Caries Activity and Ph Level Changes after Fluoride Varnish and Casein Phosphopeptides-Amorphous Calcium Phosphate Application on Children’s Saliva

Abstract

Background: Caries is a disease affecting the hard tissue of the tooth wherein the demineralization process caused by Streptococcus mutans decreases saliva pH faster than the remineralization process can maintain it. Topical fluoridation, such as fluoride varnish and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) is the most common preventive therapy for the disease. Aims: The aim of this study is to assess the difference between fluoride varnish and CPP-ACP in reducing saliva pH and caries activity. Materials and Methods: This is an experimental study with a sample population of 60 children (aged 8–9 years old), divided into two groups of 30. Group 1 was treated with fluoride varnish, Group 2 with CPP-ACP. A t-test was used to measure the effects of the different treatments. Results: The result showed that the average difference in saliva pH before and after application was −0.12933 in Group 1 and −0.14033 in Group 2 (P = 0.256). The average difference in caries activity before and after application was 3.189 log colony-forming units (CFUs)/mL in Group 1 and 2.237 log CFUs/mL in Group 2 (P = 0.275). Conclusion: The most effective treatment for increasing saliva pH and reducing caries activity can be achieved by using the varnish for 1 month. However, there is no difference between fluoride varnish and CPP-ACP with regard to altering saliva pH and reducing caries activity statistically. Future study is needed to explore this result.

Keywords: Caries activity, casein phosphopeptides-amorphous calcium phosphate, fluoride varnish, saliva pH

Introduction

The epidemiology of tooth caries indicates that the problem is major and remains a problem over time.[1] It is a progressive dissolution of the inorganic components comprising the hard tissue of the tooth and attached media. The destruction of the hard tissue of the tooth is caused by a number of interacting factors which together cause caries.[2] Cariogenic bacteria demineralize and break down the hard tissue of a tooth faster than the remineralization process can maintain it. Streptococcus mutans is the most cariogenic bacteria involved in caries,[3,4] and the more acidic saliva is the more vulnerable it makes tooth tissue to caries. An individual’s saliva can become more acidic because of its flow, viscosity, and pH.[5] Saliva also plays a main role in caries because it is an environment for bacterial activity,[6] working to clean tooth surfaces, dissolve food, lubricate the mouth, protect oral mucosa, and buffer effect.[7] A normal saliva pH is 5.75–6.5; a lower pH indicates that a patient’s oral cavity is acidic due to a lack of oral hygiene, caries, calculus accumulation, or an inappropriate diet.[8] Saliva pH tests can predict causative bacterial activity.[9]

The oral and dental health report produced by Maranatha University’s Faculty of Dentistry, Indonesia in 2016 included a sample population of 260 children aged 6–7 years old children; of these, 246 (94.6%) were positive for tooth caries and the caries index was 8.18 in the Sukajadi sub-district of Bandung, Indonesia. They were treated with topical fluoride applied via a mouth rinse, however, this treatment was failed to decrease the caries index. The next examination in the following year (2017) revealed that the percentage was still high (92.4%) with medium caries risk, indicating that these children are still at risk for developing...
caries and need a different preventive treatment to reduce caries activity.[10]

One of the most common methods of reducing caries activities is to administer systemic or topical fluoride to prevent tooth cavity. A topical fluoride application such as a varnish can be used to prevent tooth caries and has been reported by several studies and confirmed by systematic data and meta-analyses.[11] Some studies have shown that fluoride varnish can reduce a patient’s risk of developing tooth caries. Peterson showed that fluoride varnish was more effective than other forms of topical fluoridation in preventing tooth caries. Other studies have reported that fluoride is effective in preventing tooth caries in 75% of cases. Some studies also showed a positive effect of fluoride varnish on caries.[12]

Study reported that another treatment to reduce caries risk is topical casein phosphopeptides-amorphous calcium phosphate (CPP-ACP), which consists of fluoride to decrease the prevalence of S. mutans in children’s saliva, and thus reduces the risk factors associated with tooth caries.[13] Fluoride varnishes release fluoride, whereas CPP-ACP acts as a reservoir for calcium phosphate. Saliva consists of calcium and phosphate ions, and hence the application of CPP-ACP can decrease enamel demineralization and increase the remineralization process.[14]

The effectiveness of fluoride varnish and CPP-ACP has been widely researched, and how these treatments interact with saliva’s pH and reduce caries activity has become an important subject of research. Patel et al. proved that CPP-ACP application was much more effective than fluoride varnish in terms of reducing caries activity.[15]

