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Original Article

The rise in glucose concentration in saliva samples mixed with test foods monitored using a glucometer: An observational pilot study

Lea Vuletić [a,*], Stjepan Špalj [b], Dunja Rogić [c], Kristina Peroš [d]

[a] Department of Physiology, University of Zagreb, School of Dental Medicine, Šalata 3, 10000 Zagreb, Croatia
[b] Department of Orthodontics, University of Rijeka, Faculty of Medicine, Krešimirova 40, 51000 Rijeka, Croatia
[c] Clinical Institute of Laboratory Diagnostics, Clinical Hospital Center Zagreb, Kišpaticeva 12, 10000 Zagreb, Croatia
[d] Department of Pharmacology, University of Zagreb, School of Dental Medicine, Šalata 11, 10000 Zagreb, Croatia

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ABSTRACT

Objectives: The aim of this study was to quantify the changes in glucose concentration in unstimulated saliva samples mixed with finely crushed salted sticks or oat meal using a readily available portable glucometer.
Methods: Glucose measurements were taken every 10 min during a 1 h monitoring period, for a total of 14 saliva samples mixed with test foods. Salivary amylase activity was measured immediately after saliva collection (T0) and after 1h (T60). Level of salivary amylase activity was correlated with an increase in glucose concentration.
Results: We observed significant differences in the rate of increase in glucose concentration between the two different test foods, with salted sticks leading to greater increase in glucose concentration. No significant association was found between salivary amylase activity and the rate of increase in glucose concentration. The mean level of amylase activity at T60 was higher than that at T0, but this difference was not significant.
Conclusions: This pilot study presents glucose release rate characteristics from specific food particles mixed with saliva *in vitro* suggesting that the same process takes place in the oral cavity. However, the characteristics of this process when occurring in the mouth would expectedly be modified by different factors such as rinsing effect of salivary flow, oral temperature, etc. Prolonged release of low molecular carbohydrates such as maltose and glucose from food particles can be considered cariogenic and therefore unfavorable for individuals with other risk factors contributing to the development of dental caries.

1. Introduction

Salivary α-amylase (SA) is the principal digestive enzyme in human saliva. Alpha-amylases preferentially cleave the interior α-1,4-glucose linkages of amylose and amylopectin producing a mixture of maltose, maltotriose, and branched oligosaccharides. The direct nutritional advantage of the hydrolysis of dietary starch by SA is generally considered limited because of the relatively short processing time of the food in the mouth before swallowing and because of the inactivation of SA in the gastric phase of digestion due to the low pH of the gastric acid [1,2]. It is possible that the amylolytic activity in the oral cavity could be more important in the pre-absorptive phase of feeding [2–4], notably in determining enjoyment and preference for specific foods [2]. Additionally, SA could play a role in the maintenance or impairment of oral/dental health by affecting the local conditions within the oral cavity. SA is one of the protein components of the early acquired dental pellicle, in which it promotes adhesion of several species of amylase-binding oral streptococci. Along with diet composition, SA digestive activity (the activity of both salivary and dental plaque α-amylase) determines the type of energy sources available to oral bacteria. Nutrient availability affects the existing microbial balance, as well as the physiological and virulence-related properties of cariogenic microorganisms, such as *Streptococcus mutans* [5,6]. The

Abbreviations: SA, salivary amylase; [Glc], glucose concentration; FAD-GDH, flavin adenine dinucleotide-dependent glucose dehydrogenase.

* Corresponding author.
E-mail addresses: vuletic@sfzg.hr (L. Vuletić), stjepan.spalj@medri.uniri.hr (S. Špalj), dunjarogic@hotmail.com (D. Rogić), peros@sfzg.hr (K. Peroš).

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activity of SA also influences the extracellular composition and properties of dental plaque [7–9]. Lower molecular weight products of starch hydrolysis can be incorporated into the structure of glucan, a bacterial exopolysaccharide synthesized by glucosyltranferases, enzymes that are mostly produced by *Streptococcus mutans* [10]. Glucans increase the virulence of dental plaque by modulating its microbial, physical, and biochemical properties.

