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Comparative evaluation of clinical diagnostic trials for pulpal status assessment and establishment of reference values in healthy young adults

Компаративна процена клиничких дијагностичких тестова за испитивање статуса пулпног ткива и утврђивање референтних вредности код здравих младих особа

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Компаративна процена клиничких дијагностичких тестова за испитивање статуса пулпног ткива и утврђивање референтних вредности код здравих младих особа

SUMMARY

Introduction/Objective Traditional diagnostic methods for assessing pulp vitality are commonly used but lack precision and reliability, whereas recent advancements offer non-invasive alternatives, focusing on pulp tissue blood flow and oxygen saturation. This study was designed to evaluate and compare the reliability of all available clinical diagnostic methods for assessing pulpal status and to establish reference values in healthy young individuals.

Methods This cross-sectional observational study enrolled 25 voluntary participants (27.8 ± 5.2 years old), 150 upper front teeth. The participants were healthy individuals, non-smokers, teeth with a mature apex, no or minimal restorations, periodontally healthy, and radiographically visible pulp chambers. Various diagnostic tests were conducted, including electric, cold, and hot pulp testing, Laser Doppler flowmetry, and pulse oximetry (EPT, CPT, HPT, LDF, and PO); The reference values were established.

Results Statistically significant differences were observed among all tooth groups (central incisors, lateral incisors, and canines) for EPT, LDF, and PO. In contrast, CPT and HPT showed no statistically significant differences between the tooth groups. The mean values across all patients were as follows: EPT – 17.7 ± 5.4, PO – 80.6 ± 1.8%, and LDF – 4.6 ± 1.6 perfusion units. Correlation analysis showed no significant relationships between all tests.

Conclusion Modern diagnostic techniques show promise in offering more reliable results. Establishing reference values is essential for improving diagnostic accuracy. These values will not only enhance clinical decision-making but also serve as a foundation for future investigations.

Keywords: clinical diagnostic methods; laser Doppler flowmetry; pulse oximetry; electric pulp testing; dental pulp

САЖЕТАК

Увод/Циљ Традиционалне дијагностичке методе за процену сензибилитета пулпе се уобичајено користе, али им често недостаје прецизност и поузданост, док савремена достигнућа пружају неинвазивне алтернативе, фокусирајући се на проток крви и засићености пулпе кисеоником. Ова студија је осмишљена са циљем да процени и упореди поузданост свих доступних клиничких метода за процену статуса пулпе, и да успостави референтне вредности код здравих младих особа.

Методе Ова студија пресека обухватила је 25 добровољаца (27,8 ± 5,2 година,), 150 горњих предњих зуба. Учесници су били здрави, непушачи, са завршеним растом корена, без или са минималним рестауративним захватима, пародонтолошки здрави и са радиографски видљивим пулпним коморама. Извршени су различити дијагностички тестови: електро-тест, тест на хладно и топло, ласерска доплер мерач портока крви и пулсна оксиметрија (ЕТ, ТХ, ТТ, ЛДМПК и ПО). Утврђене су референтне вредности.

Резултати Статистички значајне разлике су уочене између свих група зуба (централни секутић, латерални секутић и очњак) за ЕТ, ЛДМПК и ПО. Насупрот томе, ТХ и ТТ нису показали статистички значајне разлике између група зуба. Просечне вредности за све испитанике биле су: ЕТ – 17,7 ± 5,4, ПО – 80,6 ± 1,8%, и ЛДМПК – 4,6 ± 1,6 PU. Корелациона анализа није показала статистички значајне везе између тестова.

Закључак Савремени дијагностички тестови показују велики потенцијал у пружању поузданијих резултата. Успостављање референтних вредности је од суштинског значаја за унапређење дијагностичке прецизности. Ове вредности не само да ће унапредити клиничко доношење одлука, већ ће послужити и као основа за будућа истраживања.

