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Early detection of diabetic peripheral neuropathy using EMLA-induced skin wrinkling

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Abstract

Background & Objective: Screening for early diabetic peripheral neuropathy (DN) is essential for foot ulcer prevention. The Semmes–Weinstein monofilament (SWMF) is commonly used to detect DN in Thailand. However, SWMF interpretation requires patient participation, which is susceptible to risk of error in patients with impaired cognitive function or uncooperative patients. In contrast, stimulated skin wrinkling (SSW) can be interpreted by trained investigators, which is more appropriate. This study aimed to investigate the utility of SSW using a eutectic mixture of local anesthetic (SSW-EMLA) for early DN diagnosis. *Methods:* This cross-sectional study, recruited 102 patients with diabetes (DM group), 33 with diabetes with foot ulcer (DN control group), and 30 without diabetes (non-DN control group) from the Faculty of Medicine, Vajira Hospital, Navamindradhiraj University, Thailand, from February 3, 2021 to November 30, 2021. SSW was conducted by applying EMLA on the tips of the 2nd, 3rd, and 4th fingers of both hands. SWMF, sensory perception of pain (SPP), vibration perception threshold (VPT), joint position sense (JPS), and deep tendon reflexes (DTR) were also evaluated on the same day. *Results:* The Interrater agreement of two investigators for SSW-EMLA was high with intraclass correlation coefficients of 0.87 (0.824–0.904) for the right hand and 0.874 (0.830–0.907) for the left hand. The kappa coefficients of agreement of SSW-EMLA for SPP, SWMF, VPT, JPS, and DTR testing were 0.411, 0.478, 0.714, 0.444, and 0.681, respectively. The sensitivity and specificity of SSW-EMLA testing for DN detection were 83.3%, was 85.7%, respectively.
Conclusion: The SSW-EMLA test can be considered as an alternative method for DN detection, due to its noninvasiveness, inexpensiveness (50THB), being a simple procedure, high sensitivity and specificity, and acceptable rater variation.

Keywords: Diabetic peripheral neuropathy, stimulated skin wrinkling, eutectic mixture of local anesthetic

INTRODUCTION

Diabetic peripheral neuropathy (DN) is a common complication occurring in patients with diabetes mellitus.[1] Screening for early DN is essential to prevent foot ulcers.[2] There are invasive and noninvasive techniques to detect DN.[3] The invasive technique is the skin biopsy test for determining intraepidermal nerve fiber density (IENFD) in patients[1], whereas the noninvasive techniques include diabetic neuropathy symptom (DNS) score[4], sensory perception of pain (SPP), Semmes–Weinstein monofilament (SWMF), vibration perception threshold (VPT), joint position sense (JPS), deep tendon reflexes (DTR), and stimulated skin wrinkling using a eutectic mixture of local anesthetic (SSW-EMLA).

The SWMF is commonly used in Thailand. However, results are dependent on patients' participation in accurate indication of sensory perception. In contrast, the result of SSW, the reversible undulations of the surface skin occurring 5–30 min after water immersion or exposure to an EMLA[2,3], can be interpreted by a trained investigator. Studies have shown that SSW correlates with IENFD, which can be determined as an invasive technique in patients with sensory neuropathy.[5,6] Therefore, this study was conducted to investigate the utility of SSW-EMLA for early diagnosis of DN.

METHODS

This cross-sectional study was approved by the

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medical ethical review board of the Faculty of Medicine, Vajira Hospital, Navamindradhiraj University, COA number 006/2564. Data were collected from February 3, 2021 to November 30, 2021.

Patients diagnosed with diabetes were recruited from the outpatient/inpatient clinic of the Division of Internal Medicine, Surgery Department, Orthopedic Department, Faculty of Medicine, Vajira Hospital. Patients without diabetes were recruited from the outpatient clinic of the Division of Family Medicine, Internal Medicine, Faculty of Medicine, Vajira Hospital.

Study participants

Patients with and without diabetes aged between 18 and 80 years, who provided informed consent, were enrolled in this study.

Participants were divided into 3 study groups according to the following criteria.[7]

1. Patients without diabetes with normal HbA1C and FBS levels. This was the control group representing the non-DN group.
2. Patients with diabetes that were diagnosed according to the clinical practice guideline for diabetes 2017 (DM group).
3. Patients with diabetes that were diagnosed according to the clinical practice guideline for diabetes 2017 and also having foot ulcer. This was the control group representing the DN group.