The aim of the present study was to observe the rate of glucose release in samples of unstimulated saliva mixed with finely crushed salted sticks or oat meal using a readily available portable glucometer. The observed changes in glucose concentration ([Glc]) should depend on the properties of the test food, individual differences in the activity of SA, as well as the presence and the activity of hydrolytic enzymes other than SA in the saliva. As endohydrolases, α-amylases produce only minor amounts of glucose [11] even though glycosylated SA have been reported to have an increased capacity for converting maltotriose into maltose and glucose [12]. Exohydrolases originating from oral microorganisms [13] may be responsible for most of the glucose release from starch and from the oligosaccharides cleaved from starch through the activity of SA. Monitoring the increase in [Glc] in saliva samples mixed with a uniform amount of starchy food could provide a general insight into the interindividual differences in the capacity of SA and microbial enzymes present in saliva to degrade starch. The results have been interpreted primarily with regard to how the products of starch hydrolysis in oral fluids can influence the pathogenesis of dental caries.

2. Materials and methods

2.1. Participants

The study participants were recruited from second-year dental students attending a physiology course in the School of Dental Medicine, University of Zagreb. Students were presented with the study protocol after one of their regular classes. A total of 14 students (10 females and 4 males) volunteered to participate. Students were 20–29 years of age (median 22, interquartile range 21–22).

2.2. Study design

The study participants were asked to not chew gum or smoke cigarettes, to not brush their teeth, and to fast for at least 2 h before the saliva samples were collected. Drinking water was allowed and encouraged to ensure adequate hydration. Prior to collecting a sample of unstimulated saliva via a "spitting method," the flow of stimulated saliva was induced by chewing a piece (0.3 g) of unflavored wax (Parafilm, Heinz Herenz; Hamburg, Germany) for 2 min. We wanted to collect unstimulated saliva after the salivary glands had been activated by chewing because it would better reflect the properties of saliva secreted after a meal. For each sample, a volume of 0.5 mL of the saliva was transferred from the collection cup to Eppendorf tubes (Safe-Lock Tubes 1.5 mL, Eppendorf; Hamburg, Germany) containing 1) powdered salted sticks (Saltas, Koestlin; Bjelovar, Croatia) or 2) finely crushed oat grains (SPAR Natur*pur, Bio-Hafermark; Salzburg, Austria). Due to the fact that the weight of the test foods used was very small (<0.1 g), the amount of each type of food was equalized using pipette tips that had been slightly modified (their sharp tip was cut off to gain a wider base, and after closing the opening, the tip was filled with test food up to the labeled line indicating a volume of 50 all). Test food was thoroughly mixed with saliva using a vortex mixer. Glucose measurements were performed at 10 min intervals within a 1 h long monitoring period at room temperature (approximately 23 °C). The samples were stirred using a pipette prior to each measurement to ensure uniform [Glc] throughout the sample. A total of 43 random glucose measurements were performed in duplicate to assess the reproducibility of the results. The glucometer was calibrated at the beginning of the study and several times during the study.

Sample collection and the experimental procedures described above were performed at the School of Dental Medicine during the period of 10:30 a.m. to 1:30 p.m. The saliva samples were additionally used to measure the [Glc] in pure saliva (n = 14), as well as SA activity (n = 8) at two time points: immediately after the collection of the sample (T0), and at the end of the 1 h monitoring period (T60). Saliva aliquots (1 mL) needed for these measurements were conveyed from the collection cup into Eppendorf tubes and frozen (−20 °C). Sialochemical analysis was performed at the Clinical Institute of Laboratory Diagnostics, Clinical Hospital Center Zagreb. SA activity and [Glc] were measured in saliva samples after centrifugation (10 min, 3000×*g*) utilizing spectrophotometric methods recommended by the International Federation of Clinical Chemistry on a Roche Cobas C 501 clinical chemistry instrument (Roche Corporation; Indianapolis, USA).

