Poor neck posture and longer working duration during root canal treatment correlated with increased neck discomfort in dentists with <5-years' experience in endodontics

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Abstract

Objective: This study investigated the effects of neck posture and working duration during each step of root canal treatment (i.e. opening the canal [OC], length determination, mechanical instrumentation, try main cone, and filling the root canal) on neck discomfort (ND) in dentists with <5-years' endodontic experience.

METHODS: Twenty-four dentists performed a one-visit endodontic treatment of an upper molar in a phantom head model. A video was recorded to evaluate the dentists’ neck postures using the Modified-Dental Operator Posture Assessment Instrument (M-DOPAI) and treatment duration. The M-DOPAI divides the dentists’ neck postures into three categories: acceptable, compromised, or harmful posture. The participants rated their ND using Borg’s CR-10 scale every 10 min. and at the end of each treatment step. The relationships between neck posture/treatment duration and Borg’s CR-10 scores were examined using partial correlation.

RESULTS: The number of compromised and harmful neck postures during the endodontic procedure ($r = 0.43$, $P = .04$) and treatment duration ($r = 0.58$, $P = .005$) significantly correlated with ND at the end of treatment. The number of compromised and harmful neck postures during the OC step ($r = 0.75$, $P < .001$) and the duration of the OC step ($r = .70$, $P < .001$) significantly correlated with ND at the end of the step.

CONCLUSION: Poor neck postures and long working duration during endodontic treatment correlated with ND among inexperienced dentists. Neck pain interventions should focus on neck postures and work duration during root canal treatment, particularly in the OC step.

KEYWORDS: neck discomfort, endodontic task, endodontist, musculoskeletal disorder, posture
INTRODUCTION

Musculoskeletal disorders (MSDs) are a significant health problem for dentists. Approximately 69% of dentists experienced musculoskeletal symptoms in the past 12 months. The annual prevalence of MSDs among Thai dentists was 78–83%. A previous study of 2531 Iranian dentists reported neck pain (52%) as the most common MSD followed by lower back pain (37%) and shoulder pain (33%). A study of 750 dentists in New Zealand found that the most common sites of MSD were the neck (59%), followed by the lower back (57%) and shoulder (45%). Approximately 15% of dentists left clinical practice or reduced working time due to MSDs.

Endodontics is a dental specialty concerning the diseases of the dental pulp and tissues surrounding the tooth roots. Endodontic or root canal treatment performed by endodontists involves treating the soft pulp tissue inside the tooth and is physically demanding, involving repetitive and prolonged precise movements with the use of vibrating and ultrasonic instruments. Root canal treatment can be divided into five steps. The first step is opening the canal using high- and low-speed handpieces to gain coronal access and locate the root canal entrances. This step is one of the most challenging aspects of endodontic treatment, but is key to successful treatment. The second step is canal length determination using an electronic apex locator followed by confirmation of the canal length with an undistorted periapical radiograph to measure the tooth length. The third step is mechanical instrumentation using hand files or rotary instruments to clean and remove the necrotic pulp tissue and infected dentin. The fourth step is trying in the main cone to select a master cone that fits the shape of the apical part of the root canal. The fifth step is filling the root canal using core material with various sealers and techniques to 3-dimensionally fill the entire root canal system to prevent microorganisms from entering and reinfecting the root canal system. While performing root canal treatment, endodontists need to maintain their head in a forward posture and bend their neck for a long period.

Musculoskeletal disorders are common among endodontists. A Greek study of 120 endodontists indicated that the 1-year prevalence of MSDs was 61% and the sites of symptoms with the highest prevalence were the neck (30%) and lower back (30%). Previous studies in China and Thailand found that the neck was the body region most commonly reported with MSDs in endodontists. Pain and dysfunction due to MSDs greatly affect endodontists by incurring lost workdays and decreased productivity.

Several work-related factors are associated with MSDs in endodontists, including awkward positions, static postures, work experience, body mass index, frequency of using loupes and a microscope, and psychosocial work environment. A cross-sectional study that evaluated the ergonomic conditions of 60 dental students using the rapid entire body assessment method (REBA) found that endodontic treatment was associated with the highest REBA score, while reconstructive treatment was associated with the lowest score. Endodontists have been found to adopt poor neck postures for long periods (~1 h per case) during treatment. Being in the forward-head posture and bending their neck for a long period increases stress and strain on the musculoskeletal structures in the neck, which leads to ischemia and neck pain. Working in the same position for longer than 40 min significantly increases the risk of MSDs.

