Diabetes alters the pain threshold of the oral mucosa

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Abstract

Background

According to the National Health and Nutrition Survey, the prevalence of diabetes mellitus (DM) among men aged 65 years or higher is increasing. In the dental field, denture wearers diabetes more frequently have denture ulcers in the oral mucosa than without diabetes. The author hypothesized that formation of denture ulcers and erosions relate to reduced consciousness by hypoesthesia of diabetic neuropathy, and introduce the delay of visits to a dental professional, and induce further ulcers. The objectives of this study were to compare the sensory nerve function of the oral mucosa of patient with and without diabetes and to seek the relationship between the severity of diabetes and the oral sensation threshold.

The study subjects need to examine their blood glucose level by needle puncture along with the current perceptual threshold (CPT) and pain threshold (PT) measurement. However, the influence of needle puncture on the CPT and PT values is uncertain. Thus, to investigate the effect of blood collection on CPT and PT measurement in the oral cavity, hands, and foot of healthy subjects; research one was planned. Based on research 1, research 2 examined the effect of DM on PT value.

Materials and methods

Research 1

Eighteen volunteers studying or working at the Nihon University School of Dentistry at Matsudo were recruited for the study. Measuring point for CPT and PT were left side greater palatine foramen, hand and foot, by using Neurometer CPT/C® device (Neurotron Inc., Baltimore, MD, USA) delivering electrical stimulation at frequencies of 5, 250 and 2000 Hz at the respective measuring point. Blood sampling was taken from the subjects' index finger using a small Aipitto needle® (ASAHI POLYSLIDER CO., Okayama, Japan). Blood glucose was analyzed by Antsense Duo® (HORIBA CO., Tokyo, Japan). Subjects underwent all of three test conditions (sensory measurements followed by blood sampling: SB, blood sampling followed by sensory measurements: BS, and control: sensory measurements only without blood sampling: CO) at one-week intervals, to test the interaction between blood sample sampling and sensory thresholds. The reliability of the CPT and PT values obtained from the three procedures (BS, SB, and CO) and analyzed by Cronbach's alpha coefficient. A one-way analysis of variance (ANOVA) with post hoc tests with Bonferroni correction was used to test the influence of measurement region (oral cavity, hands, and feet) on CPTs and PTs.

Research 2

Fifty-six volunteers, including 21 individuals with DM, and 35 individuals without DM were recruited from individuals were patients at the Nihon University School of Dentistry at Matsudo Affiliated Hospital or were workers or students at the Nihon University School of Dentistry at

Matsudo for the study. PT measurement followed the methods to research 1. Analysis of covariance with the Bonferroni post-hoc test was applied to test the influence of DM existence, age, stimulate frequency and measuring point to PT values.

Result

Research 1

Alpha values of the CPT and PT measurements ranged 0.78 to 0.82 for all three procedural sequences (BS, SB, and CO), which can interpret as acceptable reliability. The subjective rating of anxiety before blood sampling and pain at after blood sampling were low. Furthermore, CPT / PT had region - specific values. The highest CPT / PT values were observed in the foot, followed by the hand, and the oral region had the lowest CPT / PT value of the tested areas.

Research 2

DM had a significant influence on PT values, which showed significantly low values than those without DM. Frequency and measuring point also had a significant influence on the PT values. Age, however, did not influence the PT.

Conclusion

This study suggests that CPT and PT measurements in parallel with blood sampling have reliability. Furthermore, individuals with DM are more sensitive to pain induced by electrical stimulation than those without DM. Pain sensation was region-specific, most sensitive in oral region, and frequency-specific as well.

I. Introduction

According to the World Health Organization, the number of individuals with diabetes mellitus (DM) has risen from 108 million in 1980 to 422 million in 2014 [1]. In Japan, according to the National Health and Nutrition Surveys of 2003–2012, which included data from 51,128 individuals aged ≥ 20 years, the age-standardized prevalence of diabetes remained constant at approximately 8% and was significantly higher in men and individuals aged ≥ 65 years. The proportion of individuals receiving treatment for DM increased significantly from 41.8% in 2003 to 54.9% in 2012, and the proportion of those with controlled hemoglobin A1c (HbA1c) levels increased significantly from 13.4% in 2003 to 28.8% in 2012 [2].

Peripheral neuropathy is a major cause of disability and reduced quality of life, due to sensory loss, pain, gait disturbance, fall-related injuries, and foot ulceration and amputation [3]. Collin et al. reported that, similar to foot ulceration, which is induced by repeated pressure [4], occlusal force-induced denture-related lesions on the pressure-supporting oral mucosa occur more often in denture wearers with DM than in those without DM [5]. This report was in agreement with our clinical experience with denture-related treatment of patients with DM. Dysesthesia, known to be related to diabetic peripheral neuropathy, might partially explain the susceptibility of these patients to ulcers in the oral mucosa that is covered by dentures. In an aging society, where denture wearing, as well as the prevalence of DM, is increased, it is therefore important to investigate oral sensation as it relates to DM.

