and nerve repair programmes.[3]

Chronic hyperglycaemia and increased levels of HbA1c lead to peripheral nerve injury through an array of complex mechanisms that include the overactivation of the polyol pathway and accumulation of sorbitol,[4] alterations of myoinositol,[5] protein synthesis deficiency (myelin amongst others),[6] formation of advanced glycation end products and oxidative stress.[6,7] These interlinked metabolic pathways converge activating the transcription factor NF-kB, which induces the expression of genes involved in inflammation, triggering pro-inflammatory and immune responses.[3,8-10] In addition, under hyperglycaemic conditions, microvascular alterations result and contribute to the development of DPN via tissue hypoxia and nerve ischemia.[10-12]

Chemokines are a family of low molecular weight "chemotactic cytokines" (8-10 kDa) that, after binding to their specific G-protein coupled receptor, regulate immune cell migration. By inducing immune cell migration, they play a pivotal role not only in innate immunity but also in the adaptive immune response as well as in the maintenance of chronic inflammation.[13] While some chemokines have been described as proinflammatory mediators and are related to infection and inflammation, others are homeostatic and participate in the control of cell migration during tissue development and maintenance processes.[14,15]

Chemokines are classified into four main subfamilies according to the position of the cysteine residues on their N-terminal region: CXC, CC, C and CX3C.[14,15] The CXC subfamily is the largest one and is subdivided into CXC chemokines that contain a glutamic acid-leucine-arginine motif (ELR) in the vicinity of the CXC residues and CXC chemokines that do not contain it.[14,16] Chemokines belonging to the latter subgroup include CXCL9, CXCL10 and CXCL11 chemokines. They are produced by lymphocytes, monocytes and endothelial cells from the small vessels. These chemokines act through the CXCR3 receptor and have inflammatory functions and antiangiogenic effects, thus, playing an important role in the control of immunity, inflammation and angiogenesis.[17,18]

The implication of chemokines in the pathogenesis of various neuropathies and neuropathic pain processes has been studied previously.[16,19] More specifically, in diabetic neuropathy, the role of CXCL 9,10 and 11 has been investigated in an animal model. In streptozotocin-induced diabetic mice presented raised spinal levels of these CXC chemokines following intrathecal administration and increased neuropathic pain. The authors suggested that these chemokines might participate in the development of diabetic neuropathy, which they described as a neuroinflammatory disorder and that further research is warranted to complete the understanding of the underlying mechanisms.[20]

As mentioned, CXCL9, CXCL10 and CXCL11 have been related to the regulation of angiogenesis and local vascular inflammation, therefore, suggesting a pivotal role in the development of microvascular lesions that could affect myelin and axons in DPN. Therefore, the aim of this pilot study was to evaluate the association between the plasma levels of CXCL9, CXCL10, CXCL11 with the presence of DPN in a cohort of type 2 diabetes (T2D) patients.

What's known

- Screening of chronic complications is essential to begin an early treatment and to prevent the progression. However, many times chronic complications of diabetes are diagnosed in an advanced phase.
- Diabetic peripheral neuropathy is a chronic complication of diabetes mellitus associated with high morbidity and mortality.
- Chemokines have been previously implicated in the pathogenesis of various neuropathies and neuropathic pain processes.

What's new

- Increased circulating levels of CXCL10 were associated with diabetic polyneuropathy in type 2 diabetic patients.
- Determination of CXCL10 levels could be used as a marker for early detection and for implementing therapeutic strategies in order to reverse and prevent the diabetic polyneuropathy.

2 | MATERIALS AND METHODS

2.1 | Study population

All the participants were patients with T2D aged between 40 and 70 year old who were recruited from the Diabetes Unit of the Hospital Clínico Universitario of Valencia (HCUV) in Spain, by simple random sampling using random sampling tables. Patients with type 1 diabetes mellitus, MODY or secondary diabetes were excluded. Other exclusion criteria were the presence of heart failure defined as New York Heart Association (NYHA) class II or above, kidney disease (estimate glomerular filtration rate <30 mL/min/1.73 m^2), liver cirrhosis or serum GPT levels at least twice the upper reference limit, hypothyroidism (TSH values >5 μU/L), neuropathies or a family history of neuropathies different from diabetic neuropathy, neoplastic diseases or any type of inflammatory disease. Alcohol consumption greater than 20 g per day in men or 15 g per day in women and Charcot arthropathy or having undergone any previous amputation were also exclusion criteria.

A total of 73 T2D patients were included: 36 patients with DPN and 37 patients without DPN. All participants included in the study continued with their usual antidiabetic treatment and their medications for other associated risk factors. The ethical committee of the HCUV approved the study and all the included participants provided written informed consent.