Musculoskeletal discomfort is a strong predictor of MSDs. A prospective cohort study found that a discomfort level of 2 or more, assessed by Borg’s CR-10 scale, predicted future neck, shoulder, and lower back pain in healthy office workers. However, to date, there has been no study regarding the correlation between work-related factors, that is, neck postures and working duration, and perceived neck discomfort during each step of root canal treatment. The aim of this study was to investigate the relationship between neck postures as well as working duration and perceived neck discomfort during each root canal treatment step. A high-risk group of dentists was selected, that is dentists with <5-years’ endodontic experience.

MATERIALS AND METHODS

2.1 Study population

The sample size was calculated using the correlation sample size formula. The minimum sample size needed to guarantee a statistical power of 95% was 22 participants and 10% of the sample size for compensation was 2. The final sample size required was 24 participants. A convenience sample of 27 dentists enrolled in the endodontic postgraduate program at the Faculty of Dentistry, Chulalongkorn University, received and accepted the invitation to participate in the study. They were screened for eligibility.

Participants were included if they were dentists, 25–35 years old, had <5-years’ endodontic work experience, and worked as endodontists more than 4 days per week. Participants were excluded if they had a history of trauma, accidents, or surgery in the neck region, had been diagnosed with MSDs or mental problems in the previous 6 months, had a body mass index (BMI) greater than 25 kg/m², or were pregnant. A screening questionnaire was used to determine the eligible participants.
The study protocol was approved by the Faculty of Dentistry Human Ethics Committee (No.087/2019). The participants were provided with details of the study, including the objectives of the study, data collection process, and their right to withdraw from the study at any time. The participants provided informed consent prior to data collection.

2.2 | Experimental procedure

The participants performed a single root canal treatment session (ranging from 1–3 h), starting from accessing the canal to obturating the root canal, on a dental simulator mannequin that was an adult-sized phantom head with an upper dental arch. The model had an oral cavity that included natural teeth, allowing for the replacement of the maxillary left first or second molars each time that the experiment was performed, because these teeth were set in individual models. Twenty-four maxillary left first or second molars extracted from patients at the Department of Maxillofacial Surgery at the Faculty of Dentistry, Chulalongkorn University were used in this study. The teeth were radiographed to ensure that they had a canal without calcifications. The teeth had the surface calculus removed with ultrasonic scalers and polished using a brush with pumice paste (Figure 1). The teeth were fixed in 3 layers of materials. The inner layer was water-soaked gauze, the middle layer was alginate, and the outer layer was pink wax and set in a self-cured acrylic resin block that was prepared in a silicone matrix.

Prior to data collection, the participants were allowed to familiarize themselves with the phantom head model and to adjust the dental chair as they desired. The participants’ acromion process and tragus were marked. A video camera (JVC Everio™, icamplus, Japan) placed on a tripod with a 3-meter distance between the camera’s focal lens and the center of the participants’ body was used to record the neck postures during treatment and the treatment duration. The participants were asked to inform the researcher when they finished each step of root canal treatment; that is, opening the canal, length determination, mechanical instrumentation, trying in the main cone, and filling the root canal. The participants were instructed to use an apex locator to confirm the working length without a radiograph during endodontic treatment to prevent them leaving the workstation and were not allowed to use a microscope or loupes.

2.3 | Questionnaire

A questionnaire was used to gather data on personal, professional, and psychosocial factors. Personal data were sex, age, BMI, and exercise frequency in the past 12 months. Professional data comprised endodontic experience, working hours per day, working days per week, loupe using frequency in the past 12 months and microscope using frequency in the past 12 months. Psychosocial factors consisted of work-related psychosocial factors assessed by the Thai Job Content Questionnaire, which comprises 45 questions in the following six domains: job control (9 items; score ranging from 18–72; higher job control scores represent higher job control), psychological job demand (13 items; score ranging from 18–72; higher demand scores represent higher psychological demands), physical job demand (3 items; score ranging from 3–12; higher demand scores represent higher physical demands), job security (8 items; score ranging from 4–16; higher scores represent higher job security), social support (4 items; score ranging from 18–72; higher scores represent higher social support), and hazards at work (8 items; score ranging from 8–24; higher scores represent higher hazards at work).

