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The effectiveness of electroneuromyography in the early diagnosis of diabetic foot development

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ABSTRACT

Objective: Diabetic foot is one of the basic causes of lower extremity amputation. The aim of this study is to determine which examination method of nerve conduction disorders may be used predominantly for early diagnosis of diabetic foot development in the follow-up of diabetic patients.

Material and Method: The study consists of 3 different groups (n=150) of patients diagnosed with type 2 diabetes (DM). Group 1; 50 patients with diabetic foot complications (DFC+), Group 2; 50 patients without diabetic foot complications and with polyneuropathy (DFC-/PNP+), Group 3; 50 patients without diabetic foot complications and without polyneuropathy (DFC-/PNP+). Diabetic foot wounds were grouped by PEDIS classification. A total of 150 DM patients were included. The age, sex, diabetes duration, blood glucose levels, HbA1c measurements, and standard electroneuromyography (ENMG) findings were compared.

Findings: Age, sex, diabetes duration, blood glucose, HbA1c values and electroneuromyography (ENMG) for nerve amplitude, velocity and latency results were compared among the groups. A significant statistical difference was found between three groups when age, sex, HgbA1c, fasting blood glucose, diabetes duration was evaluated (p<0.05). All DFC+ patients had PNP+. In the DFC+ group, unlike DFC-/PNP+ group, the motor nerves of the lower extremities were also involved. Tibial nerve velocity was lower than normal in DFC+ patients and normal in other groups (p<0.05). A statistically significant difference was found in peroneal nerve conduction velocity between the DFC+ group and the DFC- groups (p<0.05). Peroneal nerve conduction velocity was not statistically significant between DFC-/PNP+ and DFC-/PNP- groups (p>0.05). Peroneal nerve conduction velocity was lowest in the DFC+ group. This factor was considered as a risk factor for DFC development.

Conclusions: The slowdown in peroneal nerve conduction velocity and the increase in diabetes duration were the primary risk factors for diabetic foot development, and the decrease in tibial nerve velocity was also considered as significant. This study showed that the involvement of motor nerve conduction in the lower extremity was considered as a signal for diabetic foot development.

Keywords: Diabetic foot, polyneuropathy, Electroneuromyography

INTRODUCTION

The prevalence of diabetes and diabetes complications has become an increasing global health problem in the world (1). Peripheral neuropathy may cause several complications, including chronic pain, foot ulcers, foot infections and amputations. The prevalence of diabetic foot ulcers in the world is 6%. Approximately 25% of diabetic people experience foot ulcers at least once in their lifetime (2). Diabetic foot ulcers are one of the main causes of lower extremity amputation. Diabetic peripheral neuropathy, history of foot ulcers, structural foot deformity, peripheral artery disease, visual impairment, diabetic nephropathy, poor glycemic control and smoking history are considered as high risk for the development of foot ulcers. It is estimated that approximately 14–24% of people with foot ulcers will require amputation. Patient training has been shown to decrease the incidence of foot ulcers and amputations by up to 50%. Therefore, early diagnosis and treatment is fundamental (3).

The role of neuropathy as a diabetes complication in the development of diabetic foot ulcers is important. Diabetic peripheral neuropathy is the most common form of neuropathy worldwide. Neuropathy is associated with pain, sensory impairment, impairment in quality of life, restrictions in daily activities and depression (4). There are several forms of diabetic peripheral neuropathy. The



most common type is distal symmetrical polyneuropathy. It constitutes approximately 75% of all diabetic neuropathies (5). Distal symmetrical polyneuropathy causes neuropathic pain symptoms in approximately 10-30% of affected patients (6). It may be characterized as pain, burning, drowsiness, hyperesthesia. It usually affects the lower legs and feet (7). Other forms of diabetic peripheral neuropathy include mononeuropathies and radiculopathy. Mononeuropathies may affect the median, ulnar, radial, tibial, sural, and peroneal nerves (8).

The effect of nerve conduction impairment in diabetic foot development is known, however, a few studies have examined the severity of the impairment on the nerve. The aim of this study is to compare electroneuromyography (ENMG) findings in diabetic patients, to determine the condition of the most affected nerve due to diabetic foot development and to follow up the electrophysiological changes indicating a risk and to take the required measures.