Кључне речи: клиничке дијагностичке методе; ласер доплер проток крви; пулсна оксиметрија; електро-тест; зубна пулпа

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INTRODUCTION

Dental caries remains one of the most prevalent diseases in the orofacial region and significantly contributes to dental pulp disease [1]. Pulpitis, the inflammation of the pulp tissue, is a common

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sequela of dental caries and can lead to significant discomfort in affected individuals [2]. Although there is growing interest in the need for improved classification and understanding of pulp tissue health [3] pulpitis is currently categorized as reversible or irreversible, depending on the extent of tissue damage [4].

Reversible pulpitis involves initial inflammation that can heal and restore. Patients typically have mild to moderate, localized pain triggered by stimuli, though some may experience no pain, diagnosed by subjective or objective complaint [4]. In contrast, irreversible pulpitis is marked by permanent pulp damage. Acute irreversible pulpitis presents with rapid-onset, intense pain, often spontaneous and hard to localize, with possible heat sensitivity, swelling, and chewing difficulties. Chronic irreversible pulpitis progresses slowly with dull, recurrent pain, usually triggered by hot or cold stimuli [4].

Establishing an adequate diagnosis is one of the keys to successful therapy. However, the lack of objective criteria that indicate actual state of the pulp tissue presents a challenge in making adequate decisions during treatment [5]. Historically, there has been a tendency to opt for vital pulp extirpation, which has contributed to the relatively low success rates of endodontic procedures and increased need for retreatment [6].

To address this issue, the European Society of Endodontology initiated a project called "S3 Clinical Guidelines," aimed to develop evidence-based guidelines for the diagnosis and treatment of pulp and periapical diseases [7]. As part of this project, Donnermayer et al. [8] conducted a systematic review that evaluated the effectiveness of diagnosing pulp diseases using various diagnostic tests [7]. The authors concluded that the existing scientific knowledge provides very low level of evidence on the accuracy and reproducibility of the current diagnostic tests and lack of reliable prognostic indicators that enable a reliable preoperative assessment of the outcome of vital pulp treatment. This further complicates the diagnostic process and makes it difficult to predict the success of therapy [8]. Before any test, meticulous and detailed anamnesis is conducted to reveal pain history, suggesting potential inflammatory state of the pulp. The most frequently used diagnostic tools for evaluating the state of the pulp were sensitivity tests (electric pulp testing, cold pulp testing, and heat pulp testing (EPT, CPT, HPT)) [9]. Despite the widespread use, their lack of precision and reproducibility represent their main limitations, primarily due to the subjective response of the patient, the interpretation of the dentist and the inability to detect blood flow in the pulp [10, 11]. In order to overcome those limitations, modern diagnostic tools, Laser Doppler blood flowmeters (LDF) and Pulse oximeters (PO), were introduced into the practice. These methods are based on the assessment

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of the blood flow in the pulp and its oxygen saturation without relying on subjective responses of the patients and personal interpretation of the dentist [12–15].

While many studies have assessed individual diagnostic tools for pulp tissue [8], none have simultaneously compared all conventional and modern methods or established reference values in healthy young individuals.

This study aimed to compare the reliability of all clinical diagnostic methods (EPT, CPT, HPT, LDF, PO) for pulpal assessment and to establish reference values in healthy young individuals for future correlation with pulp-derived laboratory tests.

METHODS

This observational cross-sectional study was conducted at the Department of Restorative Odontology and Endodontics, School of Dental Medicine, University of Belgrade, Serbia. All data were collected from January to September 2024. This study was approved by the Local Ethical Committee (protocol number: 36/36). Prior to any testing, all participants fulfilling the inclusion criteria were informed about the study's objectives and procedures, and provided written informed consent. This investigation enrolled 25 voluntary participants (27.8 ± 5.2 years old, 15 females and 10 males) comprising a total of 150 upper front teeth. The sample size was calculated using a power analysis to ensure sufficient statistical power for detecting significant differences. The calculation was based on a previous pilot study and the variability of the key measures.