![Figure 1](image-url) (A, B) Setup of a left maxillary molar tooth in the cast in the phantom head. (C) Setup of a left maxillary molar tooth in self-cured acrylic resin block.
2.4 | Outcome measures

2.4.1 | Neck posture

The Modified-Dental Operator Posture Assessment Instrument (M-DOPAI) was used to assess the dentist’s neck postures during work. The profile view of the neck working postures during root canal treatment was assessed for each participant (Figure 2). The M-DOPAI score ranges from 1–3 and higher scores indicate a greater biomechanical risk of developing injuries. Ideal postures received 1 point (<20° neck flexion), compromised postures received 2 points (20°–45° neck flexion), and harmful postures received 3 points (>45° neck flexion). Neck working postures were assessed using the M-DOPAI at baseline (before starting the treatment) and every 1 min during root canal treatment until the end of treatment.

Prior to data collection, the intra-rater and inter-rater reliability of the neck working posture assessment using M-DOPAI were determined using 150 randomly selected pictures of neck working postures of the participants. The intra-rater reliability was determined by the researcher evaluating neck working postures using M-DOPAI twice 7 days apart. The inter-rater reliability was conducted by the researcher and a clinician with 20-years’ experience in musculoskeletal physiotherapy evaluating neck working postures using the M-DOPAI.

2.4.2 | Duration of treatment

The duration of each endodontic treatment step (i.e. opening the canal, length determination, mechanical instrumentation, trying in the main cone, and filling the root canal) (min) and total treatment duration (min) were retrieved from a video camera for each participant.

2.4.3 | Perceived neck discomfort

Perceived neck discomfort was determined using Borg’s CR-10 scale, which ranges from a score of 0 (no discomfort) to 10 (extremely strong discomfort). Neck discomfort was collected at baseline, every 10 min during root canal treatment, at the end of each treatment step, and at the end of root canal treatment.

2.5 | Statistical analysis

The data were analyzed using the IBM SPSS statistical program for Windows version 22.0. The M-DOPAI intra-rater and inter-rater reliability were calculated by Cohen’s Kappa coefficient. The normal distribution of the continuous data was evaluated with the Shapiro–Wilcoxon test. The participants’ characteristics and outcome measures were described as means or proportions.

The relationships between the number of compromised and harmful postures during endodontic treatment and Borg’s CR-10 scores, and between the duration of endodontic treatment (min) and Borg’s CR-10 scores were examined using partial correlation. A Pearson’s correlation analysis was performed to evaluate the influence of each confounding factor on the Borg’s CR-10 scores at the end of treatment. As a result, job control and Borg’s CR-10 scores at baseline were adjusted in the partial correlation. The correlation coefficients >0.5 were considered as a strong correlation, those from 0.3–0.5 as a moderate correlation, and those from 0.2–0.3 as a weak correlation.

One-way analysis of covariance (ANCOVA) using the number of compromised and harmful neck postures and total endodontic treatment duration as covariate was conducted to investigate the differences between Borg’s CR-10 scores at baseline and the end of treatment. Statistical significance was set at the 5% level.

3 | RESULTS

Twenty-four of 27 of those who agreed to participate in the study were eligible. Three participants were excluded because they had a BMI >25 kg/m². Cohen’s Kappa coefficient of the intra-rater and inter-rater reliability was 0.83 and 0.81, respectively, indicating good intra-rater and inter-rater reliability for the M-DOPAI outcomes.

The sample population comprised mainly young adult females with an average endodontic experience of 2.8 years (Table 1). Their average BMI was in the normal range for Asians. Most participants (19 out of 24 participants) reported residual neck discomfort.

The average duration of the root canal treatment was 63.6 min with the average duration of the various treatment steps ranging from 8.2–15.2 min.

The average number of acceptable, compromised, and harmful neck postures during endodontic treatment was 47, 61, and 39, respectively. The mode of the M-DOPAI scores during the endodontic treatment steps, except for the filling the root canal step, was 2 (Table 2).