2.3. Using glucometers with saliva samples

[Glc] was measured using a Contour® XT glucometer and Contour® NEXT test strips (Bayer Consumer Care AG; Basel, Switzerland). This method has several advantages, including the glucometer is hand-held, portable, easily available, easy to use, and provides results quickly. However, it is intended for measuring [Glc] in the blood. Therefore, we tested its accuracy in comparison to a laboratory method by mixing 200 μL, 300 μL, and 400 μL of two saliva samples with 50 μL of a glucose solution of known concentration (10 mmol/L). The [Glc] measurements with the glucometer were higher than expected in each of the three dilutions (averagely two times higher in comparison to the results of a laboratory method). However, the decrease in [Glc] in subsequent dilutions appeared to occur at a similar rate regardless of the method used. At the same time, repeated measurements of salivary [Glc] using the glucometer showed excellent reproducibility. Inaccurate readings (falsely elevated glucose levels) could be partly attributed to the analytical imprecision of the device [14], as well as to some factor(s) of interference present in saliva. It should be noted that we obtained erroneous values (E11 denoting an abnormal result) when the glucometer was used to measure [Glc] in solutions of known glucose concentrations in water as well as in mixtures of test food and water. These erroneous results were never observed when using saliva as the specimen. We speculate that the reason behind the problem in measuring [Glc] in water solutions might be related to the lower viscosity of water compared to the more viscous saliva and blood. The higher viscosity of saliva enables us to use glucometer to measure [Glc] in saliva, but differences in the composition and properties of individuals' saliva (including differences related to the water binding capacity of salivary mucins) could be the reason for inaccurate readings in comparison to laboratory method. The excellent reproducibility of the glucometer measurements and the congruous rate of decrease in [Glc] in subsequent dilutions are suggestive of the glucometers' ability to track changes in glucose concentration in a specific salivary sample.

Presence of maltose in the sample is one of the factors that can affect glucometer accuracy. Maltose interference is of clinical importance in conditions where the maltose concentration in the blood is artificially increased, such as in case of patients undergoing peritoneal dialysis using icodextrin as an osmotic agent. During high maltose conditions, testing blood [Glc] using monitoring systems which are not glucose-specific may result in falsely elevated glucose readings. The Contour® NEXT test strips used in this study contain two active ingredients, the enzyme flavin adenine

dinucleotide-dependent glucose dehydrogenase (FAD-GDH) and a proprietary phenothiazine electron mediator. Their FAD-GDH is fungus-derived (*Aspergillus* spp.) and therefore lacks activity toward maltose [15]. This feature is important for this study because enzymatic degradation of starch by SA produces oligosaccharides including maltose, so a rise in maltose concentration in the samples was expected.

2.4. Statistical analysis

The reproducibility of the [Glc] measurements, i.e., the consistency of the two [Glc] measurements obtained using a glucometer at the same time point, was assessed using the intraclass correlation coefficient (ICC). Paired *t*-tests were used to analyze the differences in [Glc] between duplicate samples. Paired *t*-tests were also used for examining whether the mean [Glc] at separate time points during the 1 h monitoring period were equal for the two different test foods. The glucometers used in this study were not able to measure [Glc] lower than 0.6 mmol/L. In order to perform statistical calculations, samples that measured <0.6 mmol/L were assigned a [Glc] value of 0.5 mmol/L. We used Pearson's correlation to test the association between the rate of increase in [Glc] at 10 min intervals both within and between the two test food groups. The mean SA activity at two time points (T0 and T60) were compared using paired *t*-tests. The association between SA activity and the rate of increase in [Glc] was tested using Pearson's correlation. Data were analyzed using the commercial software IBM SPSS V22.0 (IBM Corp.; Armonk, New York, USA), with significance defined as $\alpha < 0.05$ for a two-sided test.

3. Results

Contour® XT glucometers are intended for self-testing by people with diabetes and by healthcare professionals to monitor the [Glc] in blood. We were unable to compare the results of the measurements performed on saliva samples mixed with food to a recommended laboratory method due to operative difficulties. The absolute [Glc] values presented in this study are likely biased by factors related to saliva composition because measurements of [Glc] in saliva samples mixed with control glucose solution showed that glucometers overestimate [Glc]. However, excellent reproducibility was confirmed by repeated measurements of salivary [Glc] using glucometers (ICC = 0.998; 95% CI: 0.996–0.999, $p < 0.001$). The difference between [Glc] in duplicate samples was small (0.02 ± 0.08 mmol/L) and statistically insignificant ($p = 0.11$). Therefore, measurement of the relative changes in [Glc] within a 1 h monitoring period can be considered credible for the samples used in this study.

Paired *t*-tests revealed statistically significant differences in the [Glc] between two test foods for all time points during a 1 h monitoring period ($p < 0.01$), except for the measurement performed at 10 min (T10, $p = 0.11$) (Fig. 1). If glucometers could measure [Glc] below 0.6 mmol/L, it is possible that the difference between the two test foods would have also been significant at T10 because of the faster increase in [Glc] in saliva samples mixed with salted sticks compared to the oats. Fig. 2 presents the [Glc] for each individual sample at each time point during the 1 h monitoring period for both test foods.