In all three study groups, the following were the exclusion criteria: Patients with a history or laboratory evidence of cancer, previous or current chemotherapy, alcohol abuse, cirrhosis, hepatitis B virus (HBV) infection, human immunodeficiency virus infection, spinal cord and root disease, thyroid dysfunction, stage 5 chronic kidney disease (stage 5 CKD), uremia, thiamine deficiency, cobalamin deficiency, inflammatory neuropathy, hereditary neuropathy, connective tissue disease, severe accident of the hand or foot[5,8], anesthetic drug allergy, and open wounds over the fingertips where the EMLA cream would be applied.

Test procedures

The DNS score, SPP, SWMF, VPT, JPS, DTR, and SSW-EMLA were evaluated on the same day for each patient.

DNS score: This score confirms the following symptom items: (i) numbness in legs or feet; (ii) prickling sensations in legs or feet; (iii) pain, burning, or aching in legs or feet; and (iv) unsteadiness in walking. A score of "1" was given for each symptom that occurred several times a week during the past 2 weeks, and a score of "0" was given if the symptom did not occur. A score of 1–4 indicated abnormal, and a score of 0 indicated absent.[4]

Sensory perception of pain: The Wartenberg wheel was used in this test on patient extremities. The investigator instructed the patient to close his/her eyes and observe the feeling of the applied Wartenberg wheel on their extremities. Patients were subsequently asked to describe whether the feeling was sharp or dull. If the answer was sharp, it was interpreted as normal, otherwise abnormal.

Semmes–Weinstein monofilament: The monofilament testing was conducted on the plantar aspect of the hallux and the base of the 1st, 3rd, and 5th metatarsals using a 10g monofilament. The tests were conducted three times by applying the monofilament twice and not applying once. Patients were asked to close their eyes and answer "sense" or "not sense." If the answers were correct less than two times, the test was interpreted as abnormal.[2,9]

Vibration perception threshold: The 128-Hz tuning fork was placed perpendicular to the pulp of the terminal phalanx of the thumb, over the pulp of the hallux, and over the medial malleoli. Time was recorded simultaneously. Patients were instructed to close their eyes and asked to report when the buzzing stopped. If the time was >10 s, it was recorded as normal.[3] Age-adjusted reference values were used, and abnormal values were considered as those that were at least 2 SD higher than the mean value.[2]

Joint position sense: This test confirms JPS. The investigator grasped the lateral side of the digit proximal to the joint with the thumb and forefinger and then placed the thumb and forefinger of the other hand distal to the joint and parallel to the plane of movement. Patients were shown up or down movement, instructed to close their eyes, and asked to answer "up" or "down," respectively.[10] Result was interpreted as normal when ≥70% of the answers were correct.

Deep tendon reflexes: The triceps, biceps, brachioradialis, patellar and Achilles tendon

were tapped briskly with the reflex hammer. Results were graded from 0 to 4+, wherein 2+ was identified as normal and the other cases were recorded as abnormal.[10]

Stimulated skin wrinkling: Patients must not have used any hand cream before 1 h of performing this test, The skin temperature was controlled at 35°C and measured using a skin surface temperature probe sensor. The initial appearance of each fingertip was inspected and photographed. EMLA 5% cream (lidocaine 2.5% and prilocaine 2.5%; AstraZeneca) was applied on the 2nd, 3rd, and 4th fingertips of both hands, and then each finger was wrapped using a food-grade plastic wrap. After 30 min, the investigator photographed the wrinkling on the distal digit pulp.[2,3] The grade was given by two investigators based on the degree of wrinkling, as shown in Figure 1, as follows: Grade 0, wrinkling absent; Grade 1, slight wrinkling and fingertip is unsmooth; Grade 2, few lines of wrinkling on the fingertip; Grade 3, three or more lines of wrinkling on the fingertip; and Grade 4, wrinkling distorts on the fingertip. If the total grading of each hand was ≥9, the result was considered as normal.

Statistical analysis

SPSS 28.0 for Windows was used for statistical analyses. Descriptive data, which were presented as frequency and percentages, were compared between groups using the chi-squared test or Fisher's exact test. Quantitative data, which were expressed as mean ± standard deviation or median and interquartile range, were compared between groups using one-way ANOVA or the Kruskal–Wallis test.

RESULTS

A total of 165 patients were recruited and categorized into three groups, including 102 patients in the DM group, 33 in the DN group, and 30 in the non-DN group. The following patients were excluded: in the DM group, five patients had thiamine deficiency, six patients had missing laboratory results, and two patients had HBV infection; in the DN group, two patients had HBV infection, and one patient had stage 5 CKD; and in the non-DN group, one patient had thiamine deficiency, and one patient had HBV infection. There were 89, 30, and 28 patients remaining in the DM, DN, and non-DN groups, respectively. The demographic and clinical characteristics of the patients are presented in Table 1.

