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Original article

Saliva analysis in children with active caries before and after dental treatment

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ABSTRACT

Background: *The amount and quality of saliva play important roles in maintaining an intraoral bacterial balance. The quality of saliva is defined by its buffering capacity, viscosity, pH and protein content. The amount of saliva is usually related to the flow rate.* ***Purpose:*** *This study aimed to compare the flow rate, pH, viscosity and buffering capacity of saliva as well as plaque formation in children before and after dental treatment.* ***Methods:*** *Saliva samples were taken from paediatric patients before their treatments and one month after their dental treatments had ended, and these saliva samples were then analysed. For each sample analysis, the GC Saliva-Check Buffer kit (GC Corporation, Tokyo, Japan) was used to evaluate buffering capacity, pH and flow rate, and the GC Saliva-Check Mutans kit (GC Corporation, Tokyo, Japan) was used for the determination of Streptococcus Mutans. GC Tri Plaque ID gel (GC Corporation, Tokyo, Japan) was applied to evaluate plaque maturation.* ***Results:*** *The pre-treatment buffering capacity, pH and viscosity sample values were found to be significantly lower than the post-treatment values ($p<0.05$). No statistically significant difference was determined in the amount of saliva pre- and post-treatment ($p>0.05$). When examining plaque maturation, it was determined that all of the post-treatment plaque was pink.* ***Conclusion:*** *This study showed that the pH, viscosity and buffering capacity of saliva had increased significantly post-treatment and that the formation of plaque had decreased in children with active caries after all their dental treatments had been completed.*

Keywords: *child; dental treatment; saliva analysis; GC Saliva-Check buffer*

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INTRODUCTION

Fighting tooth decay, a common disease for dental, is vital as it impacts people's social lives.[1] Dental decay is an infectious and multifactorial disease that develops through the proliferation and colonisation of bacteria inside the mouth and through the interaction of diet and host factors over time.[2] Saliva enables oral functions such as chewing, swallowing and talking by wetting the tissues inside the mouth.[3] Saliva has a high diagnostic potential for the study of human pathologies. The pH, circulation rate, calcium content and microbial profile of saliva can be used to predict the risk of developing dental caries.[4,5]

High saliva quantity and quality are essential in balancing demineralisation and remineralisation of the enamel in a cariogenic environment. Specific changes, such as increases in pH, buffering capacity and flow rate, can contribute to decreased sensitivity to dental decay.[6] After the intake of sugar-containing foods, cariogenic bacteria decrease the pH in dental plaque, causing demineralisation of the teeth. The neutralising effect of salivary flow, the buffering capacity of bicarbonate and the impact of salivary proteins on microorganisms' movements on dental plaque are essential for the prevention of dental caries.[7] Bacterial species associated with dental caries have been detected in higher rates in the saliva of children with severe dental caries.[8,9]

Kits such as the GC Saliva-Check Buffer kit (GC Corporation, Tokyo, Japan) can be used to determine the saliva buffering capacity to evaluate the risk of decay.

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This kit works on the principle of reverse titration.[10] Monoclonal antibodies specific to Streptococcus Mutans (S. mutans) have recently been used to determine the number of S. mutans in saliva, and by using the GC Saliva-Check Buffer kit, the antigen–antibody reaction can be evaluated in only 15 minutes.[11]

This study analyses and compares saliva samples taken before and after ten paediatric patients with dental complaints had completed their dental procedures (fillings, extractions, root canal treatments, fissures and fluoride applications). In contrast to studies on the effect of caries on saliva, the aim of this study is to evaluate whether improved oral hygiene and health in children affect saliva flow, pH, buffering capacity and plaque maturation.

MATERIALS AND METHODS

Children between the ages of seven and eight who had presented to the Dicle University, Faculty of Dentistry, Department of Pediatric Dentistry were included in this study. Specifically, paediatric patients who did not have any systemic diseases that may have affected saliva flow and who had not used any medication in the last four weeks were included in the study. Information was given to each child and their parents about the study, and a consent form was obtained. The patient's age, gender, brushing frequency and dental caries index values (DMFT) were recorded in the information form prepared for the patients. In the first session, the correct method of brushing teeth was shown. The World Health Organization recommends using the DMFT index (total number of decayed, extracted and filled teeth due to caries) to assess the status of caries in permanent teeth. The DMFT index was used to assess the children's primary teeth. Pre-treatment saliva samples were obtained from fifty children with active caries with a DMFT index of five and above. DMTF indexing and all treatments were performed by two paediatric dentists. The treatments could only be completed in ten (10) patients. Saliva samples were retaken one month after the completion of treatment, which had consisted of fillings, root canal treatments, extractions, periodontic treatments and protective applications.

The patients were instructed not to eat, drink, brush their teeth or chew gum for at least one hour before the examination during which the saliva sample was to be collected. The saliva samples were collected between 09.00 and 12.00. Each patient rinsed their mouth with distilled water and was then instructed to lean forward and spit into a saliva collection tube for five to seven minutes. Then, to stimulate saliva flow, each child chewed a paraffin tablet for five minutes, and the stimulated saliva was then collected.