Several methods are used to evaluate impaired sensory perception in DM [6-11], including electrical current stimulation. The current perception threshold (CPT) and pain threshold (PT), as determined using a NeurometerR, has been proposed to be a useful quantitative parameter for assessing peripheral sensory nerve function [12]. The large myelinated A-beta fibers, small myelinated A-delta fibers, and unmyelinated C fibers are selectively evaluated at frequencies of 2000, 250, and 5 Hz, respectively. The validity of the method has been proven by comparison to standard sensory testing techniques that have popularly been used for diagnosis of neuropathy in DM, including thermal and vibration detection thresholds [13, 14]. Furthermore, we have developed an approach to apply electrical current stimulation for oral sensory measurements and have previously reported that the PT can be useful for assessing changes in oral sensation in denture wearers[15-17]. The PT, as measured by the NeurometerR, may also be useful for oral sensory measurements in patients with DM.

However, sensation in individuals with DM can, in fact, change measurably, in a manner dependent on glycemic control (as evaluated by blood glucose and HbA1C values). Thus, it is important to check the state of a patient's glycemic control when measuring possible changes in perception due to DM. However, checking glycemic control at the same time as measuring CPT/PTs creates more uncertainty, as a needle puncture is required for blood sampling; the irritation, pain, and anxiety of the needle stick can themselves influence perception measurements [18, 19)]. Despite our knowledge of these potential confounding factors, no reports are assessing whether the act of blood sampling interacts with CPT and PT measurement. The objective of the study is to investigate whether DM increases sensory loss in the oral cavity of denture wearers relative to those denture wearers without DM. The initial study to achieve the objective was planned to clarify whether CPT/PT measurement obtained from the oral cavity, hand and foot were reliable if measured concurrently with blood sampling. The successive study was to clarify intraoral pain sensation of individuals with DM differs from that without DM in the body.

II. Methods and Results

1. Influence of blood sampling to perception and pain threshold (Research 1)

1) The participants

This study was approved by the Human Ethics Committee of Nihon University School of Dentistry at Matsudo (IRB project number: 15-003). Eighteen volunteers (10 males and 8 females, average age = 26.3 ± 1.7 years) studying or working at the Nihon University School of Dentistry at Matsudo were recruited for the study and provided written informed consent prior to enrollment. Only healthy individuals were included in the study. Thus, individuals with the following conditions were excluded: (a) general health problems that could affect the measurement of nerve activity (e.g., trigeminal neuralgia or postherpetic neuralgia); (b) signs and symptoms of orofacial pain disorders; (c) pacemaker wearers; (d) obvious cognitive impairment; and (e) a lack of understanding of written or spoken Japanese.

2) CPT/ PT testing

（1） CPT test

The participant was seated comfortably in a dental chair in a quiet room during testing. A single operator obtained CPT measurements from around the left greater palatine foramen and the tips of both index finger and big toe using the Neurometer CPT/C® device to deliver electrical stimulation at frequencies of 5, 250, and 2,000 Hz. Between each individual CPT measurement, the targeted area was checked using a dental mirror, and the participant was asked whether they still felt any residual irritation from the stimulus they had just received. As per the manufacturer's instructions, the electrical current was slowly increased from 0.01 mA until the subjects reported sensation for a given frequency. A preliminary perception threshold level was then determined. Then, a microprocessor-controlled forced-choice method, which used 6 to 20 cycles of randomly selected true and false stimuli above and below the preliminary perception threshold level, was implemented. This was double-blinded (to both the operator and participant) until the exact CPT (the perception threshold) was determined. To ensure contact between the mucosa and stimulation electrodes, a measurement apparatus with Φ 1 mm thermoforming discs was developed for each participant. Plates (18 × 9 × 6 mm) with stimulation electrodes (Φ 2 mm) mounted on an intraoral removable appliance were utilized (Figure 1).

(2) PT test

This was executed using the same methods for the CPT test described in the previous section, except that the electrical current was slowly increased until the subjects reported pain for each frequency (the PT). As described in Nakashima's report (Nakashima, Kimoto, & Kawai, 2014), the operator gave a verbal explanation of the process and exposed the participants to each of the three frequencies used to assess PT prior to administering the actual PT test. This prior exposure can negate the startle reflex, thus improving the collected data.

3) Blood sampling and analyses

Participants took blood from their index finger using a small Aipitto needle® (ASAHI POLYSLIDER CO., Okayama, Japan). The operator collected 5 μl of blood from the surface of the index finger with a capillary tube. Blood glucose was analyzed using Antsense Duo® (HORIBA CO., Tokyo, Japan). The participants were asked not to eat any foods or to drink beverages 2 hr before blood sampling.

4) Subjective anxiety and pain assessments

Participants were asked about their anxiety level before blood sampling and their perceived pain after blood sampling. Participants' perceptions of their anxiety and pain ratings were assessed by responding to questions on a 100-mm visual analogue scale anchored by the phrases “not at all anxious” and “extremely anxious” for anxiety, and “not at all painful” and “extremely painful” for pain. The questions asked were as follows: (a) How much anxiety do you feel before blood sampling? (b) How much pain did you feel at blood sampling?