3.1 | Neck working posture and perceived discomfort

The results demonstrated that the participants adopted compromised neck postures, according to the M-DOPAI, most of the time during endodontic treatment (Figure 3).
The participants adopted harmful neck postures during the opening the canal, mechanical instrumentation, trying in the main cone, and filling the root canal steps. The percentages of acceptable, compromised, and harmful posture (i.e. the number of acceptable, compromised, or harmful posture divided by total number of all postures) during endodontic treatment was 23%, 43%, and 34%, respectively.

Partial correlation analysis revealed a significant association between the number of compromised and harmful posture during treatment and Borg’s CR-10 score at the end of the endodontic treatment \((r = 0.43, P = .04)\). A significant correlation was found between the number of compromised and harmful postures during length determination \((r = 0.13, P = .57)\), mechanical instrumentation \((r = 0.25, P = .26)\), trying in the main cone \((r = 0.18, P = .42)\), or filling the

![Image](55°)

**Figure 2** The endodontic working posture. A grid was added into the images of the dentists’ neck postures. The green line indicates the ideal head and neck axis (Y axis) or straight head and neck that was perpendicular with the floor. The red line connecting the tragus to the acromion process illustrates the working head and neck axis. The angle between the green and red lines was measured.

| Characteristic                              | Mean (SD) | Min–Max |
|--------------------------------------------|-----------|---------|
| Personal data                              |           |         |
| Sex: male (%)                              | 12.5      |         |
| Female (%)                                 | 87.5      |         |
| Age                                        | 28.5 (2.5)| 20–35   |
| BMI (kg/m²)                                | 20.1 (2.6)| 16.6–25 |
| Exercise frequency in the past 12 months (%)|           |         |
| Never                                      | 58.3      |         |
| Regularly                                  | 41.7      |         |
| Professional data                          |           |         |
| Endodontic experience (yr.)                | 2.8 (1.0) | 1–4     |
| Working hours per day (hours per day)      | 5.7 (1.4) | 3–8     |
| Working days per week (days per week)     | 5.5 (0.7) | 5–7     |
| Loupe using frequency in the past 12 months (%)|           |         |
| Very often                                 | 0         |         |
| Often                                      | 12.5      |         |
| Rarely                                     | 12.5      |         |
| Very rarely                                | 40        |         |
| Microscope using frequency in the past 12 months (%)|           |         |
| Very often                                 | 4.2       |         |
| Often                                      | 42.2      |         |
| Rarely                                     | 8.3       |         |
| Very rarely                                | 8.3       |         |
| Psychosocial factors                       |           |         |
| Job control                                | 50.0 (5.0)| 42–60   |
| Psychological job demand                  | 41.1(6.0)| 27–57   |
| Physical job demand                       | 7.6 (1.5) | 5–11    |
| Job security                               | 5.8 (1.1) | 4–8     |
| Social support                             | 35.5 (8.4)| 18–51.8|
| Hazards at work                            | 13.8 (4.5)| 9–30    |
| Duration of endodontic treatment (min)     | 63.6 (19.9)| 28–100 |
| Duration of each treatment step (min)      |           |         |
| Open canal                                 | 15.2 (12.1)| 3–57   |
| Length determination                       | 9.3 (5.3) | 3–23    |
| Mechanical instrumentation                 | 14.3 (5.2)| 6–27    |
| Try main cone                              | 8.2 (6.3) | 2–28    |
| Filling root canal                         | 13.8 (8.6)| 2–40    |
| Borg’s CR-10 score at the neck             |           |         |
| At baseline                                | 2.2 (1.8) | 0–5     |
| at the end of treatment session            | 5.7 (2.2) | 1.5–9   |
| Number of acceptable posture (time)        | 47 (38.6) | 0–168   |
| Number of compromised posture (time)       | 61 (33.8) | 18–181  |
| Number of harmful posture (time)           | 39 (22.2) | 2–90    |

Abbreviation: BMI, body mass index.
root canal \((r = 0.23, P = .31)\) steps and Borg's CR-10 scores at the end of the corresponding steps.

### 3.2 Working duration and perceived discomfort

The results revealed that neck discomfort increased over time (Figure 3). Partial correlation analysis revealed a significant association between the total duration of endodontic treatment and Borg’s CR-10 scores at the end of treatment \((r = 0.58, P = .005)\). A significant correlation was found between the duration of the opening the canal step and Borg’s CR-10 scores at the end of the opening the canal step \((r = .70, P < .001)\). No correlation was found between the duration of the length determination \((r = 0.10, P = .66)\), mechanical instrumentation \((r = 0.17, P = .44)\), trying in the main cone \((r = 0.16, P = .48)\), or filling the root canal \((r = 0.36, P = .10)\) steps and Borg’s CR-10 scores at the end of the corresponding steps.

