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Measurement reliability of current perception threshold and pain threshold in parallel with blood sampling

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Abstract

The irritation and pain associated with oral blood sampling necessary to monitor glycemic control can alter oral sensation, but no studies have measured the reliability of oral sensation testing when performed concurrently with blood sampling. The primary and secondary aims of this study were to verify the measurement reliability of current perception threshold (CPT) and pain threshold (PT) tests performed before and after blood sampling and to investigate the differences in CPTs/PTs obtained from the oral cavity, hand, and foot, respectively. CPT/PT measurements were obtained from the oral mucosa and the tips of fingers and toes of 18 volunteers (10 males and 8 females, average age = 26.3 years) using electrical stimulation at frequencies of 5, 250, and 2,000 Hz. Participants also provided blood samples by pricking their own index fingers with a small needle. All subjects completed the following 3 conditions at once-weekly intervals as follows: (a) sensory measurements followed by blood sampling (SB block); (b) blood sampling followed by sensory measurements (BS block); (c) sensory measurements without blood sampling (CO control block). Cronbach's α coefficients were over 0.78 for the SB, BS, and CO blocks, and are considered to be acceptable for assuring measurement reliability. The oral cavity had significantly lower CPT/PTs than both hands and feet, such that the threshold increased in the order oral cavity < hand < foot. CPT and PT measurements performed concurrently with blood sampling are both reliable and region specific, and for which the oral cavity is the most sensitive testing region.

KEYWORDS

blood sampling, Cronbach's a, current perception threshold, pain threshold, reliability

1 | BACKGROUND

Diabetes mellitus (DM) is a worldwide public health issue that occurs in 40% of Japanese men and 30% of Japanese women over 70 years of age (Morimoto, Nishimura, & Tajima, 2010). DM can cause sensory loss related to peripheral nerve damage, known as neuropathy (Noor, Zubair, & Ahmad, 2015); the loss of sensation is associated with foot ulcers when repetitive pressure is applied (Alavi et al., 2014). Similarly, in dental research, Collin et al. (2000) have reported that denture wearers with DM have more frequent denture-related lesions than those without DM (Collin et al., 2000). This report agrees with our

own clinical experience. This susceptibility to ulcers of the oral mucosa covered by dentures might be a result of DM-induced sensory loss and could prevent denture wearers from consulting dentists for necessary denture adjustments. Given the large and still increasing size of this population and the potential health risks, it is very important to be able to measure oral sensation in individuals with DM.

There are several methods used to evaluate impaired perception in DM (Donaghue, Giurini, Rosenblum, Weissman, & Veves, 1995; Feng, Schlosser, & Sumpio, 2009; Kenny, Sigal, & McGinn, 2016; Stein, Eibel, Sbruzzi, Lago, & Plentz, 2013; Thakral et al., 2013; Thomsen, Cederlund, Speidel, & Dahlin, 2011). Of those methods, electrical

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current stimulation has been used for diagnosis of DM-induced sensory loss. The current perception threshold (CPT) and pain threshold (PT), evaluated with a Neurometer®, has been proposed as a quantitative method for assessing peripheral sensory nerve function (Katims, Naviasky, Rendell, Ng, & Bleecker, 1987). Using the standardized method of CPT, large myelinated A-beta fibers, small myelinated Adelta fibers, and unmyelinated C fibers are evaluated selectively at 2,000, 250, and 5 Hz frequencies, respectively. The validity of the method is comparable to the more popular standard sensory testing techniques, including thermal and vibration detection thresholds (Masson & Boulton, 1991; Pitei, Watkins, Stevens, & Edmonds, 1994). Furthermore, we have developed electrical current stimulation for use in oral sensory measurements and have reported that both CPT and PT can be useful in assessing changes in oral sensation in denture wearers (Ito, Kimoto, & Kawai, 2014; Kimoto et al., 2013; Nakashima et al., 2015). The CPT and PT measured by the device could potentially applied to oral sensory measurements in patients with DM as well.

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 of 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 (Meagher, Arnau, & Rhudy, 2001; Rhudy & Meagher, 2000). Despite our knowledge of these potential confounding factors, there are no reports assessing whether the act of blood sampling interacts with CPT and/or PT measurement. Therefore, to lay the foundation for a future study investigating whether DM increases sensory loss in the oral cavity of denture wearers relative to those denture wearers without DM, we first had to determine whether CPT/PT measurements obtained from the oral cavity, hand, and foot were reliable if done concurrently with blood sampling; this was the primary aim of the study. The secondary aim of this study was to investigate differences in CPTs and PTs obtained from the oral cavity, hand, and foot, respectively. We hypothesized that the CPT/PT measurements would be reliable (i.e., have acceptable Cronbach's a coefficients) in this patient population, although CPT/PT values would also likely be different between the oral cavity, hand, and foot.