Participants met the following inclusion criteria: i) healthy individuals (ASA I), ii) non-smokers, iii) mature apex, iv) no or minimal restoration on upper front teeth, v) periodontally healthy, and vi) radiographically visible coronal pulp chamber. Exclusion criteria included: i) presence of any systemic disease, ii) smokers, iii) caries or massive restorations, iv) discolorations of the tooth, v) radiographically invisible coronal pulp chamber, vi) radiographical signs of periapical lesion and vii) orthodontically treated teeth.

Patients were warned to abstain from foods and beverages for at least 2 hours before attending. All measurements were performed in a temperature-controlled room using the same unit and keeping the patient's head in the same position, resting for 10 min in the unit before the measurements.

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Each patient underwent five pulp tests. EPT, CPT, HPT, and PO were performed with 15-minute intervals. Tests were repeated twice by the same operator (EKL) to reduce bias, and results were presented as mean values. Any unclear responses prompted retesting.

For CPT no. 2 cotton pellet with a refrigerant spray (1, 1, 1, 2-tetrafluoroethane) (Cold Spray [ROEKO Endo-Frost]; Coltene Whaledent, Cuyahoga Falls, OH, USA) was used and placed onto the middle third of the buccal surface for 18 seconds or until the participant raises a hand to indicate a cold sensation, with the standardized temperature of -26°C [16].

As for the HPT heated gutta-percha rod was placed on the middle third of the buccal tooth surface for 18 seconds or until the participant raises a hand to indicate a hot sensation, with a standardized temperature of 80°C [16].

EPT was performed using a digital Pulp Tester (Analytic Technology Pulp Tester; Analytic Technology, Redmond, WA, USA). The tooth was air dried and isolated with cotton rolls to prevent false responses from surrounding tissues. The lip clip was attached to the lip, and the electrode was placed on the middle third of the buccal surface. A slow increase in the current, calibrated at medium speed, permitted patients to report any pain sensation. Detected stimulus threshold was recorded as a positive response, and the exact value was noted [16, 17].

LDF is a semi-quantitative non–invasive method used for the assessment of the pulpal blood flow (PBF). Red light with a wavelength of 632.8 nm was produced by 1 – mW helium – neon laser diode within the flowmeter (PeriFlux System PF 5001, Perimed, Jarfalla, Sweden) and transmitted to the tooth surface along the fiber-optic conductor inside a round probe (407-2, Perimed) with a cross-sectional diameter of 1 mm. The probe simultaneously received the reflected and scattered light via afferent optical fiber, which was then registered by a photodetector in the flowmeter. According to the Doppler effect, light reflected from moving red blood cells shifts in frequency, allowing calculation of their concentration and velocity. This shift indicates tissue blood flow, expressed in semiquantitative perfusion units (PU). Before data collection, a colloidal suspension of latex particles (Perimed Motility Standard) was used to calibrate the flowmeter on the wide band to a specific value of 250 PU. The artifact filter was activated with a sampling frequency set at 32 Hz. PBF values were stored on a computer software (Perisoft, Perimed) for further analysis. To stabilize and obtain reproducible position of the probe on the tooth surface, custom-made splints were prepared (Zhermack dental Hydrorise putty and regular body impression material, Faclon Medical impression trays, Vacum former EV2 3A Medes and Gasket for splint 080 2mm 1/12), and used to secure the position of probe holder (PH07-6, Perimed). Aperture for the probe holder was positioned labially on the

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cervical third of the tooth crown, at least 1 mm from the gingival margin (Figure 1). Measurements were repeated three times with 1 minute pause, and the mean value was obtained as a final result, all performed in a quiet room with constant ambient temperature [13, 14, 18].