SPP, SWMF, VPT, JPS, DTR, and SSW were evaluated on the same day for each patient; the results are shown in Table 2. All patients in the DN group had abnormal findings for SPP, SWMF, VPT, JPS, and DTR, which were 83.3%, 93.3%, 90%, 66.7%, and 90%, respectively. All of the non-DN group patients showed no abnormal results. The DM group showed variable results of test procedures which suspected some patients had early DN.

Table 3 showed the results of SSW-EMLA which had 85.7% specificity, 83.3% sensitivity, and 84.5% accuracy by using DN and Non-DN groups as reference.

The interrater agreement calculated using the intraclass correlation coefficient for both investigators was 0.87 (0.824–0.904) for the right hand and 0.874 (0.830–0.907) for the left hand. Cohen's kappa of 0.986 for both investigators demonstrated excellent level of interrater

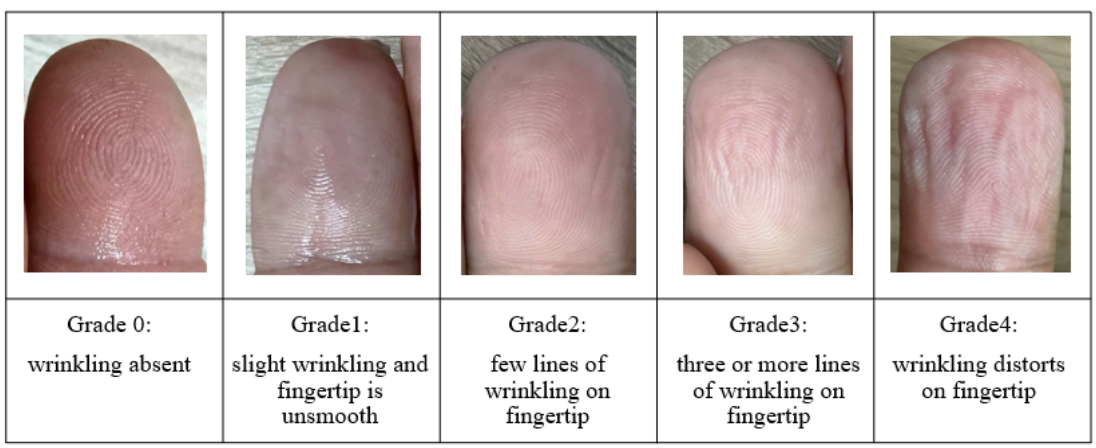

Figure 1. Degree of wrinkling and the corresponding grade levels

Table 1: Demographic and clinical characteristics of patients. (n = 147)

Characteristics	DN (n = 30)	DM (n = 89)	Non-DN (n = 28)
Age (years)	64.83 ± 10.05	57.43 ± 12.29	47.64 ± 15.59
Gender			
Male	19 (63.3)	46 (51.7)	11 (39.3)
Female	11 (36.7)	43 (48.3)	17 (60.7)
Weight (kg)	70.48 ± 14.33	71.17 ± 14.14	62.06 ± 11.86
Height (cm)	163.87 ± 11.17	161.67 ± 8.79	161.39 ± 7.65
Body mass index (kg/m2)	26.24 ± 4.70	27.26 ± 5.17	23.82 ± 4.01
Smoking	11 (36.7)	21 (23.6)	5 (17.9)
Hypertension	24 (80.0)	64 (71.9)	7 (25.0)
Dyslipidemia	19 (63.3)	69 (77.5)	12 (42.9)
Ischemic heart disease	5 (16.7)	7 (7.9)	0 (0.0)
Peripheral artery disease	13 (43.3)	16 (18.0)	0 (0.0)
Diabetes mellitus			
Diabetic retinopathy	8 (26.7)	19 (21.3)	
Duration of diabetes mellitus	10.5 (5.5 - 20)	9 (5 - 15)	
Insulin			
Not used	16 (53.3)	67 (75.3)	
Used	14 (46.7)	22 (24.7)	
Diabetic neuropathy symptom			
Normal (0)	6 (20.0)	51 (57.3)	
Abnormal (1-4)	24 (80.0)	38 (42.7)	
HbA1C level	7.6 (6.6 - 9.2)	7.3 (6.3 - 9.3)	5.4 (5.3 - 5.8)
Fasting blood sugar level	133.5 (112 - 157)	140 (124 - 191)	97 (93 - 102)

Data are presented as number (%), mean ± standard deviation or median (interquartile range).
P-value corresponds to Mann-Whitney U test, One-way ANOVA, Kruskal-Wallis test,
Chi-square test or Fisher's exact test.
Abbreviations: DN, Diabetic peripheral neuropathy; DM, Diabetes mellitus.