### 3.3 Perceived discomfort at baseline and the end of treatment

ANOVA determined a significant difference between the Borg’s CR-10 scores at baseline \((\text{mean} \pm \text{SD} = 2.19 \pm 1.83)\) and at the end of treatment \((\text{mean} \pm \text{SD} = 5.73 \pm 2.24)\) \((F_{1,22} = 4.35; P = .05)\).

### 4 DISCUSSION

The present study demonstrated that dentists with <5-years’ endodontic experience frequently adopted either compromised or harmful postures during endodontic treatment. The number of compromised and harmful postures while providing treatment significantly correlated with their neck discomfort at the end of the treatment session. There was a strong correlation between treatment duration and neck discomfort at the end of the treatment session. We also found that the number of compromised and harmful neck postures during the opening the canal step and duration of the opening the canal step strongly correlated with neck discomfort at the end of this step.

Neck discomfort increased over time during endodontic treatment in dentists with <5-years’ endodontic experience and reached an average of 5.7 on the Borg’s CR-10 scale at the end of the treatment session. To our knowledge, no study has investigated neck discomfort in dentists performing endodontic treatment. A survey of dentists, dental assistants, and dental technicians with neck pain revealed that they reported an average pain intensity of 4, ranging from 2–8, on a visual analogue scale during the past year. A study in 27 Swedish dentists with musculoskeletal complaints revealed that they reported a pain intensity of 2.8–7.3 on a visual analogue scale during

| TABLE 2 Mode M-DOPAI scores (the score that occurs most frequently) at the neck during endodontic treatment \((n = 24)\) |
|---------------------------------------------------------------|
| M-DOPAI scores during endodontic treatment (points)           |
| Mode   | Min—Max |
|--------|---------|
| 2      | 1–3     |
| M-DOPAI scores in each step of endodontic treatment (points) |
| Opening canal     | 2       |
| Length determination | 2       |
| Mechanical instrumentation | 2       |
| Trying main cone  | 2       |
| Filling root canal | 3       |

M-DOPAI: modified-dental operator posture assessment instrument.
Our results demonstrated a positive correlation between the number of compromised and harmful postures during endodontic treatment and neck discomfort at the end of the treatment session. These findings are in line with a previous study showing that 33% of endodontists regularly adopted awkward postures while working.8 Endodontists who regularly adopted awkward postures during clinical practice have been found to be at risk of musculoskeletal disorders compared with those who did not.8 A study of 65 dentists with neck pain found that pain intensity at the neck increased as their posture worsened while restoring upper teeth.31 A systematic review and meta-analysis study indicated that insufficient training, unsuitable working facility design, lack of frequent supervision of correct ergonomic working positions, and work-related stress may cause the adoption of unsuitable postures during work, leading to the development of MSDs.6

It was reported that relatively few endodontists in Thailand use a microscope (28.6%) and loupes (17.5%) in their practice,9 partly because they are expensive. Previous studies found that endodontists who used microscopes and loupes reported a lower prevalence of MSDs9,32 The authors hypothesized that the work distance created by the loupes allowed for maintaining optimal posture.9,32 Thus, the participants in this study did not use a microscope and loupes to reflect the clinical practice of most dentists in Thailand. A study of the prevalence of painful MSDs in Chinese dentists indicated that ergonomic training courses and physical activity might be beneficial for dentists in alleviating or preventing MSDs.33 Based on our findings, effective measures are required to prevent dentists from adopting compromised and harmful neck postures during endodontic treatment. Possible effective interventions may include the use of microscopes and loupes as well as an ergonomic training program.