Materials and Methods

Design and research subjects

This randomized clinical trial with the research subject population for this study comprised elementary students aged 8–9 years old being treated by the Faculty of Dentistry of Maranatha Christian University in Bandung, West Java, Indonesia. This research was approved by the Ethics Committee of the Faculty of Dentistry, Trisakti University (EC number 228/S2-Sp/KEPK/FGK/11/2018). The research subject number was obtained using multistage cluster random sampling. Saliva samples were obtained from 60 children before and after procedures. The children received topical fluoride once a week for 1 month. The sample was divided into two groups, each consisting of 30 children: Group 1 received fluoride varnish (Clinpro® white varnish, manufactured by 3M ESPE, Morley Street Loughborough Leicestershire) containing 5% sodium fluoride), whereas Group 2 received CPP-ACP (MI varnish, manufactured by GC Corporation, Japan).

The examinations included saliva pH test (conducted using an Orion Star A221/Stara 2215 digital pH meter, manufactured by Thermo Scientific) and a caries activity test that involved measuring the quantity of S. mutans quantitative polymerase chain reaction (qPCR) present in the subjects’ oral cavity using a 16srDNA primer genes of S. mutans (Macrogen USA 1330 Piccard Drive Rockville, MD, 20,850 United States).

Saliva sampling methods

Subjects were asked not to eat or drink 2 h before collecting unstimulated saliva. Saliva is collected between 9 a.m. and 11 a.m. Saliva sampling was carried out by the spitting method collected using a 5 ml sterile falcon tube as much as 5 ml. Salivary pH measurements were carried out using a digital pH meter (Orion Star A221/Stara 2215-Thermo Scientific) on a test tube containing salivary samples. Oral hygiene practice standards are explained to patients and reinforced at each visit. Saliva samples were collected and processed for the calculation of S. mutans quantity.

DNA extraction methods

DNA extraction methods were using the heat-shock method. Material preparation derived from saliva samples, phosphate buffer saline (PBS). Salivary samples were measured optical density (OD) to determine their concentration, and then as much as 1 ml of saliva was centrifuged at 4500 ×g for 15 min. Pellet rinsed with 1 ml PBS, then vortexed again. Centrifugation returns at a speed of 10,000 ×g for 10 min. After that, the pellet is resuspended with 100 μL ddH₂O plus bead then vortexed for 5 min. After that, it is then heated in a 100°C water bath for 20 min, then incubated in ice for 10 min, then in the vortex. Centrifugation returns at a speed of 10,000 ×g for 2 min. Supernatant containing DNA is transferred to a new tube and stored at −20°C.

Quantitative polymerase chain reaction methods

The primary sequence was chosen to amplify specific parts of the S. mutans gene is reverse 5’-GGTTTC(G/C) TTGTTAGCCTT-3’ and forward 5’-AGAGTGTGATC(A/C) TGTCCTC-3’. Amplification and quantification were carried out on a 96 well PCR plate. Each reaction contained 5 μL DNA template from disc extract, 10 μL Light Cycler 480 SYBR Green I Master (Roche diagnostics Gmbh, Germany), 1 μL each 10 μM forward and reverse primer. Double distilled water was added to adjust the reaction mixture to a final volume of 20 μL. Amplification and detection were carried out using Applied Biosystems Step One qPCR. Real-time PCR System with the following cycle profiles: Initial denaturation at 95°C for 10 min, followed by 40 amplification cycles at 95°C for 10 s, 65°C for 5 s, and 72°C for 11 s. Finally, the analysis of the melting curve is performed using the following cycling parameters: 95°C for 5 s, 65°C for 1 min and a slow increase in temperature until the final temperature of 97°C is reached. Each sample is run in
duplicate. Standard curves are constructed using threshold cycle computed tomography values of the corresponding qPCR and colony forming units (CFU)/mL. The amount of CFU/mL in the saliva sample is then quantified using this curve.

Statistical analysis

Salivary pH measurements were performed two times the measurement of salivary pH in each sample group (Group 1 and Group 2) before and after application, then the average values were taken. Caries activity measurement is done by measuring the quantity in two groups of applications obtained from saliva samples, each sample is measured before and after application with the qPCR measuring tool and converted into log CFU/mL units (using standard curves) then an average S quantity is assessed S. mutans before and after treatment. The data for comparing saliva pH and caries activity following treatment with fluorid varnish were analyzed using a t-test.