Paired *t*-tests also revealed that the rate of increase in [Glc] for each 10 min interval between T10 and T50 was significantly different between the two food groups ($p < 0.01$). We identified a significant positive correlation between the rate of increase in [Glc] in each 10 min interval for the salted sticks group, beginning at the T20-T30 interval (Table 1). These results were considered to be reliable because at T20, three [Glc] readings were <0.6 mmol/L, and

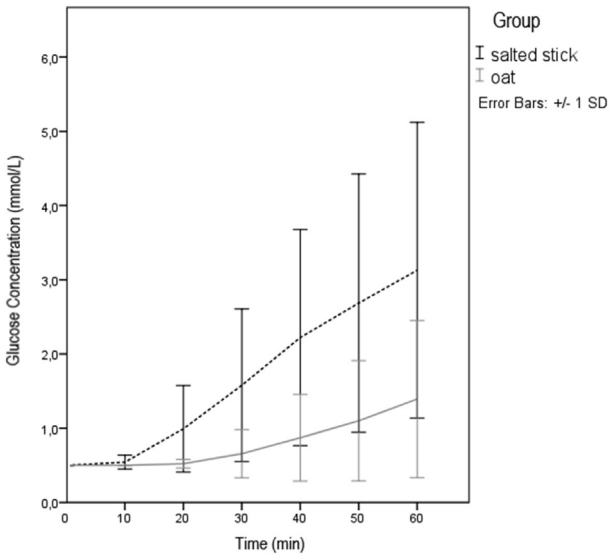

Fig. 1. Dynamic changes in glucose concentrations during a 1 h observation period for the two test food groups. T0-T60 = time points separating 10 min intervals within the 1 h monitoring period in which glucose measurements were performed; SD = standard deviation.

at T30, only two readings were <0.6 mmol/L. It should also be noted that for one of the saliva samples, the [Glc] was not measurable (did not reach 0.6 mmol/L) throughout the entire 1 h monitoring period for both test foods. Although these data suggest a linear increase in [Glc] over time, the results are not precise enough because a provisional value of 0.5 mmol/L had to be estimated for measurements <0.6 mmol/L in order to perform the statistical analyses. This limitation of the study particularly refers to the interpretation of positive correlations determined in the oats group as, due to the slower rate of [Glc] increase over time, half of the samples had unmeasurable [Glc] even at T40; and at T60, three results were still <0.6 mmol/L. The mean level of SA activity at T60 (48.98 ± 14.64 U/mL) was higher than that at T0 (47.54 ± 13.41 U/mL), but the difference was statistically insignificant.

4. Discussion

This study presents the quantitative changes in [Glc] measured using standard glucometers when unstimulated saliva samples were mixed with two test foods. We expect that the absolute [Glc] were higher than measured using glucometer due to unknown factors involved with using saliva as a sample. However, the change in [Glc] over time was able to be reliably measured due to the excellent reproducibility in repeated measurements of salivary [Glc] using glucometers. The demonstrated increase in [Glc] from the initial concentration of <0.11 mmol/L in pure saliva samples indicates enzymatic degradation of dietary starch. It can be assumed that the initially higher amount of sugar in salted sticks in comparison to oats (2.4 g versus 0.8 g per 100 g of the product, respectively) might contribute to the difference in observed absolute [Glc] between the two test foods. However, due to the use of a very small amount of test food in the experiments (<0.1 g), and the fact that the term, "sugars" on "Nutritional Facts" labels refers to both monosaccharides and disaccharides, the effect of the release of free glucose from the test food on [Glc] measurements was most likely negligible. Using mixtures of test foods in water as controls might have confirmed this hypothesis, but we were unable to conduct these controls because the glucometers regularly retrieved erroneous results when water was used as a solvent.