2.2 | Clinical data and anthropometric parameters

A comprehensive medical history was taken including age and sex, smoking habit, alcohol consumption, time of onset of diabetes, history of known cardiovascular disease, associated cardiovascular risk factors such as hypertension or dyslipidaemia and current medications.

A physical examination including vital tests such as patient's blood pressure and anthropometrical data such the body mass index and abdominal circumference was performed using standardised methods.[21] Ankle-brachial index (ABI) was obtained using a protocolised method calculating the ratio between the ankle systolic pressure and the brachial systolic pressure measured with a mercury sphygmomanometer and a Doppler ultrasound (Bi-directional Smartdop 20) after 5-10 minutes of rest. Trained health care staff from the Research Unit at the HCUV performed all the procedures according to research protocol.

2.3 | Diagnosis of DPN

The criteria used to make the diagnosis of DPN have been described previously.[21] DPN was established through the Semmes-Weinstein test (SW) by applying 10 g of force with a 5.07-gauge monofilament.[22] A total of six plantar sites were explored avoiding hyperkeratotic areas, three on each foot: the hallux, the 1st and the 5th metatarsals. A score of 1 was given when the patient perceived the force applied by the monofilament at each one of the sites and in at least two of three attempts. A score of 0 was given when the patient had no perception. Considering all six sites, a total score between 0 and 4 was considered pathological whereas a score of 5 or 6 was reported as non-pathological.

The Neuropathy Symptom Score (NSS) and the Neurological Disability Score (NDS) were also performed and used to assess the severity and clinical criteria for symptomatic DPN.[23]

2.4 | Biochemical examinations

Biochemical data were obtained from a blood test following a 10 hours overnight fasting period and performed in the Functional Testing Unit within the Endocrinology Department at the HCUV in optimal conditions. Blood samples were analysed in the Central Laboratory at the HCUV and in the Research Institute INCLIVA according to previously reported protocols.[24] Laboratory examinations were measured using standard procedures and included: full blood count, standard coagulation, liver enzymes, creatinine, glomerular filtration, fasting glucose, high sensitive C-reactive protein and lipid profile (total cholesterol, LDL cholesterol and triglycerides). High-density lipoprotein cholesterol was determined following polyanion precipitation method and ApoB and ApoA1 were measured by immunoturbidimetric tests. Glycosylated hemoglobin (HbA1c) was measured using a high-performance liquid chromatography (HPLC) assay NGSP certified.[25]

Plasma levels of circulating chemokines CXCL9, CXCL10 and CXCL11 were determined using DuoSet ELISA kits (Abingdon, UK) (see details in Ref. 18).

2.5 | Statistical analysis

Normal distribution of the variables was evaluated with the Kolmogorov-Smirnov test. Means for quantitative variables were compared between the two groups with the Student's t-test for unpaired data for variables with normal distribution and, for non-normal distribution variables, the Kolmogorov-Smirnov test was used as well as the Wilcoxon test for independent samples. The Variance test and the one-way ANOVA test were used to analyse three variables. Results are expressed as the mean ± the standard deviation (SD). *P* values < .05 were considered significant. Statistical analysis was performed using the Statistical Package for Social Sciences (SPPS) software v.12.1.3 (SPSS Chicago, IL).

3 | RESULTS

We included a total of 73 patients with T2D, 47 men and 26 women. Patient characteristics according to gender are shown in Table 1.

TABLE 1 General characteristics of type 2 diabetic patients according to gender

	Male (n = 47)	Female (N = 26)	*P*
Age (years)	63.4 ± 11.3	64.9 ± 10.4	NS
Evolution of diabetes (years)	12.5 ± 10.7	10.7 ± 9.1	NS
BMI (kg/m^2)	33.3 ± 5.6	30.3 ± 4.9	.034
Waist circumference (cm)	108.4 ± 10.9	106.6 ± 11.1	NS
SBP (mm Hg)	149.6 ± 16.9	147.0 ± 25.3	NS
DBP (mm Hg)	83.3 ± 13.5	82.4 ± 11.7	NS
Fasting glucose (mg/dL)	160.3 ± 49.7	160.9 ± 50.9	NS
HbA1c (%)	7.6 ± 1.6	7.9 ± 1.7	NS
eGFR (mL/min/1.73 m^2)	105.2 ± 47.1	94.7 ± 50.9	NS

Note: Results are expressed as the mean ± the standard deviation.

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimate glomerular filtration rate; NS, not statistically significant; SBP, systolic blood pressure.

All included patients were divided into two groups according to the presence or absence of DPN, diagnosed by the SW monofilament test: 36 patients with DPN (22 men and 14 women) and 37 without DPN (of which 25 men and 12 women).

Comparing patients' characteristics between the two groups, no statistically significant differences were found except for age (Table 2). Patients in the DPN group were older than patients in the group without DPN (68.5 ± 7.9 and 59.4 ± 11.6, respectively; $P < .001$). Patients in the group without DPN used predominantly oral hypoglycemic agents compared with patients in the group with DPN.