5) Experimental protocol

In order to test the interaction between blood sample sampling and sensory thresholds, subjects underwent all of three test conditions at once-weekly intervals according to the following sequences: (a) sensory measurements (CPT and PT measurements) followed by blood sampling (“SB”); (b) blood sampling followed by sensory measurements (“BS”); and (c) sensory measurements only without blood sampling (control, “CO”). To minimize anxiety related to impending blood sampling, the CO block was performed after the participants were told they would not have to do blood sampling during that session.

6) Statistical analysis

Before other statistical analyses were performed, the normality of the data was tested using the Kolmogorov–Smirnov test. The data were found to be normally distributed, so parametric statistical methods were applied. The reliability of the CPT and PT values obtained from the three procedures (BS, SB, and CO) was analyzed using Cronbach's α coefficient. A one-way (analysis of variance) ANOVA with post hoc t tests with Bonferroni correction was used to test how CPTs and PTs were affected by measurement region (oral cavity, hands, and feet). Statistics were performed using SPSS® Statistics 21 (SPSS-IBM, MD,USA), with $p < .05$ representing significant differences.

7) Result

(1) CPT and PT

Table 1 shows the CPT and PT values according to each of the three procedures (SB, BS, and CO) and three measurement regions (oral cavity, hand, and foot) at each current frequency. The values of SB, BS, and CO were very similar. However, it was noted that the foot, hand, and oral cavity had completely different CPT and PT values.The Cronbach's α coefficients for the procedures are shown in Table 2. The values were all over 0.78, which represent acceptable reliability of the values obtained from the SB, BS, and CO conditions. The effect of measurement region (oral cavity, hand, and foot) on CPT values at each current frequency (5, 250, and 2,000 Hz) is shown in Figure 2. The mean CPT values averaged across BS, SB, and CO conditions at 2000 Hz were 41.3 ± 1.5 (oral cavity); 180 ± 10.6 (hand); and 283 ± 13.9 (foot) × 10^{-2} mA. Those at 250 Hz were 23.7 ± 2.5 (oral cavity); 88 ± 13.1 (hand); and 127 ± 2.0 (foot) × 10^{-2} mA. Those at 5 Hz were 13.0 ± 2.6 (oral cavity); 56 ± 6.2 (hand); and 80.7 ± 1.2 (feet) × 10^{-2} mA. The one-way ANOVA revealed a significant main effect of measurement region on CPT values ($p < .05$). Post hoc, Bonferroni corrected t tests demonstrated that the foot had a significantly greater CPT value (i.e., higher threshold) than the hands and oral cavity, and the hands had a significantly greater CPT value than the oral cavity; both differences were statistically significant increases ($p < .05$ for oral cavity vs. hands, and hands vs. feet). The effect of measurement region (oral cavity, hand, and foot) on PT values at each current frequency (5, 250, and 2,000 Hz) is shown in Figure 3. The mean PT values averaged across BS, SB, and CO conditions at 2000 Hz were 130.3 ± 1.2 (oral cavity); 409.7 ± 4.6 (hand); and 547.3 ± 46.5 (foot) × 10^{-2} mA. Those at 250 Hz were 70.0 ± 3.2 (oral cavity); 177.7 ± 3.2 (hand); and 238 ± 16.0 (foot) × 10^{-2} mA. Those at 5 Hz were 69.0 ± 4.6 (oral cavity); 165.3 ± 21.7 (hand); and 197.7 ± 4.2 (foot) × 10^{-2} mA. The one-way ANOVA revealed a statistically significant main effect of measurement region on PT values ($p < .05$). Post hoc Bonferroni-corrected t tests showed that the foot had a significantly higher PT value than the hands and oral cavity, and the hand had a significantly higher PT value than the oral cavity, except at 5 Hz frequency, at which the foot had significantly greater CPT value than the oral cavity. The hand has a significantly higher PT value than the oral cavity at the 250 and 2000 Hz frequencies, but not at 5 Hz, at which the foot had a significantly greater PT value than the oral cavity; however, no significant differences were observed between hands and foot.

(2) Subjective anxiety and pain related to blood sampling

The rating of anxiety before blood sampling was 2.8 ± 3.3, and the rating of pain at blood sampling was 1.1 ± 1.6; these values were indicative of low pain and anxiety levels

2. Influence of drawing blood to perception and pain threshold (Research2)

1) The participants

This study was approved by the Human Ethics Committee of Nihon University School of Dentistry at Matsudo. Fifty-six volunteers, including 21 individuals with DM (DM group, 12 men and 9 women, average age = 72.1 ± 4.7 years), and 35 individuals without DM (NDM group, 17 men and 18 women, average age = 51.2 ± 23.9 years), were recruited for the study. All subjects with DM were diagnosed by the physician and persistent uncomfortable sensations in the mouth of the subjects were evaluated. These individuals were patients at the Nihon University School of Dentistry at Matsudo Affiliated Hospital or were workers or students at the Nihon University School of Dentistry at Matsudo, respectively, and provided written informed consent prior to enrollment. Individuals meeting the following conditions were excluded: (i) the presence of general health problems that could affect the measurement of nerve activity (e.g., trigeminal neuralgia or postherpetic neuralgia); (ii) signs and symptoms of orofacial pain disorders; (iii) pacemakers; (iv) obvious cognitive impairment; and (v) a lack of understanding of written or spoken Japanese