For PO testing, the fifth-generation Nellcor OxiMax 550 pulse oximeter (Tyco Healthcare Group LP) was used. The OxiMax system's "sensor message" function analyzes data from the sensor, using a proprietary algorithm that interprets parameters stored on the sensor's memory chip and evaluates real-time signal characteristics from the patient. A Nellcor OxiMax™ Dura–Y D-YS multisite oxygen sensor (Tyco Healthcare Group LP) was chosen due to its smaller dimensions, fitting better to the mesio-distal width of human permanent teeth. To ensure consistent and accurate sensor placement, a custom-designed pulse oximeter sensor holder was developed, designated as the Pulse Oximeter Dental Probe (Figure 2). It was positioned on each tooth, aligning so that the sensor's light beam traveled from the buccal to lingual surfaces through the middle third of the tooth crown. After 30 seconds of monitoring each tooth, values were recorded. An oxygen saturation reading within the range of 75–90% indicated a positive response, while any value below 75% indicated a negative response [11, 12, 19].

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 29.0 (IBM Corp., Armonk, NY, USA). Data were expressed as means ± standard deviations for continuous variables and as frequencies and percentages for categorical data. The normality of data distribution was assessed using the Shapiro–Wilk test. For normally distributed data, one-way analysis of variance (ANOVA) with Bonferroni post hoc correction was used to compare mean values across the three tooth groups. Non-normally distributed variables were analyzed using the Kruskal–Wallis test, and pairwise comparisons were performed using the Mann–Whitney *U* test with Bonferroni adjustment (corrected significance threshold: $p < 0.016$). Categorical variables were compared using the chi-square test. Pearson correlation coefficients were calculated to assess relationships between diagnostic test parameters (EPT, PO, and LDF), with statistical significance set at $p < 0.05$.

RESULTS

The mean values of the investigated parameters for each group of teeth are presented in Figure 3. All teeth have responded positively on CPT and HPT. When analyzing the mean values

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across all patients for each of the three tests (EPT, LDF, and PO), distinct patterns emerged. For EPT, the average threshold was 17.7 ± 5.4, indicating a typical level of electrical stimulus required to provoke a sensory response from the pulp. This reflects the overall excitability of pulpal nerve fibers across the sample. For PO, the mean oxygen saturation value was $80.6 \pm 1.8\%$, suggesting consistent readings of pulpal blood oxygen levels among participants. Regarding LDF, the mean value was 4.6 ± 1.6 PU, representing the average pulpal blood flow. This measure reflects microcirculatory activity within the pulp and serves as an important indicator of tissue vitality. Together, these mean values provide a reliable baseline profile for pulp sensitivity, oxygen saturation, and vascular perfusion, forming the basis for comparisons between individual tooth groups. Statistically significant differences were observed between the three investigated tooth groups (central incisors, lateral incisors, and canines) for all parameters, except for cold and hot pain threshold.

To identify which tooth group differed significantly, we performed multiple pairwise comparisons. To account for the increased risk of Type I errors due to multiple testing, we applied the Bonferroni correction by dividing the standard p-value threshold (0.05) by the number of comparisons, 3. Consequently, a difference was considered statistically significant at $p < 0.016$. Significant differences were found between the canine and both incisors in EPT ($p < 0.001$), with no difference between the central and lateral incisors ($p = 0.563$). In PO, only the central incisor and canine differed significantly ($p = 0.006$). For LDF, significant differences were seen between the central and lateral incisors and between the lateral incisor and canine (both $p < 0.001$), indicating distinct pulpal blood flow in the lateral incisor. All pairwise comparison results for the investigated tests (EPT, PO, and LDF) are presented in Figure 4. The correlation analysis revealed no significant relationships between the EPT, PO, and LDF. Specifically, the Pearson correlation between the EOT and PO was -0.067 ($p = 0.457$), indicating no significant association. Similarly, no significant correlation was found between the EPT and LDF ($r = 0.013$, $p = 0.891$) or between PO and LDF ($r = 0.029$, $p = 0.751$). The only statistically significant correlation observed in the analysis was between LDF and Doppler flow variability, which indicates that higher flow is associated with greater variability in flow.

DISCUSSION

Achieving an accurate assessment of dental pulp conditions poses a considerable challenge in clinical practice, underscoring the need for improved tools to enhance diagnostic precision. To our knowledge, this is the first study to assess and compare all available clinical diagnostic tests

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for testing pulp vitality within the same research [20]. The analysis of the mean values across different tooth groups revealed distinct patterns in EPT, PO, and LDF, while all teeth showed positive responses to CPT and HPT.