Table 2: Results of each test procedures between DN, DM, and Non-DN groups

Test	DN (n = 30) n(%)	DM (n = 89) n(%)	Non-DN (n = 28) n(%)
Vibration perception threshold			
Normal	3 (10.0)	54 (60.7)	28 (100.0)
Abnormal	27 (90.0)	35 (39.3)	0 (0.0)
Joint position sense			
Normal	10 (33.3)	71 (79.8)	28 (100.0)
Abnormal	20 (66.7)	18 (20.2)	0 (0.0)
Deep tendon reflexes			
Normal	3 (10.0)	60 (67.4)	28 (100.0)
Abnormal	27 (90.0)	29 (32.6)	0 (0.0)
Sensory perception of pain			
Normal	5 (16.7)	64 (71.9)	28 (100.0)
Abnormal	25 (83.3)	25 (28.1)	0 (0.0)
Semmes–Weinstein monofilaments			
Normal	2 (6.7)	73 (82.0)	28 (100.0)
Abnormal	28 (93.3)	16 (18.0)	0 (0.0)

Abbreviations: DN, Diabetic peripheral neuropathy; DM, Diabetes mellitus.

Table 3: Correlation between SSW-EMLA and Control groups* with diagnostic accuracy indices

SSW-EMLA	DN	Non-DN	Total
Abnormal	25	4	29
Normal	5	24	29
Total	30	28	119
Sensitivity	83.3%	(95%CI: 65.3 - 94.4)	
Specificity	85.7%	(95%CI: 67.3 - 96.0)	
Accuracy (overall fraction corrects)	84.5%	(95%CI: 72.6 - 92.7)	

*Control groups refer as DN and Non-DN
Abbreviations: DN, Diabetic peripheral neuropathy; DM, Diabetic mellitus; CI, confidence interval; SSW-EMLA, stimulated skin wrinkling by using eutectic mixture of local anesthetic.

agreement.[11,12]

Cohen's kappa Statistic was calculated using the kappa coefficient of agreement. The kappa coefficients of agreement between SSW-EMLA test and the other tests in both the DM and DN groups were as follows: SPP 0.411 (0.249–0.573), SWMF 0.478 (0.325–0.631), VPT 0.714 (0.589–0.840), JPS 0.444 (0.293–0.595), and DTR 0.681 (0.549–0.812), which indicated moderate-to-substantial agreement, as presented in Table 4.

In this study, early DN was defined by an abnormal DNS score with an additional abnormal physical examination. Thirty eight participants in the DM group had abnormal DNS score. The result of abnormal DNS score and SSW-EMLA was 55.3%, which is significantly higher than abnormal DNS score and SWMF, which was 34.2%, as shown in Table 5.

Among the study variables, an abnormal SSW-EMLA test was associated with age, peripheral arterial disease, duration of diabetes mellitus, and history of insulin use, as presented in Table 6.

Table 4: The Cohen's Kappa coefficient and the percentage agreement

Test	SSW-EMLA				PA (%)	k (95%CI)	p-value
	Abnormal		Normal				
DN and DM Group (n = 119)							
Vibration perception threshold							
Abnormal	52	(43.7)	10	(8.4)	85.7	0.714	<0.001
Normal	7	(5.9)	50	(42.0)		(0.589 - 0.840)	
Joint position sense							
Abnormal	32	(26.9)	6	(5.0)	72.3	0.444	<0.001
Normal	27	(22.7)	54	(45.4)		(0.293 - 0.595)	
Deep tendon reflexes							
Abnormal	48	(40.3)	8	(6.7)	84.0	0.681	<0.001
Normal	11	(9.2)	52	(43.8)		(0.549 - 0.812)	
Sensory perception of pain							
Abnormal	37	(31.1)	13	(10.9)	70.6	0.411	<0.001
Normal	22	(18.5)	47	(39.5)		(0.249 - 0.573)	
Semmes–Weinstein monofilaments							
Abnormal	36	(30.3)	8	(6.7)	74.0	0.478	<0.001
Normal	23	(19.3)	52	(43.7)		(0.325 - 0.631)	

Cohen's Kappa Statistic
Abbreviations: DN, Diabetic peripheral neuropathy; DM, Diabetes mellitus; PA, Percentage agreement; k, Cohen's Kappa Coefficient; SSW-EMLA, stimulated skin wrinkling by using eutectic mixture of local anesthetic.