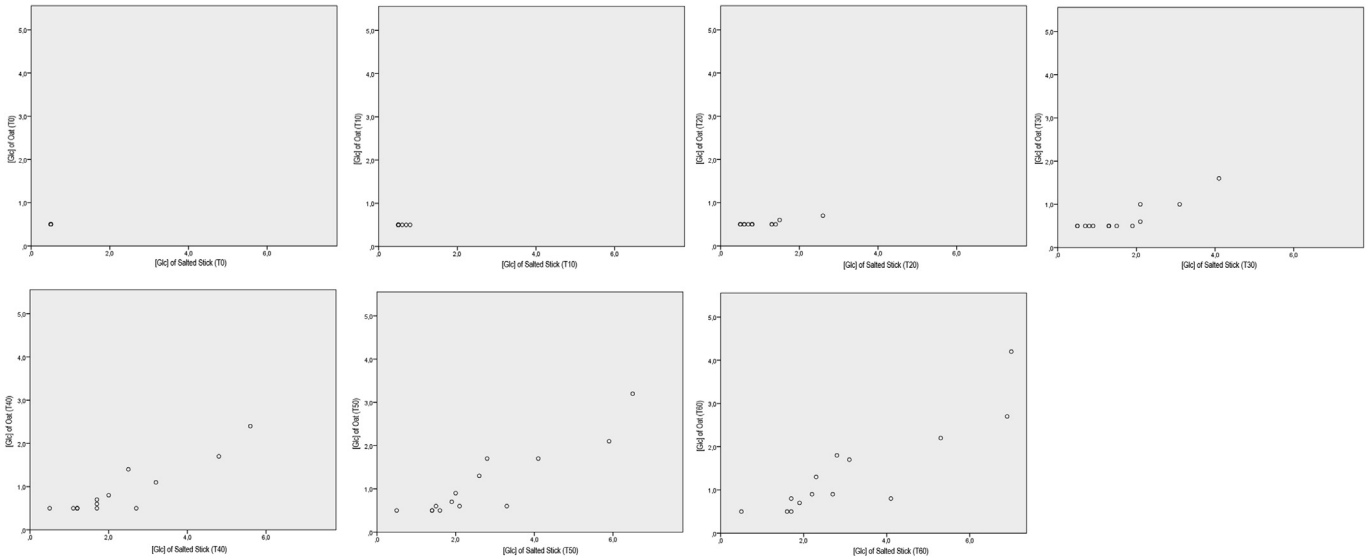

Fig. 2. Individual glucose concentrations for each saliva sample during the 1 h monitoring period. Glucose concentrations (mmol/L) in saliva samples mixed with salted sticks (X-axis) and oats (Y-axis) for each individual sample at different time points within the 1 h monitoring period (T0-T60).

The difference in the rate of increase in [Glc] between the two test foods can primarily be related to the differences in macrostructure and properties of the test foods themselves, including their physicochemical structure, their starch characteristics at the granule and molecular levels, and the presence of other dietary components (e.g. fibers and lipids) [16]. Analysis using Pearson's correlation suggested a linear increase in [Glc] over time, particularly starting at T20, which could suggest a relatively steady pace of enzymatic starch degradation. Our results did not reveal a significant association between the level of SA activity and the rate of [Glc] increase. Although these results should be cautiously interpreted as due to the small sample size of this study, they could possibly relate to the differences in the total amount of SA, differences in SA activity caused by environmental factors (e.g. the presence of calcium, various anions such as chloride, salivary pH, presence of dietary inhibitors), and the limiting influence of the physicochemical structure and properties of starch on the catalytic effectiveness of amylase, and thus the rate of digestion [16]. Additionally, a lack of a significant association between SA activity and the rate of increase in [Glc] could possibly be explained by differences in the glycosylation of different SA isoforms (different glycosylation identities may influence the capacity of SA to form glucose from malto-oligosaccharides [12]) and the number of salivary microorganisms capable of degrading starch and its hydrolytic products into glucose. The presence of a larger amount or higher activity of microbial glucosidases would enhance the rate of glucose release [17]. The activity of these enzymes would also be dependent on various environmental factors including the relative concentrations of the substrates and products of the enzymatic reactions. The faster increase in [Glc] observed in the salted sticks group might be due to higher digestibility of their starch and due to the fact that amyloglucosidases act faster on the products of amylolytic starch digestion than on the native starch itself [17].

Table 1
Results of Pearson's correlation for the increase in [Glc] at 10 min intervals for saliva samples mixed with salted sticks (n = 14).