Pulp sensitivity tests (EPT, CPT, HPT) assess the nerve response of the pulp rather than its blood flow [20]. Because nerve tissue is highly resistant to inflammation, it may still respond even when surrounding tissues have been degraded, leading to potential false-positive results [19]. Our EPT results showed variability among the tooth groups, with canines requiring significantly higher stimulation than central and lateral incisors, likely due to the greater pulp chamber size [21].

To ensure stable placement of PO sensor on each tooth, specialized dental pulse oximeter holder was designed and fabricated based on Gopikrishna's model [12]. It maintained a consistent alignment between the sensor components and the tooth, ensuring a fixed path length for the light emitted by the LED sensor and received by the photoreceptor sensor, allowing for accurate readings. The relatively low standard deviation indicates limited variability, pointing to a stable physiological range in pulpal oxygenation for the tested teeth. On the other side, the obtained reference values differ from those reported by Calil et al. [22], due to their use of signal amplification 2.5 times higher than standard levels.

Previous research indicated that LDF is among the most reliable methods for detecting pulp vitality, although its routine application revealed technical challenges that must be considered (patient head movement, interference, and the need for custom-made probes) [20]. They may also lead to inaccurate representations of pulp health in cases where the pulp is diseased yet maintains a viable blood supply [23]. The heterogeneity of blood flow within the dental pulp [24] may further complicate the acquisition of consistent measurements. In this study, all blood flow measurements were standardized by positioning the probe at least 1 mm away from the gingiva, isolating the area with a black rubber dam, and placing the probe within a custom-made splint. This setup aimed to enhance the accuracy and consistency of the readings by minimizing movement and isolating the measured area. Our results are consistent with those obtained using the same type of probe by Ingolfsson et al. [14]. However, significant differences between the central and lateral incisors, as noted by Seasano et al. [25], can be attributed to the thicker dentin, which impedes deep photon penetration and direct transmission to the receiving fiber.

A key factor contributing to the differences between the results of this and previous studies may lie in variations in research procedures and control of influencing factors, such as sample size.

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Dastmalchi et al. [20] examined 24 pairs of teeth, Gopikrishna et al. [12] examined 80 teeth, Karayilmaz et al. [13] 59 pairs, and Janani et al. [19] assessed only 29 teeth. On the other side, in our study, 150 teeth (75 pairs) have been examined which may account for some of the observed differences compared to the previous investigations.

Gopikrishna et al. [10] studied patients aged 15 to 40 years, Calil et al. [22] examined individuals between 26 and 38 years, Karayilmaz and Kirzioğlu [13] focused on those aged 12 to 18 years, and Dastmalchi et al. [20] examined individuals from 18 to 50 years. Our study included patients aged 20 to 40 years, which is in accordance with most of the previously mentioned studies.

The main strengths of this study include the use of a single, calibrated operator, as well as the implementation of duplicate measurements for all tests to minimize measurement errors. All available clinical diagnostic tests were performed on the same tooth, allowing for a comprehensive assessment. The sample size was determined based on a pilot study conducted within the same experimental framework.

A limitation of this study may be the restricted selection of tooth types. Including all tooth types could improve the study, but may complicate diagnosis due to multi-root variability in diagnosis. Future research should place greater emphasis on molecular analyses of inflammatory mediators, which may serve as predictive markers for different stages of pulpal pathology [26, 27, 28].

Establishing reference values is essential for improving diagnostic accuracy and supporting clinical decision-making, serving as a foundation for future investigations. Before initiating any kind of dental treatment, a clinician should collect all information on pulp disease, combining this with clinical examination and results from all available pulp tests. With recent scientific advancements and with the integration of new technologies into dentistry, the endodontics field have had a great benefit from updated diagnostic tools, such as PO and LDF, which should ideally become standard in everyday practice.