Results

Each subject’s saliva pH was measured twice, before and after the course of treatment, to measure caries activity. The quantity of S. mutans was measured at the MiCORE Laboratory, used by the Faculty of Dentistry of Trisakti University. The caries activities test was performed by assessing each sample before and after treatment using qPCR; the data were then converted into log CFU/mL using a standard curve.[16]

The statistical analyses of the saliva pH and caries activity (quantity of S. mutans) measurements gathered from the sample population are presented in Table 1. For Group 1 (those treated with fluoride varnish), the data showed that there was a difference in saliva pH before and after treatment (P < 0.05). The data for Group 1 indicated that most of the subjects experienced a reduction of caries activity. Only five participants experienced a slight increase and one subject experienced a significant increase following treatment with fluoride varnish (Figure 1). The data presented in Table 2 showed that there was a difference in the caries activity before and after treatment with fluoride varnish (P < 0.05).

The data for Group 2 (CPP-ACP) indicated that there was a difference in the subjects’ saliva pH before and after treatment with CPP-ACP (P < 0.05) as shown on Table 3. The caries activity test results for both groups indicated a decrease of S. mutans in most subjects, although nine participants experienced an increase of S. mutans after treatment with CPP-ACP (Figure 2). There was a difference in caries activity before and after treatment with CPP-ACP application (P < 0.05) [Table 4]. The statistical analyses of the data indicating the different effectiveness of fluoride varnish and CPP-ACP on saliva pH and caries activity were performed using a t-test [Tables 5 and 6].

Discussion

This study lasted for 1 month and compared the effects of fluoride varnish and CPP-ACP on saliva pH and caries activity. The subjects were aged 8–9 years old. The caries activity test was performed by measuring the quantity of S. mutans present in a patient’s oral cavity using qPCR. The fluoride varnish used in this study was a single dose of Clinpro white varnish, which contained 5% sodium fluoride in addition to calcium fluoride and released fluoride ions.[17]

The results presented in Table 1 indicate that the participants’ saliva pH increased after 1 month of treatment with fluoride varnish; this was confirmed by the statistical data. This treatment is effective in inhibiting the demineralization process, thereby slowing the progression of carious damage. The study results indicate that intensive treatment with a slow-releasing fluoride varnish is promising in increasing patients’ saliva pH and slowing the demineralization of teeth.[16] Patil found that fluoride varnish releases a large amount of fluoride, thereby significantly enhancing the remineralization process and reducing caries.[18] The other treatment used in this research was CPP-ACP. Reynold stated that the anticariogenic property of CPP-ACP is substantially localized in the calcium and phosphate ions in plaque, thus creating a reservoir for calcium phosphate ions to

Table 1: Saliva pH before and after treatment with fluoride varnish

| Fluoride varnish  | n  | Saliva pH, x±SD | P       |
|-------------------|----|---------------|---------|
| Before treatment  | 30 | 6.9260±0.0378 | 0.000*  |
| After treatment   | 30 | 7.0553±0.0344 |         |

*Significance P<0.05. SD: Standard deviation

Table 2: Caries activity before and after treatment with fluoride varnish

| Fluoride varnish  | n  | Caries activity (log CFU/mL), x±SD | P       |
|-------------------|----|---------------------------------|---------|
| Before treatment  | 30 | 7.518±2.165                    | 0.000*  |
| After treatment   | 30 | 4.329±2.637                    |         |

*Significance P<0.05. SD: Standard deviation; CFU: Colony-forming units

Figure 1: Caries activity (Streptococcus mutans number in the saliva) before and after treatment with fluoride varnish using quantitative polymerase chain reaction method
dissolve on the tooth surface. In an acidic environment, CPP binds with ACP and releases calcium and phosphate ions. This process is ideal for preventing enamel demineralization because of the reverse relation between calcium level, plaque, and the phosphate concentration in the saliva.\textsuperscript{[19]}

The statistical test results of this research indicate that the subjects’ saliva pH increased following treatment with CPP-ACP [Table 3], indicating that treatment once a week for 1 month can increase saliva pH in children aged 8–9 years old. Saliva has a minimal potential for remineralization, as it acts only on the enamel surface. Mineral deposition on enamel lesions starts with the calcium and phosphate ions penetrating the enamel surface: CPP is an efficient means of remineralizing enamel because it removes acid from the enamel lesion. These results confirm the finding of Kargul et al. that 1 month of treatment with CPP-ACP decreased caries lesions by 77\%.\textsuperscript{[20]}

The different effects of fluoride varnish and CPP-ACP on the saliva pH of 8–9-year-old children in Sukajadi were statistically tested and found to be comparable [Table 5], indicating that both varnishes have advantages and that fluoride varnish remains the gold standard treatment for protecting teeth against white spot lesions and preventing demineralization. The statistical test indicated that treatment with fluoride varnish affected the quantity of S. mutans present in the subjects’ oral cavities [Table 2], but not all of the subjects experienced the same degree of reduction [Figure 1]. A few of the participants experienced an increase in S. mutans activity for a variety of reasons, including inappropriate tooth brushing and following a cariogenic diet.