Biological parameters of the two groups are described in Table 3. No statistically significant differences were observed between the two groups when comparing fasting glucose and glycosylated hemoglobin. However, the estimated glomerular filtration rate was significantly lower amongst patients with DPN than amongst patients without DPN (87.5 ± 35.4 and 115.4 ± 55.7, respectively; $P = .013$). No differences were found in the lipid profile between the two groups. Considering the classification of patients with DPN, no significant differences were observed between the classification performed using the monofilament test results and the ones carried out with the NSS and NDS scales (Table 2).

The analysis of serum concentrations of chemokines is shown in Table 3. Serum levels of chemokine CXCL10 were significantly higher amongst patients with DPN than amongst patients without DPN (57.6 ± 38.3 and 38.1 ± 33.4 pg/mL, respectively; $P = .034$). To the same extent, serum levels of chemokine CXCL9 were also higher amongst patients with DPN (188.1 ± 72.7 and 150.4 ± 83.6 pg/mL, respectively). However, in this case, no statistical significance was reached ($P = .06$). No significant differences were found between the two groups in relation to CXCL11 serum levels. Furthermore, patients with DPN presented significantly higher plasma values of high sensitivity C reactive protein compared with that observed in patients without DPN. We did not find differences in all these parameters when we analysed them according to the gender.

TABLE 2 Clinical and anthopometrical parameters in type 2 diabetic patients according to the presence of diabetic polyneuropathy

	DPN + (n = 36)	DPN − (n = 37)	*P*
Male (n)	22	25	NS
Female (n)	14	12	NS
Age (years)	68.5 ± 7.9	59.4 ± 11.6	<.001
Evolution of diabetes (years)	13.6 ± 10.8	9.8 ± 9.4	NS
BMI (kg/m^2)	30.6 ± 4.6	31.9 ± 5.8	NS
Waist circumference (cm)	106.7 ± 9.6	108.7 ± 12.7	NS
SBP (mm Hg)	151.7 ± 20.5	145.7 ± 19.7	NS
DBP (mm Hg)	80.7 ± 12.7	85.2 ± 12.6	NS
Treatment for hypertension, n (%)	26 (75)	23 (65)	NS
Hypoglycemia treatment			
Diet (n)	2	3	
Oral hypoglycemic agents (n)	15	27	
Insulin (n)	9	2	
Insulin + OHA (n)	10	5	.021
Documented myocardial infarction, n (%)	18 (50)	12 (32)	NS
ABI	0.84 ± 0.17	0.88 ± 0.15	NS
Statins, n	22	25	NS
NDS n, (%)			
0	10 (27.8)	29 (78.4)	
1	11 (30.6)	7 (18.9)	
2	12 (33.3)	1 (2.7)	
3	3 (8.3)	0 (0.0)	<.001
NSS n, (%)			
0	19 (52.8)	32 (86.5)	
1	6 (16.7)	3 (8.1)	
2	3 (8.3)	2 (5.4)	
3	8 (22.2)	0 (0.0)	<.001

Note: Results are expressed as the mean ± the standard deviation.

Abbreviations: ABI, ankle brachial index; BMI, body mass index; DBP, diastolic blood pressure; DPN, diabetic polyneuropathy; NDS, neurological disability score; NS, not statistically significant; NSS, neuropathy symptom score; SBP, systolic blood pressure.

TABLE 3 Biological parameters in type 2 diabetic patients according to the presence of diabetic polyneuropathy

	DPN + (n = 36)	DPN − (n = 37)	*P*
Fasting glucose (mg/dL)	162.5 ± 48.6	158.6 ± 51.6	NS
HbA1c (%)	7.9 ± 1,6	7.5 ± 1.6	NS
eGFR (mL/min/1.73 m^2)	87.5 ± 35.4	115.4 ± 55.7	.013
TC (mg/dL)	191.1 ± 37.0	185.5 ± 46.4	NS
TG (mg/dL)	151.4 ± 71.3	144.1 ± 89.3	NS
cHDL (mg/dL)	49.6 ± 13.3	47.6 ± 11.3	NS
cLDL (mg/dL)	112.1 ± 28.6	109.4 ± 42.5	NS
hsCRP (mg/L)	10.1 ± 2.7	3.9 ± 3.3	.01
CXCL10 (pg/mL)	57.6 ± 38.3	38.1 ± 33.4	.034
CXCL9 (pg/mL)	188.1 ± 72.7	150.4 ± 83.6	.06
CXCL11 (pg/mL)	59.0 ± 50.8	43.0 ± 36.5	NS

Note: Results are expressed as the mean ± the standard deviation.