The GC Saliva-Check Buffer kit was used for each saliva analysis to evaluate buffering capacity, pH, viscosity and flow rate. To determine S. mutans in the saliva, the GC Saliva-Check Mutans kit (GC Corporation, Tokyo, Japan) was used. GC Tri Plaque ID gel (GC Corporation, Tokyo, Japan) was applied to evaluate plaque maturation. The applications were made as per the manufacturer's instructions.

A drop of saliva taken from the tube was dropped onto each of the three pads on the test strip in the GC Saliva-Check Buffer kit, and the saliva was spread onto the absorbent surface. The wait time was two minutes for an accurate result. The result was then scored by evaluating the colour of each pad: 0–5 points indicated very low (red), 6–9 points indicated low (yellow) and 10–12 points indicated normal/high (green) (Figure 1).

A saliva sample was collected from each patient when the patient was in a resting state, and the pH level was measured using the pH strips in the GC Saliva-Check Buffer kit. The colour shown on the strip was checked against the colour control chart in the kit. The pH values were evaluated with 5.0–5.8 indicating low, 6.0–6.6 indicating moderate and 6.8–7.8 indicating healthy (Figure 1).

The amount of saliva was measured in ml as marked on the cup in which the stimulated saliva had been collected. According to the data of Lund University Faculty of Odontology Department of Cariology, Sweden,[7] the amount of saliva collected in five mins was evaluated with <3.5 ml indicating very low, 3.5–5.0 ml indicating low and >5.0 ml indicating normal. Stimulated saliva at the rate of 1–6 ml is seen as normal in healthy individuals. Plaque maturation was evaluated with the application of GC Tri Plaque ID gel. After application, the gel was removed from the surface of

Figure 1. Application of the GC Saliva-Check Buffer kit.

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the teeth with a small swab. The colouration of the teeth was evaluated with pink or red indicating newly formed plaque, blue or purple indicating plaque of at least 48 hours maturity and light blue indicating mature plaque producing acid (Figure 2).

The measuring of saliva viscosity was made visually while the patient was resting. Sticky foaming saliva was evaluated as high viscosity (red), foaming saliva with bubbles as increased viscosity (yellow) and watery clean saliva as normal viscosity (green).

The GC Saliva Check Mutans kit measured the level of S. mutans in saliva within 15 minutes without the need for a special device and bacteria culture (Figure 3). One drop of Reagent #1 was dropped into the collection cup of stimulated saliva; after ten seconds, four drops of Reagent #2 were added, and the mixture was shaken. Using a graded pipette, saliva that had turned light green was placed in the window of the test device and left for 15 minutes. The S. mutans level was evaluated as high (>5 x 10^5 CFU/ml saliva) with the determination of a red line in the control (C) window and as low (<5 x 10^5 CFU/ml saliva) if no red line was formed.

Approval for the study was granted by the Ethics Committee of Dicle University Dental Faculty (decision no: 2021-26). Data obtained in the study were statistically analysed using IBM SPSS version 21 software. Due to unit numbers, the Shapiro Wilk test was used in the assessment of conformity of the variables to normal distribution. To examine the differences between two dependent categorical variables, the marginal homogeneity test was applied according to the levels. When examining the difference between two dependent variables not showing normal distribution, the Wilcoxon test was applied. A value of $p<0.05$ was accepted as statistically significant in all tests.

Figure 2. Intraoral application of GC Tri Plaque ID gel.

RESULTS

Saliva buffering capacity was significantly lower after treatment than before treatment ($p<0.05$) (Table 1). No statistically significant difference was determined between the pre-treatment and post-treatment amounts of saliva (flow rate) ($p>0.05$) (Table 2). The salivary pH value was determined to be statistically significantly lower before

Figure 3. GC Saliva-Check Mutans kit.

Table 1. Results of the analysis of the difference between the pre-treatment and post-treatment saliva buffering capacity values

Saliva buffering capacity	n	Mean ± SD	Median (Min–Max)	p
Pre-treatment	10	7.60 ± 2.27	6 (6–12)	0.024*
Post-treatment	10	9.40 ± 2.12	9 (6–12)	

*Wilcoxon test

Table 2. Results of the analysis of the difference between the pre-treatment and post-treatment saliva amount values [n (%)]

Saliva amount		Pre-treatment		
		<3.5 ml	3.5–5 ml	>5 ml
Post-treatment	<3.5 ml	0 (0)	0 (0)	0 (0)
	3.5–5 ml	1 (50)	5 (71.43)	0 (0)
	>5 ml	1 (50)	2 (28.57)	1 (100)

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Table 3. Results of the analysis of the difference between the pre-treatment and post-treatment saliva pH values

Saliva pH	n	Mean ± SD	Median (Min–Max)	p
Pre-treatment	10	6.89 ± 0.44	6.8 (6.4–7.6)	0.007*
Post-treatment	10	7.56 ± 0.23	7.6 (7.2–7.8)	

* Wilcoxon test

Table 4. Results of the analysis of the difference between the pre-treatment and post-treatment plaque scores [n (%)]

Plaque score value	Pre-treatment			
	Pink	Purple	Blue	Purple-Blue
Post-treatment	2 (100)	5 (100)	1 (100)	2 (100)
	2 (100)	5 (100)	1 (100)	2 (100)

treatment compared with the post-treatment value ($p<0.05$) (Table 3).