Table 5: Comparison between SSW-EMLA and SWMF in abnormal DNS score of DM group

Test	Abnormal	Normal
DM Group with abnormal DNS score (n=38/89)		
SWMF	13 (34.2%)	25 (65.8%)
SSW-EMLA	21 (55.3%)	17 (44.7%)
P value 0.0209		

Abbreviations: DM, Diabetes mellitus; DNS, Diabetic neuropathy symptom score; SWMF, Semmes–Weinstein monofilament; SSW-EMLA, stimulated skin wrinkling by using eutectic mixture of local anesthetic.

DISCUSSION

DN can affect both small and large nerve fibers.[13] Damage to small fibers results in the loss of thermal and pain perception, whereas large fiber impairment results in the loss of joint position and vibration perception.[14] The most common type of DN is a chronic, symmetrical, length-dependent diabetic sensorimotor polyneuropathy (DPSN) that typically involves small nerve fibers earlier than large nerve fibers.[2] The gold standard method for small fiber neuropathy diagnosis is the quantification of small fibers in skin biopsies, where a decrease in IENFD is interpreted as abnormal.[15] Nerve conduction studies are noninvasive techniques that objectively measure nerve function, but they do not identify small fiber neuropathy.[16] They also require trained technologists and/or neurologists to perform and interpret the result.[2] In Thailand, the SWMF is currently used for screening DN. Although the SWMF test is a noninvasive procedure and is easy to perform, it requires patient participation to interpret the result. The SSW-EMLA test is one of the alternative noninvasive techniques that is a simple procedure, is inexpensive, and does not require the expertise of an investigator. The result of the SSW-EMLA test can be reliable even in patients with cognitive impairment or in uncooperative patients. In this study, 89 patients in the DM group, 30 in the DN group, and 28 in the non-DN group were recruited for analysis. In all groups, no patient had limb weakness. We set the DN and non-DN groups as control criteria for data analysis. The sensitivity and specificity of SSW-EMLA were 83.3% and 85.7%, respectively, which validate its reliability. We also found a low false negative result of SSW-EMLA, which was only 16.7%. The kappa coefficient of SSW-EMLA for SPP, SWMF, VPT, JPS, and DTR also indicated moderate-to-substantial agreement. When we compared the result of SSW-EMLA with SWMF in participants of DM group who had abnormal

Table 6: Multivariable analysis for factors associated with diabetic peripheral neuropathy

Factors	Univariable analysis			Multivariable analysis		
	OR[1]	95%CI	p-value	OR_{adj}[2]	95%CI	p-value
Age (years)	1.061	(1.018 - 1.105)	0.005	1.058	(1.007 - 1.111)	0.026
Peripheral artery disease	3.489	(1.415 - 8.601)	0.007	2.634	(0.952 - 7.291)	0.062
Duration of diabetes mellitus	1.059	(1.009 - 1.112)	0.020	1.003	(0.944 - 1.066)	0.923
Insulin						
Not used/Used to	1.000	Reference		1.000	Reference	
Used	2.665	(1.123 - 6.322)	0.026	2.322	(0.806 - 6.690)	0.119
Diabetic neuropathy symptom						
Normal (0)	1.000	Reference		1.000	Reference	
Abnormal (1-4)	5.368	(1.998 - 14.423)	0.001	3.736	(1.275 - 10.948)	0.016

Abbreviations: OR, Odds Ratio; OR_{adj}, Adjusted Odds Ratio; CI, confident interval.
Variable was included in multivariable model due to have p-value < 0.050 in univariable analysis.
[1]Crude Odds Ratio estimated by Binary Logistic regression.
[2]Adjusted Odds Ratio estimated by Multiple Logistic regression.

DNS score, the result of abnormal DNS score with SSW-EMLA is higher than with SWMF because SSW-EMLA investigators interpret the result directly and the bias from participant rating was removed. The statistical results also revealed correlations of age, peripheral arterial disease, duration of diabetes mellitus, history of insulin use, and abnormal DNS score with abnormal SSW-EMLA. Based on all results, it can be interpreted that the SSW-EMLA test can be used for DN detection, and is another effective method for early DN screening, especially in patients with cognitive impairment or in uncooperative patients.

DISCLOSURE

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Conflict of interest: None

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