2) PT testing

PT was measured using the modified method described Reseach 1. In brief, participants were seated comfortably in a dental chair in a quiet room during testing. A single operator obtained PT measurements from around the left greater palatine foramen and from the tip of the left index finger and of left big toe using the Neurometer CPT/C® device to deliver electrical stimulation at frequencies of 5, 250, and 2000 Hz. The PT was measured one time at each frequency. Between each individual PT measurement, the targeted oral area was checked using a dental mirror, and the participant was asked whether they still felt any residual irritation from the stimulus they had just received. The operator gave a verbal explanation of the process and exposed the participants to each of the three frequencies used to assess PT prior to administering the actual PT test, as such prior exposure can negate the startle reflex, thus improving the quality of the collected data. For PT measurements, the participants initiated the stimulation by pushing a button on the device. As long as the participants depressed the button, the amount of stimulation slowly increased automatically. We asked participants to release the button when they perceived the stimulus as painful. To ensure contact between the mucosa and stimulation electrodes, a measurement apparatus with φ 1 mm thermoforming discs was developed for each participant. Plates (18 × 9 × 6 mm) with stimulation electrodes (φ 2 mm) mounted on an intraoral removable appliance were utilized (Figure 1)

3) Statistical analysis

Before other statistical analyses were performed, the normality of the PT values was tested using the Kolmogorov–Smirnov test, following which parametric statistical methods were applied. Participants' characteristics were analyzed by a *t*-test and the chi-squared test. In order to adjust for the difference in age between the DM and NDM groups, we used analysis of covariance (ANCOVA) with the Bonferroni post-hoc test to assess whether there were any differences in PT between the DM and NDM groups; whether the PTs obtained from the oral cavity, hands, and feet differed from each other; and whether PTs obtained using three different current frequencies for testing (2000 Hz, 250 Hz, and 5 Hz) differed from each other. Furthermore, we investigated whether the use of age as a covariate affected PTs. Statistical analyses were performed using SPSSR Statistics 21 (SPSS-IBM, MD, USA), with $p < 0.05$ representing significant differences.

4) Result

(1) Participants' characteristics

The participants' characteristics are shown in Table 3. There were significant differences in age, HbA1c levels, and blood sugar levels between the DM and NDM groups. However, there were no significant differences in the proportion of males and females or the body mass index between the DM and NDM groups.

(2) PT values

The PT value of the DM group was $171.4 \pm 164.3 \times 10^{-2}$ mA and that of the NDM group was $195.7 \pm 167.1 \times 10^{-2}$ mA (Figure 4). Individuals with DM had significantly lower PT values than those without DM (ANCOVA, $p = 0.013$ Table 4). The PT values obtained from the oral cavity, hands, and feet were $80.0 \pm 58.0 \times 10^{-2}$ mA, $205.1 \pm 142.0 \times 10^{-2}$ mA, and $274.6 \pm 200.5 \times 10^{-2}$ mA, respectively (Figure 5). The PT values of the oral cavity, hands, and feet differed significantly from each other (ANCOVA with Bonferroni post-hoc test, all $p < 0.0001$, in the order oral cavity < hand < foot. The PT values obtained using 5 Hz, 250 Hz, and 2000 Hz were $107.4 \pm 78.7 \times 10^{-2}$ mA, $125.8 \pm 82.6 \times 10^{-2}$ mA, and $326.5 \pm 201.3 \times 10^{-2}$ mA, respectively (Figure 6). The PT values obtained using 5 Hz and 2000 Hz differed significantly (ANCOVA with Bonferroni post-hoc test, $p < 0.001$), as did those obtained using 5 Hz and 250 Hz (ANCOVA with Bonferroni post-hoc test, $p < 0.001$). However, there was no significant difference between the PT values obtained using 5 Hz and 250 Hz (ANCOVA with

Bonferroni post-hoc test, p = 0.202).

III. Discussion

This study was conducted to consider two studies for the sensory nerve of the diabetic under control. The objective of research 1 was to verify the measurement reliability of CPT and PT tests performed before and after blood sampling and to investigate the differences in CPT/PT obtained from the oral cavity, hand, and foot, respectively. The objective of research 2 was to investigate whether PT differs between individuals with and without DM.

The research 1 revealed that CPT and PT measurements derived from the oral cavity, hand, and foot were reliable even during parallel blood sampling. The reliability of the measurements was confirmed by Cronbach's α coefficient, for which the acceptable range 0.70 to 0.95 [20, 21]. Alpha values of the CPT and PT measurements ranged 0.78 to 0.82 for all three procedural sequences, which translates to acceptable reliability. Furthermore, CPT/PT had region‐specific values. The highest CPT/PT values were observed in the foot region, followed by the hand region. The oral region had the lowest CPT/PT value of the tested areas. To our knowledge, this was the first report to show no interaction between blood sampling and CPT/PT values, as well as an increased threshold in the order oral cavity < hand < foot.