When examining plaque maturation, it was determined that 100% of patients with pink, purple and blue plaque pre-treatment had pink plaque after treatment (Table 4). All the cases with increased viscosity of saliva pre-treatment were seen to have returned to normal viscosity post-treatment ($p<0.05$). The presence of S. mutans in the saliva was determined to be positive in all of the patients before and after treatment.

DISCUSSION

Saliva is a complex secretion that has a significant protective effect against tooth decay through its buffering capacity, cleansing ability, antibacterial effect and its ability to preserve calcium and phosphate levels. It can also be used as a diagnostic fluid.[12] Many studies in the literature have analysed and compared saliva samples taken from children who have been categorised into two groups, with and without caries.[7,12] As a result, our study evaluated salivary flow rate, pH, buffering capacity and viscosity as well as plaque maturation and S. mutans levels in children with active caries (DMTF ≥ 5) before and after dental treatment.

The main limitation of this study was that as it was conducted during the COVID-19 pandemic, only a small number of patients (10 patients) who had completed treatment could be reached. However, the results the study obtained were invaluable and established that there is a need for further studies with greater numbers of patients in order to obtain more data for more precise results.

The amount of saliva flowing into the mouth in one minute is known as the saliva flow rate.[13] Pyati et al.[7] reported in their study that the salivary flow rate had been shown to be significantly reduced ($p<0.05$) in children with active caries. However, some studies have reported no relationship between caries activity and salivary flow rates.[12,14]

Similar to results reported in published study findings, no statistically significant difference was found regarding the saliva flow rate. However, this result could be attributed to the study group not including patients with systemic salivary gland disease that can cause hyposalivation or xerostomia, but only healthy children who had not used any drugs such as antidepressants, antihistamines, diuretics or narcotics, which can reduce saliva flow, for at least four months.

The buffer capacity of saliva is a key factor in caries prevention. When the pH level in the mouth falls below the critical pH value of 5.5, inorganic tooth matter may dissolve. The presence of bicarbonate in saliva neutralises acid formation in the mouth and dissolves it into dental plaque.[15] Therefore, in this study, it was predicted that the pH and buffer capacity of saliva that were found to be low in children with active caries would have an important roles in the formation of caries in children's teeth, which can be detected by the Saliva-Check Buffer kit method.

The results of this study determined that salivary pH and buffering capacity in children with dental caries increased significantly after treatment compared with pre-treatment values. These findings were similar to many previous studies.[16–18] Bagherian and Asadikaram[19] concluded that children who had not had early childhood caries (ECC) had higher salivary pH levels and better buffer capacity than children with ECC and children without current ECC. Pyati et al.[7] reported that in 50 children with active caries (DMFS/dfs ≥ 5) and 50 children without caries (DMFS/dfs=0) aged between six and twelve, buffering capacity, salivary flow rates and pH levels were significantly lower ($p<0.05$) in children with active caries. In light of these results, it is clear how important the results of our study are.

Plaque accumulation increases the risk of dental caries. Plaque is a thin layer on the tooth surface that contains a bacterial community. Utami[20] reported that dental plaque is a risk factor for the severity of dental caries in preschool students. The risk of dental caries is 3.3 times higher in children with high dental plaque than in children with low dental plaque. In this study, the finding that 100% of the plaque scores improved after dental treatment was thought to be due to improved oral hygiene post-treatment.

Karabekiroğlu et al.[21] reported in their study that although no significant correlation was found between saliva viscosity and the risk of decay, they were observed that patients with less intense saliva viscosity had lower

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DMTF-DMFS values than patients with more intense saliva viscosity. Voelker et al.[22] found no significant relationship between saliva viscosity and decay in the saliva analyses of 53 patients, but reported that dense saliva consistency did not help in the removal of plaque from the teeth. The results of this study demonstrated that saliva viscosity returned to normal after dental treatment. Further larger-scale studies would be needed in order to better understand the relationship between saliva viscosity and decay.

S. mutans is one of the most important bacteria in the formation of cavities in children. Recent studies have shown that a colony variation of S. mutans can form in the oral cavity at the age of 3-6 months with the horizontal transmission. The basic defence against S. mutans is provided by immunoglobulin A in the saliva, serum and gingival groove fluid.[23] Dogra et al.[24] reported that the number of S. mutans was significantly higher in children with active decay in the 7–14 age. In this study, all the patients had S. mutans positivity before and after treatment. The advantage of using the S. mutans kit is that after adding the samples to the device, the results are obtained in 15 minutes, and the kit can therefore be easily utilised in daily practice without having to use a laboratory. However, that the same result emerges for moderate risk progressing to high risk seems to be a disadvantage of the S. mutans kit.

The ready availability and positive correlations between the components of saliva are the most important advantages to using saliva as a diagnostic tool. In this study, after dental treatment and oral hygiene education, it was observed that the pH, viscosity and buffering capacity levels of the saliva in all ten children with active caries had increased significantly and that the formation of plaque had decreased.

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