MATERIAL AND METHOD

Ethics committee approval for the study was obtained from Atatürk University Faculty of Medicine Clinical Research Ethics Committee (permission granted: 28.05.2020, decision no: B.30.2.ATA.0.01.00/278). All procedures were performed adhered to the ethical rules and the Helsinki Declaration of Principles.

The study consisting of 3 different patient groups diagnosed with type 2 diabetes (DM) was designed prospectively. Group 1; 50 patients followed up in the Infectious Diseases clinic, diagnosed with type 2 diabetes and diabetic foot complication (DFC+), Group 2; 50 patients admitted to neurology outpatient clinic with polyneuropathy and without diabetic foot complications (DFC-/PNP+), Group 3; 50 patients admitted to neurology outpatient clinic without polyneuropathy and without diabetic foot complication (DFC-/PNP-). A total of 150 DM patients were included. The age, sex, diabetes duration, blood glucose levels, HbA1c measurements, and standard electroneuromyography (ENMG) findings were compared. Motor and sensory action potentials latency (peak delay time), velocity, amplitude data were noted in the median, ulnar, tibial, peroneal and sural nerves by ENMG procedure. Foot wounds of DFC + patients were divided into four groups by PEDIS classification. Demographic characteristics, blood glucose levels, and HbA1c measurements of the patients were compared between the groups. Several rating systems by the ulcer condition were used for the classification of diabetic foot ulcers. In this study, PEDIS classification was used by the "Diabetic Foot International Study Group" to evaluate ulcer perfusion, width, depth, infection and sensory loss (9).

Nihon Kohden Neuropack M1 ENMG measurement unit was used in our study. The patients were prepared by resting for 15 minutes at 22–24°C room temperature before the examination. In all cases, skin resistance was minimized by cleaning the skin using alcohol before the examination. In the electrodiagnostic study; sensory and motor nerve action potentials of the median and ulnar nerves in the upper extremities, peroneal, posterior tibial motor nerve action potentials and sural nerve action potential in the lower extremities were measured. The nerve conduction velocity, amplitude and latency values were considered as normal by the cut-off values of our local hospital and the literature (10). All nerve conduction studies were performed by the same investigator.

Statistical analysis was performed by SPSS 22.0 package program. Kolmogrof Smirnow test was used to evaluate the compliance of the data to normal distribution. When the numerical data were compared, Anova analysis was used if the number of groups was more than three in normal distributions and Kruskal Wallis analysis was used in non-normal distribution. Post-hoc Tukey analysis was used in the groups distributed homogeneously after Anova test if the data differed significantly between the groups. Mann-WhitneyU test was used for binary comparisons in the groups when data were not normally distributed. Pearason correlation test was used to evaluate categorical data. p <0.05 was considered statistically significant in all tests.

RESULTS

The study results showed that all patients with DFC development had PNP+. Given the mean age of the patients, a statistically significant difference was found between three groups. This difference was found between two DFC-/PNP + and DFC-/PNP- groups. The mean age of the DFC + patients was (62.06 ± 10.48) . The mean age of the patients with DFC-/PNP+ (62.84±11.7) was significantly higher than the patients with DFC-/ NP-(57±11.08). A significant statistical difference was found between three groups in terms of sex (p <0.05). The male ratio was higher in DFC patients than the other two groups. HgbA1c levels were statistically significantly different between three groups. HgbA1c level was the highest in the DFC+ group, and the lowest in the DFC-/ PNP- group (p <0.05). The blood glucose level was the highest in the DFC+ group, the lowest in the DM +/ PNP- group. DM duration was significantly different between three groups (p <0.05). This difference was found between DFC+ group and other two DFC- groups. Disease duration was determined as the highest causative parameter for DFC development (Table 1).