2 | METHODS

2.1 | 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.

2.2 | 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 fingers and toes 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).



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

2.3 | 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.

2.4 | 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 μ l 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.

2.5 | 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?

2.6 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.

2.7 | 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.

3 | RESULTS

3.1 | 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, Bonferronicorrected *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

TABLE 1	CPT and PT	values according to e	each of the three	procedures and thr	ee measurement r	regions
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			CPT			PT		
		SB	BS	BS CO		SB	BS	со
5 Hz	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 ± 75.0
	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 ± 40.0	Foot	201.9 ± 101.2	193.1 ± 118.6	199.7 ± 101.2
250 Hz	Oral cavity	21.2 ± 22.5	24.5 ± 28.7	26.8 ± 31.0	Oral cavity	66.7 ± 54.5	72.5 ± 46.7	71.9 ± 63.1
	Hand	64.1 ± 22.4	67.7 ± 24.4	73.0 ± 36.3	Hand	174.3 ± 91.1	180.3 ± 102.7	179.6 ± 68.0
	Foot	125.0 ± 35.8	127.8 ± 33.1	129.8 ± 44.4	Foot	222.4 ± 84.0	239.2 ± 105.6	254.2 ± 84.9
2,000 Hz	Oral cavity	43.0 ± 37.3	41.0 ± 31.8	40.0 ± 27.6	Oral cavity	129.7 ± 86.5	131.3 ± 73.5	131.4 ± 93.9
	Hand	172.0 ± 34.8	176.0 ± 49.0	192.0 ± 47.4	Hand	408.0 ± 135.5	407.5 ± 184.7	415.9 ± 137.9
	Foot	267.0 ± 82.7	291.0 ± 70.0	291.0 ± 85.9	Foot	519.6 ± 221.6	522.7 ± 211.4	601.6 ± 245.4

Note. BS = blood sampling followed by sensory measurements; CO = control procedure (sensory measurements only); CPT = current perception threshold; PT = pain threshold; SB = sensory measurements followed by blood sampling.

TABLE 2 Cronbach's alpha coefficients

	СРТ					
	Oral cavity	Hand	Foot	Oral cavity	Hand	Foot
5 Hz	0.81	0.81	0.81	0.80	0.80	0.78
250 Hz	0.81	0.81	0.81	0.80	0.79	0.78
2000 Hz	0.81	0.81	0.81	0.80	0.78	0.82

Note. The values were all over 0.78, which represent acceptable reliability. Note. CPT = current perception threshold; PT = pain threshold



FIGURE 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). CPT = current perception threshold

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 \pm 46.5 (foot) 10^{-2} mA. Those at 250 Hz were 70.0 \pm 3.2 (oral cavity); 177.7 \pm 3.2 (hand); and 238 \pm 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 \pm 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.

3.2 | 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.



FIGURE 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). PT = pain threshold

4 | DISCUSSION

The present study 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 (Bland & Altman, 1997; Bosma et al., 1997). 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 increasing 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 (Tossani, 2013). 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/or fear was never evoked.

This was the first study that directly compared CPT/PT in the foot, hand, and oral regions, and that showed the CPT/PT 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. Ro et al. (1999); Takekuma, Ando, Niino, and Shimokata (2000); Kim, Kho, Kim, Lee, and Chung (2000); and Ogura, Kimoto, Yamaguchi, and Kobayashi (2007) studied CPT at the foot/finger, finger, face, and face/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 (Kim et al., 2000; Ogura et al., 2007; Ro et al., 1999; Takekuma et al., 2000). This suggests that our results are likely to be valid and that the oral region was the most sensitive region studied. The classical report describing Penfield's homunculus showed that the precise topography of cortical localization indicates that motor and sensory phenomena affecting a particular part of the body stimulate a nonoverlapping, discrete part of the cerebral cortical motor and sensory cortex, respectively (Schott, 1993). This could partially explain our finding that the three regions had different CPT and PT values.

The ages of the participants in our study were in a narrow range (average age = 26.3 years). Because human sensory perception changes with age, it is likely to be a confounder of sensory measurement. Ogura's group reported the CPT to be slightly decreased with age (Ogura et al., 2007). Although CPT/PT seemed to be influenced little by age, this should still be considered a limitation of this study. Future studies to analyze the conditions herein across a wide range of ages are needed.

To conclude, this study found that 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.

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CONFLICT OF INTEREST

There are no conflict of interests to declare.

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