The most interesting finding of this study was that the three measurement sequences (BS, SB, and CO) in all measurement frequencies (5, 250, and 2,000 Hz) had similar CPT and PT values, resulting in acceptable measurement reliability. The lack of a difference between BS and SB values implies that the complex combination of physical and psychological factors induced by needle puncture at blood sampling does not change either threshold. Furthermore, the fact that these values did not differ between BS and CO implies that psychological stress (e.g., anxiety) associated with needle puncture or its anticipation did not change CPT and PT values, because the only difference between these conditions was knowing whether there would or would not be a needle puncture at the beginning of the trial. Human senses are said to be influenced by emotional factors induced by subjective experiences [22]. This, combined with the fact that most people have negative feelings towards needle punctures, led us to hypothesize that the needle puncture used in blood sampling would bias sensory measurements. However, this was not found to be the case, given that the subjective rating of anxiety before blood sampling was 2.8 ± 3.3 and that of pain at blood sampling was 1.1 ± 1.6, both of which are low (i.e., participants felt little anxiety and pain at blood sampling). The needle, developed to allow easy self‐collection of blood by patients, is very small, and the blood needed for analysis is very little; thus, the participants' anxiety and fear was never evoked.

This was the first study that directly compared CPT/PT in the foot, hand, and oral regions, and showed that the CPT/PT is increasing in the following order: oral cavity < hand < foot. It suggested that measurement regions closer to the head had smaller CPT values, which corresponds to a greater sensitivity to stimulation. There are several studies conducted with the same device used to measure CPT, even though the studies did not directly compare CPT/PT in the foot, hand, and oral regions. Studies [23-26)] reported current perception threshold at the foot and finger, finger, face, and face and oral cavity, respectively. Comparison between our results and those of the previous reports mentioned above indicated that our values were in line with those of all the other studies, despite the fact that the characteristics of the study populations were different in all five studies [23-26)]. Thus, this result supports a previous study and that the oral region was the most sensitive region.

The research 2 revealed that there are significant differences in PT between DM and NDM individuals; that PTs obtained from the oral cavity, hands, and feet differ significantly from each other; and that PTs obtained using three different current frequencies varied. Participants with DM had a lower PT than those without DM (Figure 4). It is well known that diabetic peripheral neuropathy causes several symptoms, such as pain, dysesthesia, and loss of sensation, which have been evaluated by several sensory tests [6-11)]. Considering that the PTs of participants with DM are lower than those of individuals without DM, the diabetic neuropathy observed in this study resulted in hyperalgesia, which was contrary to our a prior expectation of identifying pain hypoalgesia as the outcome of diabetic neuropathy. Several authors have reported that hyperalgesia is observed as a symptom of diabetic peripheral neuropathy at an early stage, while hypoesthesia is observed at an advanced stage [27)]. Umezawa et al. reported that 40% of participants with DM (mean HbA1c 8.8 ± 2.2 %) had hyperalgesia[28)]. Given that the mean HbA1c of participants in our study was lower, i.e., 6.9 ± 0.8%, than that in the study [28)], the observation of hyperalgesia in this study is plausible. However, this implies that the results are dependent on the participants under study, and thus further investigations of a wide range of participants with DM are needed.

This study showed region-induced PT diversity, with PT values in the order oral cavity < hand < foot (Figure 5). Thus, regions closer to the head had smaller PT values, corresponding to a greater sensitivity to stimulation. Studies [23-26)] reported the current perception threshold at the foot and finger, finger, face, and face and oral cavity, respectively. The present study also showed current frequency-induced PT diversity, in the following order 5 Hz, 250 Hz < 2000 Hz (Figure 6). These results suggested that different frequencies of electrical stimulation are associated with different pain thresholds. Sine waves at 2,000, 250, and 5 Hz corresponds to depolarization periods of 0.25, 2, and 100 milliseconds, respectively. The large-diameter fibers can respond to the rapid 2,000 Hz stimulus, whereas the small unmyelinated fibers require several milliseconds of a continuous depolarization period to respond. However, the large fibers repolarize faster than the 5 Hz stimulus can depolarize them. Large fibers do not achieve there threshold potential with the 5Hz sinusoid waveform stimulation. Consequently, the 2,000, 250 and 5 Hz sine wave depolarizations selectively evoke responses from the large myelinated A-beta, the small myelinated A-delta, and the small unmyelinated C fibers, respectively [29, 30]. However, when PT is measured, electrical stimulation at one frequency can evoke several fibers[31], since intense electrical current during PT measurement was stronger than that at the current perception threshold. Due to this limitation, it is possible that there was contamination among the different fibers, as current frequency-induced PT diversity cannot indicate the involvement of specific nerve fibers, such as A-beta, A-delta, C fibers.