The results demonstrated a positive correlation between the duration of endodontic treatment and neck discomfort at the end of the treatment session. A previous study reported that pain intensity increased with the amount of time spent in clinical practice.34 A survey of 356 Serbian dentists found that working in the same position for longer than 40 min significantly increased the odds of musculoskeletal pain.18 A cross-sectional study of 220 Thai dentists demonstrated that full-time dentists had higher odds of experiencing musculoskeletal pain in the past 7 days compared with part-time dentists.4 Endodontists usually fix their neck and head for a long time (1 h per case), leading to musculoskeletal complaints in several body regions.15 Prolonged muscle activation in a static sitting position may lead to localized muscle tension, muscle strain, muscle fatigue, and other soft tissue damage, causing impaired motor coordination and control as well as increased mechanical stress on ligaments and intervertebral discs.35 If damaged tissues do not have adequate rest time to repair, the tissue damage can accumulate faster than it can repair itself.14 One intervention that has been found to reduce the onset and intensity of perceived musculoskeletal discomfort is short, but frequent, active breaks.36 A randomized controlled trial revealed that taking active rest breaks reduced the pain intensity or disability level in those experiencing neck and low-back pain.37 Previous studies found that frequent active breaks with a postural change, with a break duration of approximately 3 min, were beneficial in reducing pain, discomfort, and fatigue in the neck and low back.37 A study reported that dentists worked approximately 8 h a day with only one break, while 30% of them had no break.38 Therefore, frequent active breaks of short duration should be introduced to effectively reduce perceived neck discomfort during work and thereby prevent the onset of neck pain among dentists.

The present study is among the first of its kind to investigate the relationship between various endodontic treatment steps and perceived neck discomfort. Our results revealed a positive correlation between the number of compromised and harmful neck postures during the opening the canal step and neck discomfort at the end of the canal step. A positive correlation between the duration of the opening the canal step and neck discomfort was also found. A study investigating the posture of 18 right-handed endodontists during root canal treatment of the maxillary right first and second molars using rotary and manual instrumentation revealed that the rotary technique presented greater postural demands compared with the manual technique.15 In the manual technique, greater activity was observed in the anterior and medium deltoid, as well as in the short thumb abductor muscles.15 Endodontists’ vision field is limited by the oral cavity opening, tooth morphology, poor access, and lack of a direct view.15 When dentists’ attempt to gain a direct view of the treated teeth, awkward postures are frequently adopted, such as flexing the neck.8 During the opening the canal step, dentists are required to be in a static posture for a long time because gaining access to a complex root canal system is the first, and arguably the most important, phase of any nonsurgical root canal procedure.39 Thus, the opening of the canal step requires high precision and concentration from dentists to locate the canal orifice. One solution to reduce neck bending is to use magnification lenses to increase their ability to observe the small canal opening, thereby preventing dentists from adopting awkward postures during treatment.40
4.1 Limitations of the study and further studies

Several limitations should be considered when interpreting the results. This study was conducted in healthy dentists with specific characteristics, including being 25–35 years old, having <5 years' endodontic experience, and having a high risk of neck pain. Therefore, extrapolating the results from this study to other groups of dentists should be made with caution. Further research on the effects of posture and work duration on neck discomfort in another dentist population or in endodontists with >5-years’ experience are suggested. Furthermore, discomfort is subjective, possibly leading to data inaccuracy. Some dentists may be more sensitive to discomfort than other dentists. Therefore, there is a risk of over- or under-reporting of Borg’s CR-10 scores. Thus, further studies using an objective assessment are recommended to increase data accuracy. Last, the sample population was recruited by convenience sampling and comprised mainly females. In addition, a small sample size was recruited in this study, which had the risk of baseline imbalance between males and females. Further research should include a larger sample size or pair matched randomization.

5 CONCLUSION

Dentists with <5-years’ of experience frequently adopted compromised and harmful postures during endodontic treatment. Neck discomfort among our participants increased over time. Neck discomfort significantly correlated to the number of compromised and harmful postures as well as the duration of endodontic treatment. Neck discomfort at the end of the opening the canal step highly correlated to the number of compromised and harmful neck postures during the opening the canal step as well as the duration of the opening the canal step. Interventions to reduce neck discomfort should include attempts to maintain good neck posture and to promote rest breaks during work.

AUTHOR CONTRIBUTIONS

The authors have contributed in the following ways: WA provided the concept/research design, data collection, data analysis, and manuscript writing. UC and PJ provided the concept/research design, data analysis, and manuscript writing. All authors read and approved the final manuscript.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

DISCLOSURE

Approval of the research protocol: The study was approved by the University Human Ethics Committee. Informed consent: All participants were given information about the study and signed a consent form prior to their participation. Animal studies: N/A. Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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