According to Badjatia et al., the 1\textsuperscript{st} month of treatment with fluoride varnish is significantly effective in reducing the quantity of bacteria in saliva. This effect can be sustained for the following 3 to 5 months of treatment, but after 6 months, the effect begins to deteriorate.\textsuperscript{[21]}

In this study, although the application of fluorine varnish and CPP-ACP was only done once a week for 1 month, it resulted in a decrease in the quantity of S. Mutans in

| Table 3: Saliva pH before and after treatment with casein phosphopeptides-amorphous calcium phosphate |
|-----------------------------------------------|
| **CPP-ACP** | **n** | **Saliva pH, x±SD** | **P** |
| Before treatment | 30 | 6.9327±0.0239 | 0.000* |
| After treatment | 30 | 7.0730±0.0349 | 0.000* |

*Significance P<0.05. SD: Standard deviation; CPP-ACP: Casein phosphopeptides-amorphous calcium phosphate

| Table 4: Caries activity before and after treatment with casein phosphopeptides-amorphous calcium phosphate |
|-----------------------------------------------|
| **CPP-ACP** | **n** | **Caries activity (log CFU/mL), x±SD** | **P** |
| Before treatment | 30 | 6.568±1.663 | 0.000* |
| After treatment | 30 | 4.331±1.515 | 0.000* |

*Significance P<0.05. SD: Standard deviation; CPP-ACP: Casein phosphopeptides-amorphous calcium phosphate

| Table 5: The difference between fluoride varnish and casein phosphopeptides-amorphous calcium phosphate in terms of effect on saliva pH |
|-----------------------------------------------|
| **Group** | **n** | **Mean±SD** | **P** |
| 1 (treatment with fluoride varnish) | 30 | -0.12933±0.0397 | 0.256 |
| 2 (treatment with CPP-ACP) | 30 | -0.14033±0.0343 | 0.256 |

*Significance P<0.05. SD: Standard deviation; CPP-ACP: Casein phosphopeptides-amorphous calcium phosphate

| Table 6: The difference between fluoride varnish and casein phosphopeptides-amorphous calcium phosphate in terms of effect on caries activity |
|-----------------------------------------------|
| **Group** | **n** | **Mean±SD. (log CFU/mL)** | **P** |
| 1 (treatment with fluoride varnish) | 30 | 3.189±3.72 | 0.275 |
| 2 (treatment with CPP-ACP) | 30 | 2.237±2.91 | 0.275 |

*Significance P<0.05. CPP-ACP: Casein phosphopeptides-amorphous calcium phosphate; SD: Standard deviation; CFU: Colony-forming units

number. Hashemi found that applying fluoride varnish once a week for 3 months significantly reduced caries incidence in the oral cavity.\textsuperscript{[22]}

The other topical fluoride used in this research is CPP-ACP. This material is a topical fluoride that enhances the binding between fluoride and calcium ions in the oral cavity and increases the remineralization process to protect against early stage caries. Treatment with CPP-ACP produced the same results as fluoride varnish, namely the reduction of the quantity of S. mutans. This finding was confirmed by the statistical test [Table 4]. In this study, it was found that treatment with of CPP-ACP reduced the S. mutans in children aged 8–9 years. These results are similar to those of Jafari et al., who identified that CPP-ACP has an antibacterial effect. The right dosage of CPP-ACP is associated with a reduction in the quantity of bacteria.\textsuperscript{[23]}

Figure 2: Caries activity (Streptococcus mutans number in the saliva) before and after treatment with casein phosphopeptides-amorphous calcium phosphate using the quantitative polymerase chain reaction method.
The results of the measurements and analysis indicate that there is no difference between fluoride varnish and CPP-ACP in terms of reducing the incidence of caries in children. Moreover, the data presented in Figures 1 and 2 indicate that there was a difference in quantity in the reduction of S. mutans. CPP-ACP and fluoride varnish each have their own benefits; a dentist, as a medical practitioner, can choose one treatment or use both because of their respective benefits, fluoride varnish because of its anticariogenic properties and CPP-ACP because of its ability to protect the structure of teeth. Either fluoride varnish or CPP-ACP can be administered regularly at elementary schools in Bandung to reduce the incidence of caries. Routine checking must be done to ensure that the desired preventive effect is being achieved.

Conclusion

The most effective treatment for increasing saliva pH and reducing caries activity can be achieved by using the varnish for 1 month. However, there is no difference between fluoride varnish and CPP-ACP with regard to altering saliva pH and reducing caries activity statistically. Future study is needed to explore this result and to analyze the mechanism of varnish to reduce the oral pathogens.

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Conflicts of interest

There are no conflicts of interest.

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