DFC+ n=50	DFC-/PNP+ n=50	DFC-/PNP- n=50	р
62.06 ± 10.48^{ab}	$62.84{\pm}11.7^{a}$	57±11.08 ^b	0.019*
			0.000***
13(26%)	21(42%)	40(80%)	
37(74%)	29(58%)	10(20%)	
9.63±2.27	8.32±1.56	7.56±2.11	0.000**
245.08±95.29	169.04±53.08	151.80±81.22	0.000**
11.36±5.96	7.34±6.34ª	4.80 ± 4.49^{a}	0.000*
	62.06 ± 10.48^{ab} $13(26\%)$ $37(74\%)$ 9.63 ± 2.27 245.08 ± 95.29	$\begin{array}{cccc} 62.06 \pm 10.48^{ab} & 62.84 \pm 11.7^{a} \\ & & & & \\ & & & & \\ & & & \\ & & $	$\begin{array}{c ccccc} 62.06 \pm 10.48^{ab} & 62.84 \pm 11.7^{a} & 57 \pm 11.08^{b} \\ & & & & & & \\ & & & & & & $

Our study showed that the latency values were within normal limits in all nerve parameters of the upper and lower extremities examined by ENMG in three groups, except for the prolonged median motor latency in DFC group. Nerve conduction studies showed that the patients in DFC+ group had sensorial polyneuropathy in the upper extremities and sensor-motor axonal type polyneuropathy in the lower extremities. Sensory axonal type polyneuropathy was present in the upper and lower extremities in DFC-/PNP + group. In DFC+ group, unlike DFC-/PNP+ group, the motor nerves of the lower extremities were also involved (**Table 2**).

In our study, amplitudes and velocities of all motor and sensory nerve parameters examined in the upper and lower extremities were lowest in DFC+ group and the highest in DFC-/PNP- group. A statistically significant difference was found between three groups in amplitude and velocities of all nerves except median motor amplitudes (p<0.05). Tibial nerve velocity was lower than normal in DFC+ patients and normal in other groups. Tibial nerve velocity was lowest in DFC+ group and was statistically significantly lower than the other two groups. A significant difference was also found between DFC-/ PNP+ and DFC-/PNP- groups (p<0.05). A statistically significant difference was found in peroneal nerve conduction velocity between DFC+ group and DFC-/ PNP+ and DFC-/PNP- groups (p<0.05). Peroneal nerve conduction velocity was lowest in the DFC+ group (Table 2). However, peroneal nerve conduction velocity was not statistically significant between DFC-/ PNP+ and DFC-/PNP- groups (p>0.05). No statistically significant correlation was found between peroneal velocity and DM duration, HgbA1c level, blood glucose, and age in DFC+ patients (Pearson's correlation test p < 0.05).

	DFC+ med±sd	DFC- PNP+ med±sd	DFC-PNP- med±sd	р
Median nerve motor latency (msec)	4.13±1.38	3.83±1.06	3.32±0.35	0.000**
Median nerve motor amplitude (mV)	7.63±3.57	7.57±1.84	8.39±1.55	0.106**
Median nerve motor velocity (m/sec)	47.64±10.11	54.08±5.07	60.50±8.26	0.000**
Median nerve sensory latency (msn)	2.61±4.28	2.95±1.14	3.12±0.43	0.433**
Median nerve sensory amplitude (µV)	5.22±6.27	11.02±8.53	26.86±7.85	0.000*
Median nerve sensory velocity (m/sec)	24.21±23.11	42.11±16.85	54.32±4.83	0.000**
Ulnar nerve motor latency (msec)	3.02±0.69	2.49 ± 0.34	2.25±0.20	0.000**
Ulnar nerve motor amplitude (mV)	8.43±3.93	10.71±5.90	13.06±3.39	0.000*
Ulnar nerve motor velocity (m/sec)	46.62±8.93	54.08±8.57	59.88±9.13	0.000**
Ulnar nerve sensory latency (msn)	1.64 ± 1.58	2.29±0.83	2.03±0.96	0.692**
Ulnar nerve sensory amplitude (μV)	4.40±6.25	17.02±18.02	25.60±8.86	0.000*
Ulnar nerve sensory velocity (m/sn)	25.27±24.95	46.45±18.55	58.36±7.70	0.000**
Peroneal nerve latency (msn)	3.72±1.81	3.63±0.83	3.27±0.70	0.000**
Peroneal nerve amplitude (mV)	3.11±2.34	5.30±6.89	5.32±2.26	0.017*
Peroneal nerve velocity (m/sec)	46.04±23.52	52.27 ± 10.22^{b}	56.64±15.26 ^b	0.027**
Posterior tibial nerve latency (msn)	3.73±3.16 ^a	3.84±1.32ª	3.31±0.55	0.007**
Posterior tibial nerve amplitude (mV)	2.10±2.4	5.37±2.97	11.67±3.84	0.000**
Posterior tibial nerve velocity (m/sec)	24.59±21.18	43.04±11.65	47.98±8.31	0.000**
Sural nerve latency (msec)	0.67±1.98	1.67±1.29	2.39±0.39	0.000**
Sural nerve amplitude (μV)	1.80 ± 4.92	3.95±3.64	20.58±7.69	0.000**
Sural nerve velocity (m/sn)	6.38±18.32	30.42±24.35	64.86±12.83	0.000*