	T0-T10	T10-T20	T20-T30	T30-T40	T40-T50	T50-T60
T0-T10	1	**0.775**	0.446	0.455	0.368	0.102
T10-T20	**0.755**	1	**0.786**	**0.756**	**0.708**	0.414
T20-T30	0.446	**0.786**	1	**0.878**	**0.863**	**0.605**
T30-T40	0.455	**0.756**	**0.878**	1	**0.942**	**0.730**
T40-T50	0.368	**0.708**	**0.863**	**0.942**	1	**0.841**
T50-T60	0.102	0.414	**0.605**	**0.730**	**0.841**	1

T0-T10, T10-T20, etc. indicate the 10 min intervals between subsequent [Glc] measurements within a 1 h monitoring period. The correlation values shown in bold are significant at $p \leq 0.022$.

4.1. Clinical impacts and other implications

The dynamics of starch digestion when in contact with saliva could be clinically relevant with respect to the pathogenesis of dental caries. The rate of starch digestion and glucose production could impact dental plaque by altering the supply of low molecular weight carbohydrates released from food particles remaining in the mouth [18]. These carbohydrates can then be utilized by plaque bacteria leading to the production of acid [19,20] or serving as a substrate for the construction of extracellular polysaccharides [7]. However, the potential of starch degradation to influence the formation of dental plaque, its microbial characteristics, its extracellular matrix properties, and finally, the initiation and/or progression of dental caries, is likely dependent upon multiple factors involved in the complex pathogenesis of this disease [21]. Therefore, the prolonged release of starch hydrolysates from leftover food in the mouth may have no clinical impact in individuals who generally maintain good oral hygiene and plaque control. High risk groups include children and adults who frequently consume snack foods and lack adequate dental hygiene, people with special health care needs who have difficulties with chewing and/or swallowing (as this may prolong feeding time and food clearance) [22,23], as well as elderly individuals who are vulnerable to a reduction in oral sugar clearance [24]. It is important to highlight the fact that the characteristics of *in vivo* starch hydrolysis would likely be different from the processes occurring under the conditions used in this *in vitro* study. Intraoral factors that may affect starch hydrolysis include the location of food remnants in the mouth, the presence and properties of the underlying dental biofilm, the rinsing effect of salivary flow, the pH value, and the oral temperature, among others.

The results of the present study can also have implications for the potential use of saliva as an easily accessible specimen for diagnostic testing. Saliva samples have been used to compare salivary [Glc] to blood [Glc] in diabetic patients in order to assess whether diabetic control could be monitored by the alternative, noninvasive method of salivary glucose measurement. For this purpose, biosensors are being developed which could detect low levels of glucose in saliva. However, even though saliva-based tests would be simpler and more comfortable for patients compared to classic finger prick blood tests, it is more difficult to avoid interference [25]. The presence of food remnants and the level of oral hygiene would be expected to influence the results of salivary [Glc] measurements [26]. Study designs on this subject regularly aim to neutralize the effect of feeding and poor oral hygiene on the results of [Glc] measurements in saliva, and these complicating factors must be taken into account, especially in cases where the results of studies are supportive of the use of saliva as a diagnostic fluid [27].

With certain modifications, experiments similar to those conducted in this study (using a readily available, simple, and relatively inexpensive method of measurement and foods with favorable digestion profiles) could be appropriate for use in the education of dental students during physiology practical(s) as a part of the discussion of the catalytic activity of digestive enzymes and the (patho)physiological roles of saliva.

5. Conclusions

This pilot study presents glucose release rate characteristics from specific food particles mixed with saliva *in vitro* observed using a readily available portable glucometer. The results indicate that certain food leftovers exposed to salivary enzymes may increase the availability of low molecular weight carbohydrates in the mouth shortly after food consumption and in a prolonged period of time. Biosensors sensitive to low levels of glucose and able to provide accurate readings using saliva could be a valuable tool in investigating hydrolytic activity occurring in saliva and its implications for the development of dental caries and for the use of saliva as a diagnostic fluid in patients with diabetes mellitus.

Data statement

All data from this research are available from the corresponding author upon reasonable request.

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Conflicts of interest

The authors report no conflicts of interest.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to their inclusion in the study. The study was approved by the Ethics Committee of the School of Dental Medicine, University of Zagreb, Croatia.

CRediT authorship contribution statement

Lea Vuletić: Conceptualization, Methodology, Investigation, Writing - original draft. **Stjepan Špalj:** Methodology, Investigation, Writing - review & editing. **Dunja Rogić:** Methodology, Investigation, Writing - review & editing. **Kristina Peroš:** Methodology, Writing - review & editing.

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