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**CLINICAL AND PARACLINICAL CORRELATIONS IN
DIABETIC DISTAL SYMMETRIC POLINEUROPATHY**

ABSTRACT

PhD supervisor:

Professor Maria MOȚA, MD, PhD

PhD student:

Cristina MUNTEAN

CRAIOVA

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INTRODUCTION

Diabetes mellitus (DM) is a global health problem. It is estimated that by 2030, diabetes will affect about 366 million people (1). In Romania the proportion of patients with diabetes will reach 7.8% of the population (2).

According to data published by the American Diabetes Society, more than 50% DM patients present with peripheral nervous system involvement (3). Moreover, DM is the leading cause of neuropathy, and the association between the two has been known for more than 100 years (4). Diabetic symmetric polyneuropathy (DSP) seems to be the most frequent complication found in association with peripheral nerve damage (5–7). It is known to be a distal symmetric sensory-motor, length-dependent form, caused by metabolic changes and microvascular damage.

DSP causes long-term disability firstly due to sensory symptoms, mainly neuropathic pain, and secondary due to high risk of foot ulceration, and appearance of Charcot arthropathy (8). Thus, there is a need for a better assessment of diabetic neuropathy during early stages, if possible using nerve conduction studies (6).

STATE OF KNOWLEDGE

1. PATHOGENESIS OF DIABETIC NEUROPATHY

DSP pathophysiology is incompletely elucidated, but several factors such as chronic exposure to hyperglycemia, increased level of lipids, obesity, high blood pressure, smoking, alcohol abuse and genetic factors seem to play an important role (9,10). Chronic exposure to high blood glucose levels is strongly correlated with peripheral nerve lesion severity (10,11). Hyperglycemia leads to some metabolic changes that are extremely harmful for nerve fibers: increased polyol pathway activity, upregulation of advanced glycation end products (AGEs), and increased oxidative stress. Excess glucose in nerve cells is diverted via polyol pathway, where the formation of sorbitol and fructose will reduce the level of myo-inositol and decrease

Na⁺/K⁺ ATPase activity, with secondary impairment of axonal transport and peripheral nerve structure (12–14). At the same time glucose reduction to sorbitol by aldose reductase is accompanied by depletion in nicotinamide adenine dinucleotide phosphate (NADPH), main cofactor in the reaction. Therefore, NADPH will cause decreased glutathione activity and further increase in oxidative stress. Since NADPH is also a cofactor of nitric oxide synthase, its deficit will lead to low levels of nitric oxide. Thus, in addition to damaged nerve structures, these changes will cause endothelial vasodilation and ischemia (14). Moreover, the formation of AGEs by coupling glucose to proteins, nucleotides and lipids, will contribute to disruption of neuronal integrity through interaction with nerve cell metabolism and axonal transport (15–18).

2. CURRENT CONCEPT IN DIABETIC NEUROPATHY

DSP is the main complication involving peripheral nervous system found in association with type 1 and type 2 diabetes. It starts insidious, distally in the lower limbs and slowly progresses during several years toward more proximal parts; it may involve the upper limbs, although it often remains localized to the feet.

Clinical impairment of patients is usually mild or moderate, though 60% of them present with a pain predominant form of DSP (8). Involvement of thin myelinated and unmyelinated fibres causes complaints such as "tinglings", "prickings", "burning sensations", "cold feet" or "electrical discharges". Nocturnal muscle cramps are common symptoms. Motor involvement, consisting in muscle weakness and atrophy of ankle plantar and dorsal flexors, seems to be less important than sensitive damage, and it is closely related to severity of DSP. Patients examination reveals symmetric sensory loss with a stocking-and-glove distribution in the distal extremities, abolished or diminished ankle reflexes (sometimes involving knee reflexes) and vasomotor disturbances. Sometimes allodynia may be present.

Criteria for DSP diagnosis were recently established, based on clinical history, physical examination and nerve conduction studies (5). The following types were identified:

- Confirmed DSP: presence of symptoms (tingling, stabbing, burning pain, decreased sensitivity) and signs of neuropathy (distal symmetrical

hypoesthesia or diminished/abolished ankle reflexes) in association to abnormal nerve conduction results;

- Probable DSP: presence of symptoms and signs of neuropathy with normal conduction studies;
- Possible DSP: presence of either symptoms or signs of neuropathy;
- Subclinical DSP: absence of symptoms and signs of neuropathy, though altered nerve conduction studies.