When diabetic foot wounds were grouped by PEDIS classification, the highest was PEDIS 3 (42%), the lowest was PEDIS 1 (10%). Wound classification showed that no significant difference was found in age, sex, DM duration and fasting blood glucose between four groups, however, a statistically significant difference was found in HgbA1c levels. HbA1c levels were the highest in PEDIS 4 group and this was significant as compared to PEDIS 2 (p=0.007) and PEDIS 3 (p=0.003) groups. Compared to PEDIS 1, HbA1c level was higher in PEDIS 4, but it was not statistically significant (p=0.061), this was attributed to the low number of patients in the groups (**Table 3**).

DISCUSSION

It is known that factors such as age, gender, and duration of diabetes also play a role in the development of diabetic foot ulcers and other complications. In our study, the mean age was 62.06±10.48 in DFC+ group. The male ratio was higher (74%). DM duration was significantly higher in DFC+ patients as compared to two groups with DFC-, no difference was found in DM duration between DFC- groups. We may suggest that the increase in diabetes duration is a risk factor for DFC development. HbA1c and blood glucose levels were the highest in the DFC+ group, and lowest in the DFC-/PNP- group. These results showed that a better glucose regulation may reduce the risk of PNP and DFC development in diabetic patients. The data of the previous studies showed that elderly age, male sex, impaired glycemic control, and increased diabetes duration are the risk factors for the diabetic foot development (11,12). In our study, patients with diabetic foot were in the elderly age group and male ratio was higher. Patients in the DFC+ group had longer diabetes duration and all patients had PNP. Our results were compatible with the literature. PNP incidence increases with the age and diabetes duration in DM patients. PNP may affect both large and small fibers, causing pain symptoms depending on its size. It may often be asymptomatic. As peripheral neuropathy progresses, the patient becomes insensitive due to loss of protective sensory in distal extremities. This problem may significantly increase the risk of extremity loss. Diabetic patients cannot take protective measures since they do not feel the trauma in foot. Therefore, the development of diabetic foot ulcers is easier (11). Early detection of PNP by evaluating early nerve conduction in DM patients will contribute to prevention of diabetic foot development and decrease the administration of expensive treatment applications.

In our study, there was motor nerve involvement in addition to sensory nerve involvement in the lower extremities of DFC+ patients, unlike the DFC-group. When neuropathic involvement especially affects motor nerves, muscle weakness occurs in diabetic patients, facilitating the diabetic foot development by pressure changes on feet (13).

The study conducted by Karsidag et al. (14) in 30 patients with type 1 diabetes showed that the percentage of abnormal electrophysiological parameters in different motor and sensory nerves was 86.7% in the sural nerve, 83.3% in the peroneal motor nerve, 73.3% in the posterior tibial motor nerve, and the percentage of nerve involvement in the lower extremity was 90% motor, 86.7% sensory and 76.7% sympathetic nerves. They noted that the significance of nerve dysfunction in the lower extremity is associated with the length of these nerves. Again, the neuropathy study conducted by Kakrani et al. (15) on 50 type 2 diabetic patients showed that posterior tibial and sural nerve involvement was more common in diabetic neuropathy. They concluded that the long nerves are often affected by these involvements, and the lower extremity is affected more because of long nerves, and upper extremity involvement requires a longer diabetes duration. In our study, a statistically significantly difference was found in the mean motor amplitude and velocity of posterior tibial nerve and peroneal nerve between the groups, and the values were lowest in DFC + group among 3 groups. However, the motor amplitude of the posterior tibial nerve was within the normal range, and the velocity of posterior tibial nerve was lower than normal. Similarly, the amplitude and velocity of the sural nerve were lower than normal in DFC group and the values were lowest in