CONCLUSION

Modern diagnostic methods hold promise in delivering results that are both more objective and reliable. Establishing reference values is essential for improving diagnostic accuracy. These values will not only enhance clinical decision-making but also serve as a foundation for future investigations. While EPT remains a common tool in clinical practice, the incorporation of PO

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into routine diagnostic procedures represents a significant step forward. However, future research should focus on emerging diagnostic methods, including laboratory analyses and the evaluation of inflammatory mediator levels, to further enhance diagnostic accuracy.

Conflict of interest: None declared.

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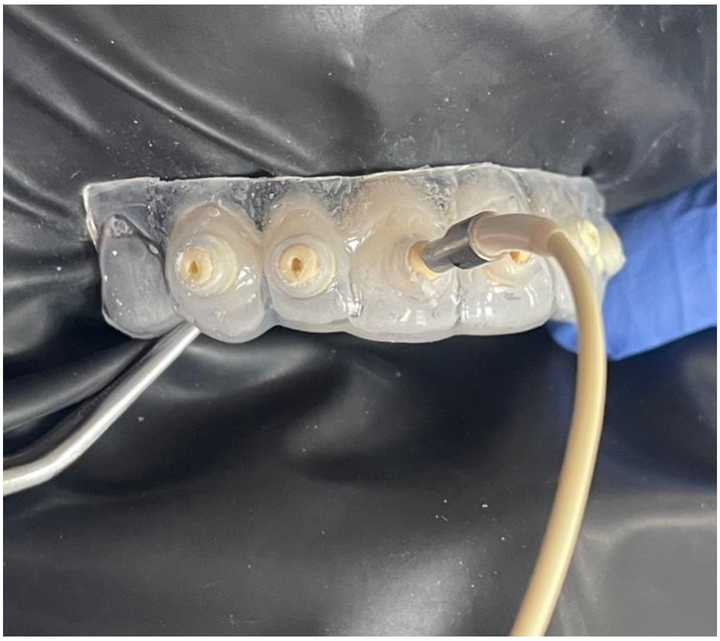

Figure 1. Laser Doppler flowmetry probe holder

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Figure 2. Custom made pulse oximeter dental probe

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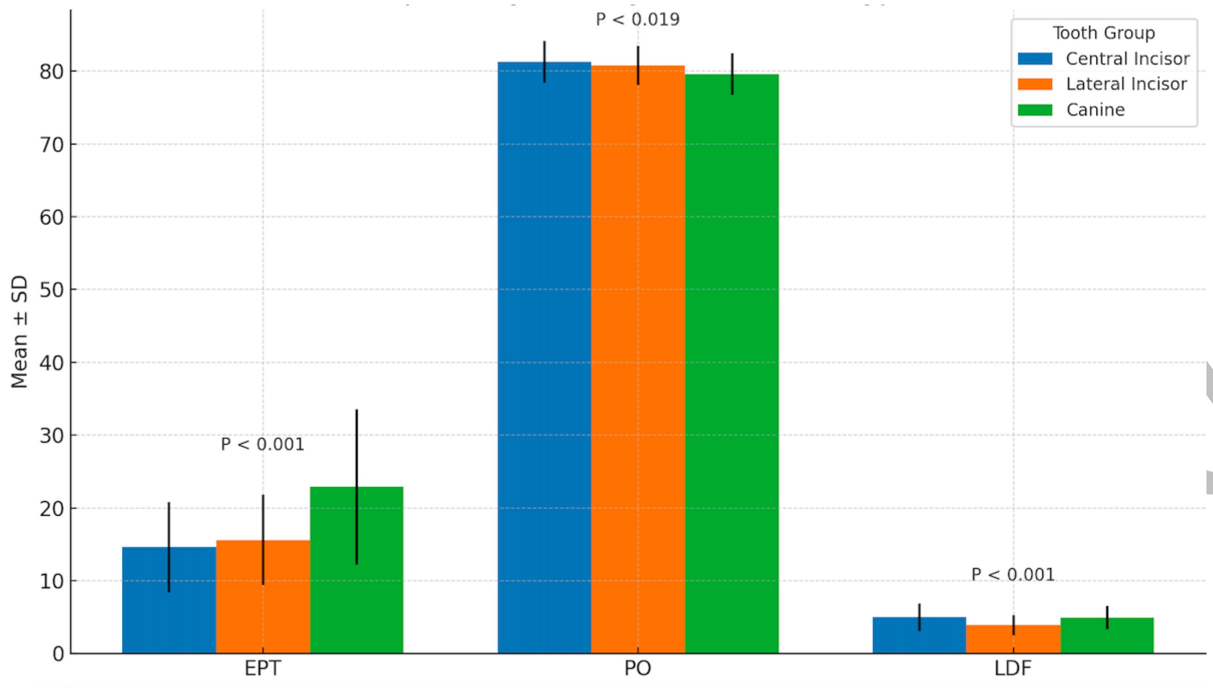