3. INVESTIGATION OF DIABETIC NEUROPATHY

The primary step in diagnosing DSP consists in excluding other possible causes of neuropathy. A study that was conducted on patients with diabetes found that 53% of investigated subjects presented other causes of neuropathy. The most frequently reported were: alcohol abuse, neurotoxic medication and vitamin deficiency (B1, B12, B6) (19).

Nerve conduction studies are a reliable and accurate measure of DSP. This measure reports the presence of diabetes axonal neuropathy at diagnosis in 29-70% type 1 diabetes patients and 45-60% type 2 diabetes. These numbers increase proportionally to diabetes duration (20) and low glycemic control (21).

Skin biopsy is another important method in DSP assessment as it provides information about intraepidermal small nerve fiber density (not accessible to electroneuromiography). Highly marked reduction of these fibers was found even in patients with normal nerve conduction studies (22–24).

Several other methods have been developed for facilitating and improving DSP diagnosis. They can be grouped as follows: tests that assess large nerve fibers such as Vibratip and Ipswich Touch Test, and tests that investigate small nerve fibers such as Sudoscan, Neuropad, corneal confocal microscopy and NeuroQuick. Although many of them turned out to be more effective than tests used in usual practice, these methods are still waiting to be validated for routine use (25).

4. TREATMENT IN DIABETIC NEUROPATHY

Metabolic control remains the only effective method for prevention and intervention in patients with DSP (26) together with neuropathic pain treatment with antidepressants and anticonvulsant drugs.

PERSONAL CONTRIBUTIONS

5. MATERIAL AND METHODS

There is a paucity of reports on DSP in Romania; no complete description and no prevalence are available at the moment. For these reasons the present study focused on finding the rate of DSP-“Toronto” diagnosis criteria were applied (4)-in our cohort, secondly in characterizing it, and thirdly in proposing a protocol for diagnosis and evaluation of diabetes associated neuropathy and finding an easy-to-apply method in everyday practice.

We conducted a cross-sectional study in the Diabetes, Nutrition and Metabolic Diseases Clinic, County Hospital of Craiova, Romania, between April 2013-September 2014. Fifty one type 2 diabetic patients (group I), followed in the clinic, were enrolled after having given written consent. At the same time 20 healthy volunteers formed the control group (group II).

Patients were selected according to the following inclusion/exclusion criteria:

Inclusion criteria: 1) patients diagnosed with type 2 diabetes (27,28), 2) written consent to answer questions and cooperate with the examiner during clinical and paraclinical examination, as well as allowing us to use data for scientific purposes.

Exclusion criteria: 1) patient refusal to sign the informed consent, 2) patients that have worked in toxic environments for a period longer than 6 months, 3) alcohol abuse, 4) end stage terminal diseases (chronic kidney disease, hepatic dysfunction, cancer, hematological disease) or infections (HIV, hepatitis C), 5) vitamin deficiency (B1, B6, B12, E, folic acid) or celiac disease, 6) treatment with antibiotics known to cause neuropathy, 7) other causes that could lead to symptoms resembling diabetic neuropathy.

The 2 groups underwent a thorough clinical examination for symptoms and signs of neuropathy. Nerve conduction studies were also performed. Overall appreciation of neuropathy was realized using 2 composite scores: Michigan Neuropathy Screening Instrument-MNSI (29–31) and Toronto Clinical Scoring System-TCSS (32). Finally, fasting blood glucose and glycated hemoglobin (HbA1c), renal and liver function, vitamin B12, thyroid hormones, protein electrophoresis together with other routine blood tests (complete blood count, erythrocyte sedimentation rate) were assessed in all patients. Retinopathy was evaluated through a funduscopy exam by an ophthalmologist.

Statistical analysis was performed using GraphPad Prism version 6.0.