	PEDIS 1	PEDIS 2	PEDIS 3	PEDIS 4	р
n (%)	5 (%10)	17(%34)	21(%42)	7(%14)	
Age (years)	63.60±12.17	60.06±9.27	64±10.32	60±13.52	0.646*
Sex n (%)					0.114***
Female	1 (%7.7)	3(%23.1)	5(%38.5)	4(%30.8)	
Male	4 (%82.3)	14(%76.9)	16(%61.5)	3(%69.2)	
DM duration (years)	11±6.51	10±5.5	12.9±6	10.29±6.8	0.481*
HbA1c (%)	9.20±2.8 abc	9.27 ± 1.74 ad	$9.07{\pm}1.91^{\rm bd}$	12.51±2.30°	0.022**
Fasting Blood glucose: (mg/dl)	224.60±37.08	237.88±79.70	239.52±120.46	293.85±67.47	0.106**

DFC+ group among three groups. This finding showed that the long nerves in DFC+ group were affected more as compared to those without DFC development. All necessary proteins synthesized in the cell body are transmitted to the distal parts of the nerves by axoplasmic flow, protecting the anatomical and functional integrity of the nerve (14). Termination of axoplasmic flow in the long nerves is more apparent than in short nerves. Also, in our study, the involvement of long nerves such as peroneal, tibial and sural nerves in the lower extremities in DFC+ group is supported by this information.

In their study, Kızıltan et al. (16) measured only the peroneal nerve and sural nerve conduction levels in the patients with diabetic foot, and they could not find a correlation between these nerve conduction levels and diabetic foot development. In their electrophysiological evaluation on the newly diagnosed diabetic patients, Kulkarni et al. (17) reported that they detected an increase in peroneal nerve latencies and a decrease in motor conduction velocity and amplitudes. As a first proof of diabetic neuropathy, they suggest following up the slowdown of motor conduction velocity to detect the subclinical dysfunctions. Similarly, in our study, the mean peroneal nerve motor velocity (even though at normal level) was statistically significantly lower in DFC+ patients as compared to DFC-/PNP+ and DFC-/PNPdiabetic patients. No significant difference was found between DFC- cases. When the results were evaluated, the slowdown of peroneal nerve velocity was considered as a risk factor for diabetic foot development.

In their study, Taşkiran et al. (18) reported that the velocity of posterior tibial nerve impairs as the duration of diabetes increases. No relationship was found in our study between the slowdown of peroneal velocity, which is considered as a risk factor in DFC+ patients, and DM duration, HbA1c, blood glucose level, and age.

When DFC+ patients were grouped by PEDIS wound classification, the highest was PEDIS 3 (42%) and the lowest was PEDIS 1 (10%). Wound classification showed that no significant difference was found in age, sex, DM duration and fasting blood glucose between four groups, however, a statistically significant difference was found in HgbA1c levels. HbA1c levels were the highest in the PEDIS 4 group. According to PEDIS 1, HbA1c level was higher in PEDIS 4, but it was not statistically significant. This was associated with the low number of patients in the groups. Literature data showed that the incidence of ulcer development and ulcer recurrence increased and the healing times were prolonged in diabetic patients with high HbA1c levels (HbA1c >9%) (19, 20). The data suggested that better glucose regulation in the long term will be effective in the prevention of diabetic foot development and the wound progression.

CONCLUSIONS

The slowdown in peroneal nerve conduction velocity and the increase in diabetes duration were the risk factors for diabetic foot development, and the decrease in tibial nerve velocity was also considered as significant. The disease duration should be considered for early determination of the risk of diabetic foot development, and nerve conduction should be measured at certain intervals. The involvement of motor nerve conduction in the lower extremity, especially, the slowdown in peroneal nerve conduction velocity should be considered as a signal for diabetic foot development.

ETHICAL CONSIDERATIONS

Ethics Committee Approval: The study was carried out with the permission of Atatürk University Faculty of Medicine Clinical Research Ethics Committee (permission granted 28.05.2020, decision no. B.30.2.ATA.0.01.00/278).