Figure 3. The mean values of the investigated parameters for each tooth group;

SD-standard deviation, EPT- electric pulp testing, PO- pulse oximetry, LDF- laser Doppler flowmetry

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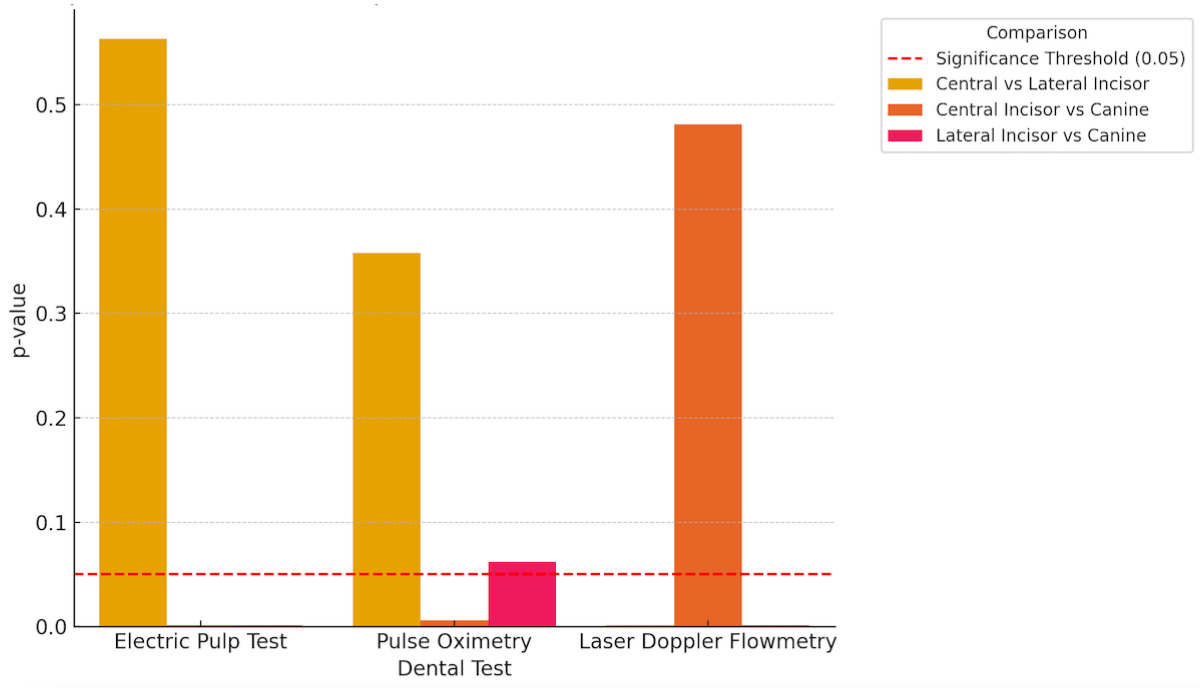

Figure 4. Multiple comparisons between tooth groups (least significant difference test) for electric pulp test, pulse oximetry, and laser doppler flowmetry

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