6. RESULTS

The study included 51 type 2 diabetic patients and 20 healthy volunteers, representing group I and group II respectively. Group I consisted of 23 men (45.10%) and 28 women (54.90%), while 9 men (45%) and 11 women (55%) were part of group II. Mean age at evaluation was 60.23 ± 13.40 years for group I and 53.05 ± 7.72 years for group II.

Mean diabetes duration was 3.78 ± 3.53 years (range 0-11). No significant difference in diabetes duration was noted between men and women (3 years for women and 4.73 years for men; $p=0.08$); 60.78% of group I were treated with oral antidiabetic agents (ADO), insulin alone or combined therapy (insulin and oral agents), while the other 39.21% were non-treated patients either with recent diagnosed diabetes <1 year (29.41%) or with treatment interruption (9.80%). Approximately 87.5% of the group received concomitant antihypertensive treatment; no other associated comorbidities were noted.

Mean neuropathic symptoms duration at diagnosis was 2.47 ± 2.17 years, with no sex differences ($p=0.21$). About half of the group (41.17%) reported presence of neuropathic symptoms before diabetes diagnosis. Most common referral sensory symptoms were: numbness-54.90%, tinglings-37.35%, burning sensation-35.29%, while a small number of patients reported presence of allodynia-31.37%. Muscle cramps were found in 37.25% of group I. Clinical examination revealed: diminished

vibratory sensation-64.70%, sensory ataxia-56.86%, tactile hypoesthesia-58.82%, and motor deficit-19.60%. Symmetrical diminished/abolished ankle reflexes were present in 56.86% cases. Sensory loss was symmetric and distally located in the lower limbs. No signs and symptoms were noted in the upper limbs. Group II had a normal clinical exam.

Median value for MNSI questionnaire was 2 and 50% patients scored between 1 and 3 points. Pathological values (≥ 4) were found in 23.54% cases. MNSI questionnaire scores were strongly correlated with neuropathic symptoms duration ($r=0.403$; $p=0.003$). MNSI exam results ranged between 0-9 points, 50% patients having scored between 1 and 4 points. Pathological values for MNSI exam (>2) were found in 50.98% of group I.

TCSS results ranged between 0-8 points in patients group. Mean value was 4.08 ± 2.38 and only 5.88% patients had results ≥ 8 , associated with the presence of DSP. None of the 2 composite scores applied in the study, TCSS and MNSI, were not correlated with diabetes duration or with HbA1c values; this result was not unexpected as diabetes duration does not coincide with its onset, and HbA1c is only a marker of short term glycemic control.

All patients underwent nerve conduction studies, and their results were compared to those obtained by the control group. Sensory and motor response were altered in both upper and lower limbs. The most important motor conduction abnormalities were decreased fibular ($p=0.01$) as well as ulnar amplitude ($p<0.0001$) and long distal latency in median nerve ($p=0.008$), the latter being compatible with the presence of carpal syndrome in 84.31% patients. Sensory nerve examination found significantly reduced amplitude, with almost 3/4 of the response recorded in the control group, for all tested nerves. Contrary to motor response that was elicited in all patients, sensory response was abolished in the lower limbs in 31.37% cases.

Interestingly, MNSI as well as TCSS results were correlated with nerve conduction studies parameters. Patients with high scores at MNSI exam had diminished motor conduction velocity for ulnar, median and fibular nerve ($r=-0.482$, $p=0.004$; $r=-0.408$, $p=0.003$; $r=-0.294$, $p=0.038$), diminished tibial amplitude ($r=-0.375$, $p=0.007$) and diminished conduction velocity and amplitude for sural nerve ($r=-0.407$, $p=0.003$; $r=-$

0.330, $p=0.019$). For upper limbs MNSI exam was also correlated with sensory conduction velocity for ulnar nerve ($r=-0.328$, $p=0.022$). TCSS results were only influenced by sensory response of the lower limbs, represented by sural nerve conduction velocity and amplitude ($r=-0.443$, $p=0.008$; $r=-0.572$, $p=0.0004$).

Laboratory results found a mean fasting blood glucose of 181.8 ± 78.22 mg/dL and a mean HbA1c level of $9.17\pm 2.60\%$ (range 5.7-14.1%); poor glycemic control (HbA1c $<7\%$) was found in 27.45% patients. When comparing clinical and electrophysiological characteristics of patients with good glycemic control (HbA1c $<7\%$) to those of patients with poor glycemic control (HbA1c $\geq 7\%$), we observed a more severe form of DSP in the latter. Main features were presence of motor deficit and allodynia together with worse sensitive and motor electric values.