Although there were significant differences in intraoral sensation between the DM and NDM groups using analysis of covariance (ANCOVA) with the Bonferroni post-hoc test, we cannot rule out the possibility that other factors, age-matched control subjects, may have played a role in the difference in scores observed. Also, our sample size is small and is restricted to individuals with relatively low or controlled diabetes. Therefore, further studies need to be conducted to verify these findings.

IV. Conclusion

The result of the summarized research revealed and suggested the following.

1. CPT and PT measurements derived from the oral cavity, hand and foot were reliable even during parallel blood sampling.
2. CPT and PT measurements in parallel with blood sampling have reliability and region-specific values, which increased in the following order: oral cavity < hand < foot.
3. The participants with DM had a lower PT than the NDM.

V. References

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□. Figures and Table

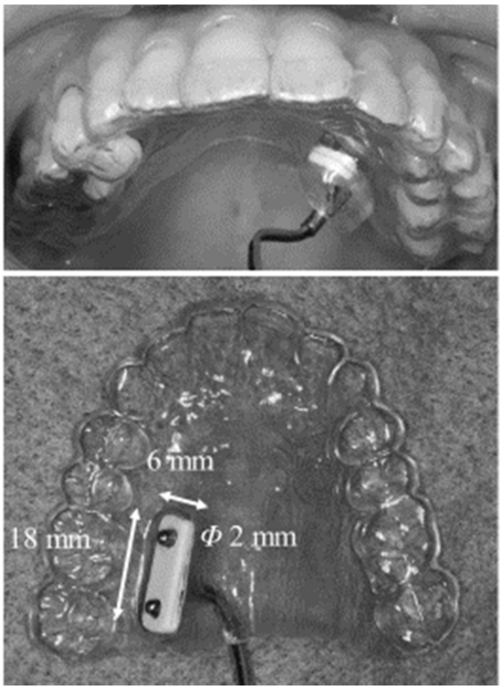

Fig 1. Intraoral removable device with stimulating electrodes.
Participants wear the measurement apparatus with Φ 1 - mm thermoforming discs to ensure contact between the mucosa and stimulation electrodes.

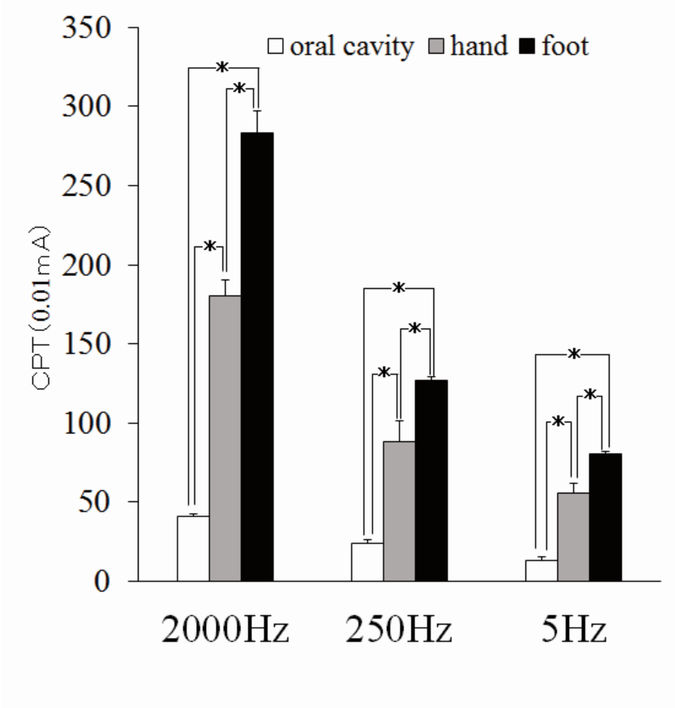

Fig 2. Current perception thresholds. The bar in each group (oral cavity, hand, and foot) represents mean CPT values across SB, BS, and CO. The one way ANOVA with post hoc t tests adjusted with the Bonferroni correction showed that CPTs increase in the following order: oral cavity < hand < foot ($p < .05$).