Informed Consent: All patients signed the free and informed consent form.

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REFERENCES

- 1. Monteiro-Soares M, Boyko EJ, Ribeiro J, Ribeiro I, Dinis-Ribeiro M. Predictive factors for diabetic foot ulceration: a systematic review. Diabetes Metab Res Rev 2012; 28: 574-600.
- 2. Zhang P, Lu J, Jing Y, Tang S, Zhu D, Bi Y. Global epidemiology of diabetic foot ulceration: a systematic review and meta -analysis. Ann Med 2017; 49: 106-16.
- 3. Markowitz JS, Gutterman EM, Magee G, Margolis DJ. Risk of amputation in patients with diabetic foot ulcers: a claims-based study, Wound Rep Reg 2006;14: 11–17.
- 4. Vileikyte L, Leventhal H, Gonzalez JS, et al. Diabetic peripheral neuropathy and depressive symptoms. Diabetes Care 2005; 28: 2378-83.
- 5. Pop-Busui R, Boulton AJM, Feldman EL, Diabetic Neuropathy: A Position Statement by the American Diabetes Association. Diabetes Care 2017; 40: 136-54.
- 6. Gregg EW, Gu Q, Williams D, Prevalence of lower extremity diseases associated with normal glucose levels, impaired fasting glucose, and diabetes among U.S. adults aged 40 or older. Diabetes Res Clin Pract 2007; 77: 485–88.
- 7. Boulton AJM, Vinik AI, Arezzo JC, et al. Diabetic Neuropathies. Diabetes Care 2005; 28: 956-62.

- 8. Smith BE, Chapter 3 Focal and entrapment neuropathies, Handbook of Clinical Neurology 2014; 126: 31-43.
- 9. Schaper NC. Diabetic foot ulcer classification system for research purposes: a progress report on criteria for including patients in research studies. Diabetes Metab Res Rev 2004; 20: 90-5.
- 10.Weiss L, Silver JK, Weiss J. Easy EMG. Elsevier, 15th April 2015, Page Count: 296.
- 11.Ahmad J, The diabetic foot, Diabetes Metab Syndr 2016; 10: 48-60.
- Mottaghi T, Khorvash F, Maracy M, Bellissimo N, Askari G. Effect of folic acid supplementation on nerve conduction velocity in diabetic polyneuropathy patients, Neurol Res 2019; 41: 364-68.
- 13. Abboud RJ, Rowley DI, Newton RW. Lower limb muscle dysfunction may contribute to foot ulceration in diabetic patients. Clin Biomech 2000; 15: 37-45.
- 14.Karsidag S, Moralı S, Sargın M, Salman S, Karsidag K, Us O. The electrophysiological findings of subclinical neuropathy in patients with recently diagnosed type 1 diabetes mellitus. Diabetes Res Clin Pract 2005; 67: 211–9.
- Kakrani AL, Gokhale VS, Vohra KV, Chaudhary N. Clinical and nerve conduction study correlation in patients of diabetic neuropathy. J Assoc Physicians India 2014; 62: 24-7.
- 16.Kiziltan ME, Gunduz A, Kiziltan G, Akalin MA, Uzun N. Peripheral neuropathy in patients with diabetic foot ulcers: clinical and nerve conduction study. J Neurol Sci 2007; 15: 75-9.
- Kulkarni AP, Saroja AO, Naik KR, Ghatnatti V, Hesarur N. Nerve conduction abnormalities in patients with newly diagnosed diabetes mellitus. J Sci Soc 2018; 45: 30-3.
- 18. Taşkıran B, Güldiken S, Turgut N, Güldiken B, Tuğrul A. Electrophysiological risk factor in the development of diabetic foot: pobterior tibial nerve conduction pathology. Yeni Symp 2009; 47: 76-9.
- 19.Singh N, Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. JAMA 2005; 12: 217-28.
- 20.Zelen CM, Serena TE, Snyder RJ. A prospective, randomised comparative study of weekly versus biweekly application of dehydrated human amnion/chorion membrane allograft in the management of diabetic foot ulcers. Int Wound J 2014; 11: 122-8.