7. DISCUSSIONS

DSP has a high prevalence worldwide. According to literature it varies widely, between 10%-75% (33–36) depending on the chosen criteria for patients selection and evaluation. Taking into consideration clinical and electrophysiological characteristics included in the so called „Toronto” diagnosis criteria (5), we found a confirmed DSP prevalence of 72.54%.

Patients enrolled in this study had classical DSP, and only 27.45% of them met optimal glycemic target (37). Main complaints were numbness, tinglings, muscle cramps and burning sensations; 54.90% presented more than 2 symptoms. Painful DSP form was only revealed in 11.76% cases. The most common sign was vibratory hypoesthesia. Pain and temperature sensations, considered to be an important risk factor for developing diabetic foot (38), were found to be normal. Motor deficit was observed only in those with HbA1c $\geq 7\%$. Diabetic retinopathy was also associated with low glycemic control, as already described in other studies (39).

Nerve conduction studies revealed notably reduced parameters in patients compared to healthy volunteers. Mainly sensory response was modified in patients with good glycemic control, while patients with poor glycemic control presented additional diminished motor response in all tested nerves. Ulnar and fibular

amplitude were preferentially diminished in patients with HbA1c $\geq 7\%$. Recently published data consider ulnar nerve as a marker of poor glycemic control (40).

Nerve conduction studies remain an expensive diagnosis tool, which is why several studies tried to compare their efficiency to that of clinical scores. One such study, conducted in Turkey, compared MNSI score to electroneuromyography and found a prevalence of 32.1% and 46.2% respectively (30). Similar prevalence was found by an Italian group that applied the same score (41). Differently from this data, we provided a higher DSP prevalence of 50.98% with MNSI. Moreover, we correlated for the first time nerve conduction parameters with MNSI values, and we identified that there seems to be a strong relation between MNSI exam and motor and sensory electric values recorded in the lower limbs.

TCSS, like MNSI, proved to be correlated with nerve conduction studies, but opposite to it, its relations was only with sensory lower limb response.

Despite the fact that we cannot translate our results to other centers and our cohort is representative only for one region, we could expect to find high rate of DSP in the whole country. Therefore we propose a similar protocol in other centers in Romania, which should include a composite score and if possible nerve conduction studies. MNSI could be the key to diagnostic and follow-up of patients with diabetes, as it is an easy-to-apply score with a strong correlation to electroneuromyography.

8. ORIGINAL PART

Little is known about DSP in Romania and there are no complete clinical and electrophysiological descriptions available at the moment. Composite scores for global appreciation of neuropathy, frequently used in international practice, are yet to be implemented in our country. Furthermore, although MNSI was often applied in several international clinical trials that investigated patients with diabetes, it was never correlated with electrophysiological parameters.

Our aim was therefore to broaden the knowledge regarding DSP in the country and abroad. We realized for the first time a thorough evaluation of DSP in patients with type 2 diabetes, including international validated clinical scores, as well as nerve

conduction studies. Thus we established DSP prevalence in our cohort and we described clinical-electrophysiological phenotypes associated with glycemic control. We identified a high prevalence of DSP and of poor glycemic control, responsible for more severe forms of neuropathy. Noteworthy was the fact that TCSS and MNSI have proven to be strongly correlated with electrophysiological values. These results complement international data regarding MNSI score.

The present study highlights the utility of DSP composite scores in daily practice for diagnosis as well as follow-up of patients presenting diabetic neuropathy.

9. CONCLUSIONS

1) When applying clinical and electrophysiological criteria to our cohort we found a DSP prevalence of 72.54%.

2) Patients with poor glycemic control present a more severe clinical and electrophysiological form of DSP; motor deficit and neuropathic pain are more frequently found in these patients.

3) Composite scores for global appreciation of diabetic neuropathy, mainly MNSI, are strongly correlated with nerve conduction studies results.

4) These scores provide a rapid and objective appreciation of DSP; they should therefore be implemented in daily clinical practice.

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