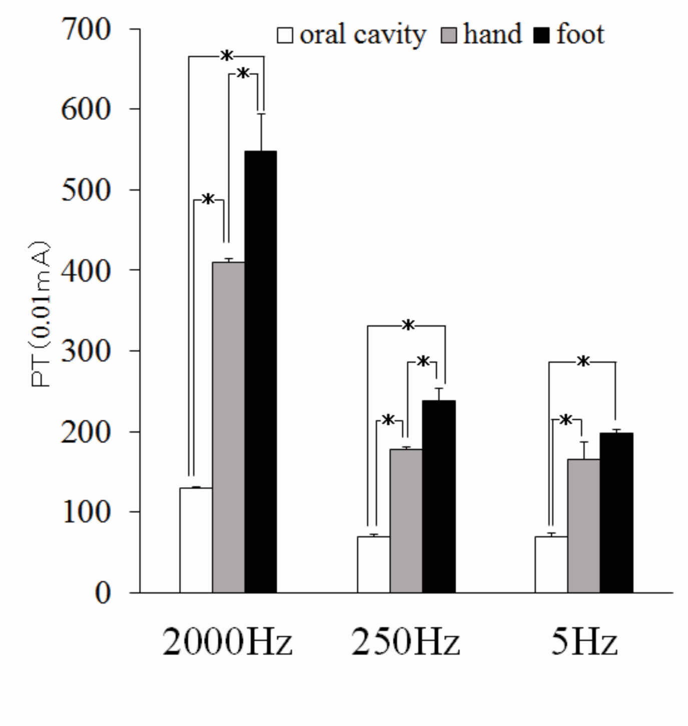

Fig 3. Pain thresholds. The bar in each group (oral cavity, hand, and foot) represents mean PT values across SB, BS, and CO. The one way ANOVA with post hoc t tests adjusted with the Bonferroni correction showed that PTs increase in the following order: oral cavity < hand < foot ($p < .05$).

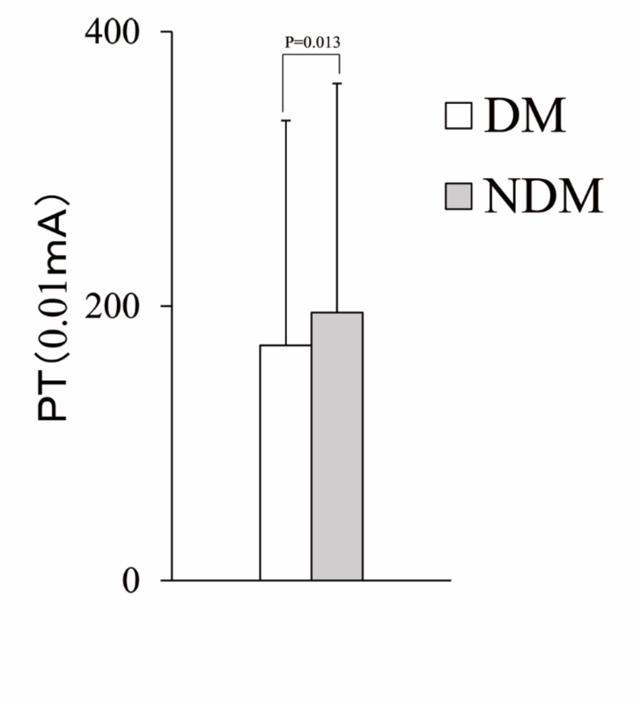

Fig 4. The difference in pain thresholds (PTs) between DM and NDM groups ANCOVA revealed that individuals with DM had lower PT values than those without DM.

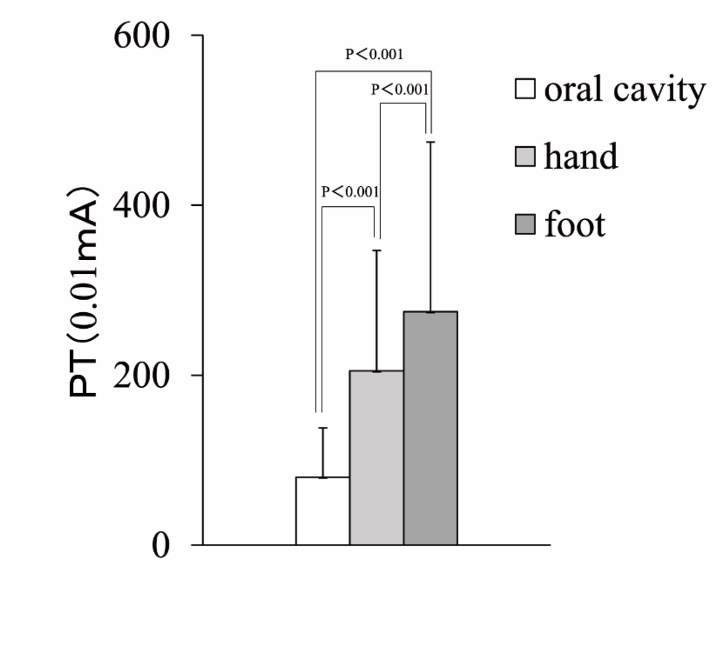

Fig 5. Differences in pain thresholds (PTs) obtained from foot, hand, and the oral cavity ANCOVA with Bonferroni post-hoc tests showed that PTs increase in the following order: oral cavity < hand < foot.