Abbreviations: DPN, diabetic polyneuropathy; eGFR, estimate glomerular filtration rate; HDLc, High-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; LDLc, low-density lipoprotein cholesterol; NS, not statistically significant; TC, total cholesterol; TG, triglycerides.

4 | DISCUSSION

This preliminary study investigating the relationship between CXCL9, 10 and 11 chemokines and DPN has some important findings. When comparing patients with T2D with and without DPN classified using the monofilament test, we found that serum concentrations of chemokine CXCL10 were significantly higher amongst patients with DPN. We also found that serum CXCL 9 and CXCL 11 levels were raised in the DPN group; however, no statistical significance was reached. To the best of our knowledge, this is an original work showing the direct link between elevated circulating CXCL10 levels and the presence of DPN.

In the present study, the diagnosis of polyneuropathy was based on the response to the monofilament test as it has already been published by our group and reported previously in the literature.[21,26,27] Lee et al described a sensitivity of 93% and a specificity of 100% when performing a 10-point monofilament examination and comparing it to nerve conduction studies. In their work, the diagnostic threshold was defined as the inability to perceive at least four sites out of the ten evaluated.[28] Nevertheless, Baraz et al reported that in terms of sensitivity and specificity, there were no significant differences between the monofilament testing in three to four points and the monofilament testing in 10 points.[29] In the present study, we carried out the DPN classification using the monofilament test in three points and its results were also in concordance with the NDS and NSS scales performed. Therefore, we retained this method as a valid tool to screen for DPN.

Regarding CXCL10, we found that serum concentrations were higher amongst diabetic patients with DPN. Chemokine CXCL10 is functionally classified as an inflammatory chemokine. The role of the CXCL10/CXCR3 axis in the immunopathogenesis of type 1 diabetes has been extensively studied with the blockade of the CXCL10/CXCR3 system considered as a promising therapeutic target.[30,31] Chemokine CXCL10 has also been suggested to be a potential biomarker of inflammation and angiogenesis in T2D patients. In fact, a previous study involving 100 patients with T2D and 150 healthy controls, found that serum values of CXCL10 and CXCL11 were increased amongst the T2D patients when compared to healthy controls.[32]

Furthermore, it was previously shown that hyperglycaemia promoted the expression of CXCR3 in activated CD8+T lymphocytes and the expression of CXCL9, CXCL10, CXCL11 on Schwann cells, leading to Schwann cells apoptosis. This study suggests that the CXCR3 and its ligands (CXCL19, CXCL10, CXCL11) participate in the development of DPN and that this axis could represent a novel therapeutic target for preventing DPN.[33]

However, fewer studies exist exploring the role of CXCL10 in the DPN in T2D patients. A recent study based on the German prospective cohort the population-based Cooperative Health Research in the Region of Augsburg (KORA) involving 127 subjects with incipient DPN and 386 without, evaluated the association between chronic inflammatory biomarkers and the development of DPN. Amongst all the biomarkers analysed, it was found that three chemokines: CCL7, CXCL9 and CXCL10 were independently associated with DPN after adjustment and correction for multiple testing. It was also found that, when the investigators tested their effect in vitro on human neuroblastoma cells, those chemokines had direct neurotoxic effects.[34] This study comes to support our findings suggesting further research evaluating the role of CXCL9 and CXCL10 in the pathogenesis of DPN should be undertaken.

Our study presented several limitations. Since it is a preliminary study, a limited number of patients were included. Additionally, conduction nerve studies were not conducted in the study participants to establish the diagnosis and severity of DPN. Moreover, because of the inherent nature of the study, no causal effect can be established. However, our findings will be hopefully complemented with further research studies.

To conclude, in our work, DPN in T2D patients was associated with a significant increase in CXCL10 circulating levels suggesting a role of this chemokine in the DPN. These preliminary results open a door to further research to gain a better understanding of the complex pathogenic pathways implicated in the development of DPN. DPN has a major clinical impact and often develops in predisposed subjects despite well-controlled diabetes. Novel biological markers that allow for early detection and therapeutic strategies in order to reverse and prevent the disease should be investigated.

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DISCLOSURES

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Laura Piqueras and José T. Real contributed to the conception and design of the study; Pilar Ascaso, Ana Palanca, Sergio Martinez-Hervás and Juan F. Ascaso contributed to the acquisition and analysis of data; María Jesús Sanz and Laura Piqueras performed the laboratory experiments; Sergio Martinez-Hervás, María Jesús Sanz, Juan F. Ascaso, Laura Piqueras and José T. Real contributed to interpretation of the results; Pilar Ascaso, Ana Palanca, Sergio Martinez-Hervás, María Jesús Sanz, Juan F. Ascaso, Laura Piqueras and José T. Real contributed to drafting and revision of the text. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed in the current study are available from the corresponding author on a reasonable request.

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