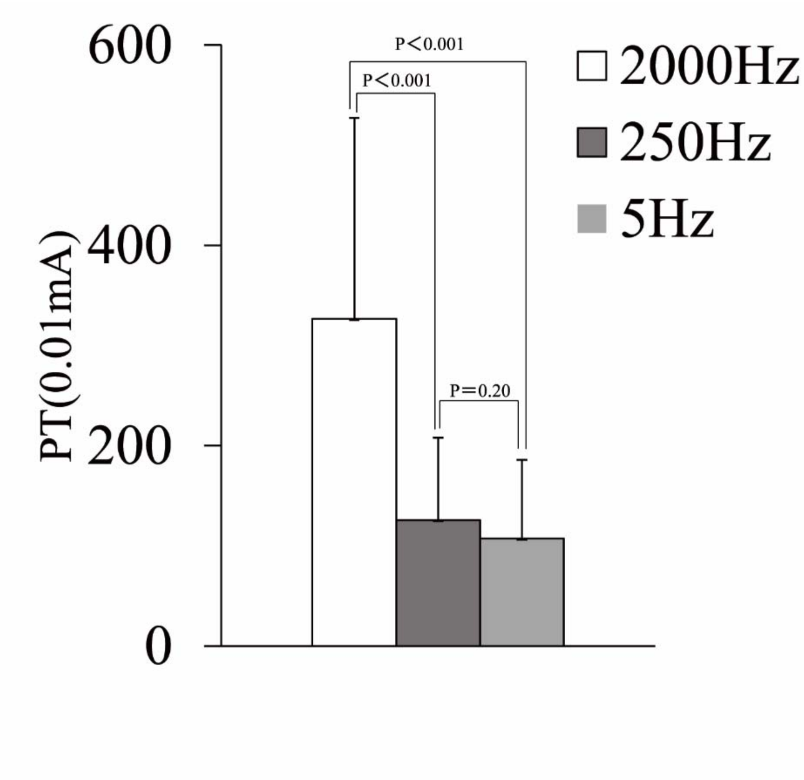

Fig 6. The difference in pain thresholds (PTs) obtained with electrical currents of 2000 Hz, 250 Hz, and 5 Hz frequencies. ANCOVA with Bonferroni post-hoc tests showed that there was a significant difference in the PT values obtained with 5 Hz vs. 2000 Hz as well as with 250 Hz vs. 2000 Hz.

Table 1. CPT and PT values according to each of the three procedures and three measurement regions

		CPT				PT		
		SB	BS	CO		SB	BS	CO
5Hz	oral cavity	11.7 ± 9.5	12.7 ± 7.3	16.3 ± 18.6	oral cavity	64.6 ± 49.9	73.8 ± 56.7	70.7 ± 75
	hand	49.3 ± 27.3	58.2 ± 29.3	61.6 ± 30.7	hand	190.9 ± 144.8	157.9 ± 96.6	149.9 ± 66.6
	foot	82.7 ± 36.8	80.6 ± 27.3	80.8 ± 40	foot	201.9 ± 101.2	193.1 ± 118.6	199.7 ± 101.2
250Hz	oral cavity	21.2 ± 22.5	24.5 ± 28.7	26.8 ± 31	oral cavity	66.7 ± 54.5	72.5 ± 46.7	71.9 ± 63.1
	hand	64.1 ± 22.4	67.7 ± 24.4	73 ± 36.3	hand	174.3 ± 91.1	180.3 ± 102.7	179.6 ± 68
	foot	125 ± 35.8	127.8 ± 33.1	129.8 ± 44.4	foot	222.4 ± 84	239.2 ± 105.6	254.2 ± 84.9
2000Hz	oral cavity	43 ± 37.3	41 ± 31.8	40 ± 27.6	oral cavity	129.7 ± 86.5	131.3 ± 73.5	131.4 ± 93.9
	hand	172 ± 34.8	176 ± 49	192 ± 47.4	hand	408 ± 135.5	407.5 ± 184.7	415.9 ± 137.9
	foot	267 ± 82.7	291 ± 70	291 ± 85.9	foot	519.6 ± 221.6	522.7 ± 211.4	601.6 ± 245.4

BS = blood sampling followed by sensory measurements; CO = control procedure (sensory measurements only); SB = sensory measurements followed by blood sampling.

Table 2. Cronbach's alpha coefficients

	CPT			PT		
	oral cavity	hand	foot	oral cavity	hand	foot
5Hz	0.81	0.81	0.81	0.80	0.80	0.78
250Hz	0.81	0.81	0.81	0.80	0.79	0.78
2000Hz	0.81	0.81	0.81	0.80	0.78	0.82

The values were all over 0.78, which represent acceptable reliability.

Table 3. Percipients' characteristics

	DM (n=21)	NDM (n=35)	P
Sex (Male/Female)	12 / 9	17 /18	0.589
Age (years)	72.1±4.7	51.2±23.6	<0.001
Body mass index(kg/m^2)	23.1±4.4	21.8±3.0	0.210
Haemoglobin A_{1c}(%)	6.9±0.8	5.7±0.5	<0.001
Blood sugar level(mg/dl)	153.4±38.2	118.2±22.1	<0.001

Proportion of male and female in DM was analyzed by Chi-squared test.

Mean differences of the sex, age, body mass index, and blood sugar level between DM and NDM were analyzed by t-test.

Table 4. Summary results of analysis of covariance

Dependent variable	Mean Square	F	P-value
Age	3.192	0.00	0.985
DM	53715.3	6.281	0.013
Frequency	2373770.2	277.6	<0.001
Region	1461048.9	170.8	<0.001
DM*Frequency	395.424	0.44	0.642
DM * Region	23032.932	2.69	0.069
Frequency * Region	330457.184	38.6	<0.001
DM * Frequency * Region	931.4	0.11	0.98

*: interaction

F:Fisher distribution