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Fluoride concentration in urine after silver diamine fluoride application on tooth enamel

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Abstract. Silver Diammine Fluoride (SDF), which contains fluoride, is known to inhibit tooth enamel demineralization and increase fluoride concentrations in saliva and urine. The aim of this study is to analyze the fluoride concentration in urine after application of SDF on tooth enamel. Urine from four subjects was collected prior to, 30 minutes after, and two and three hours after the application of SDF, and an ion-selective electrode was used to measure the fluoride concentrations. There was no significant difference between time 1 and time 2, time 1 and time 3, time 1 and time 4, time 2 and 3 ($p > 0.05$), and there was a significant difference between time 2 and time 4 as well as time 3 and time 4 ($p < 0.05$). There was a decrease in the concentration of fluoride ions in urine from the baseline to 30 minutes after application, and an increase from baseline to two and three hours after the application of SDF.

1. Introduction

Tooth decay; is a process that begins with the localized dissolution of the acidic by-products that form as a result of various metabolic processes that take place in the biofilm that covers the affected tooth surface [1]. Although enamel caries are related to the dissolution of highly mineralized tissues by bacterial acid attack, in the dentin organic matrix degradation of type 1 collagen fibers network added. [2]. Silver Diamine Floride (SDF) is one of chemical compound material that used to prevent and arrest dental caries. Silver diamine fluoride (SDF) is a colorless liquid solution containing silver ions, fluoride ions, and ammonia [3,4]. Clinical studies have shown that SDF can be used to arrest the caries process and treat dentine hypersensitivity [5,6]. SDF can also reduce the demineralization process caused by caries and may inhibit the growth of cariogenic multispesies [5]. The advantages of using SDF are that it is affordable, easy to apply, and does not require complicated training. SDF application is non-invasive, so the risk of infection is very low and the procedure is easy [2,7,8]. However, SDF has some weaknesses, as it causes black stains to appear on teeth with active caries, it can leave stains on the shirts and skin of users, and it also has an unpleasant metallic taste when applied [9]. SDF can also cause irritation of the gingiva and mucosa [8]. The silver ion contained in the SDF acts as an antimicrobial agent, which may be helpful in treating dentine hypersensitivity. Further, the fluoride ion plays an important role in supporting the remineralization process while ammonia plays a role in stabilizing the SDF solutions [2].

Excessive use of fluoride can cause toxicity in the body. This toxicity is divided into three categories: acute toxicity, chronic toxicity, and enamel fluorosis (invisible spots on the teeth). Fluorosis is irreversible and occurs only when the tooth is exposed to fluoride ions during the enamel developmental process [8]. The amount of fluoride that is administered can also affect the concentration of fluoride ions in the body. Besides water, fluoride ions are also available in gel concentrations, rinsing solutions, lacquers, and varnishes. Fluoride uptake from toothpaste considered as safe, studies have shown that exposure and excretion of fluoride are within acceptable limits at water fluoridated and non floridated areas [10,11]. Fluoride is suitable in many ways for selective use in individuals suffering from high caries activity and for trigger remineralization [3]. All fluoride ions that are applied topically or consumed systemically are absorbed by the body through the blood vessels and the digestive system. Moreover, fluoride ions are absorbed in large quantities by calcified tissues such as teeth and bones.

Every day, teeth undergo demineralization and remineralization cycles. The process of demineralization and remineralization is determined by a balance between protective and pathological factors in the oral cavity. Demineralization refers to the loss of mineral elements in teeth. The enamel and dentine surfaces that are demineralized will experience a decrease in hardness, leading to caries [12-14]. Acid in the oral cavity is derived from Streptococcus mutans bacteria. Demineralization can be influenced by several factors, such as the consumption of foods and beverages that contain high levels of acid, bacteria in plaque, sugar consumption, and salivary acidity. It can be prevented by restoring the mineral components, which is known as remineralization. Remineralization is the process of placing new minerals into a demineralized tooth. It occurs when the pH is neutral and there are sufficient calcium and phosphate ions in the saliva [12]. One of the agents that has been proven to assist in the process of remineralization and can arrest the caries process is SDF since it contains fluorine ions [12]. Research on SDF has been conducted in several countries, but no studies have examined the safety level or effects on the body by calculating the concentration of fluoride ions in the urine. In 2012, research by Vasques *et al.* analyzed the difference in silver and fluoride ion concentrations in the blood before and after SDF application. The results showed that the peak concentration of silver and fluoride ions occurred at 2.5 hours for the silver ion and 3 hours for the fluoride ion after SDF application [15]. Therefore, further research is needed on the safety of SDF as calculated by the concentration of fluoride ions in the urine.

2. Materials and Methods

The selected subjects were women from 20 to 30 years old with no history of kidney or other systemic diseases. Ten treated subjects were administered SDF (test) while ten subjects were not administered SDF (control), in accordance with the inclusion criteria, and all the subjects were given an explanation of the research to be performed and asked to sign an informed consent form indicating that they understood and agreed to participate in the study. The test and control subjects were asked to not consume high fluorine-containing foods and beverages for at least 12 hours prior to the SDF application. All subjects were asked to arrive on a pre-agreed day for urine sampling in the morning. The materials needed in the study to make TISAB IV (Total Ionic Strength Adjusted Buffer) solution were aquadem, glacial acetic acid, NaCl, and 5 M NaOH. The solution was prepared by dissolving 10 g of NaOH in 50 ml of aquadem. The preparation of 500 ml TISAB IV Solution was: A total of 250 ml of aquadem was placed in a plastic beaker with a capacity of 500 ml. Next, 10 g of NaOH was dissolved in 50 ml aquadem in a different plastic beaker, and then 28.5 ml of glacial acetic acid was combined with 29 g of NaCl and put in a plastic beaker containing 250 ml of aquadem. The plastic beaker was placed in a water basin to cool down. A pH meter was used, and the pH of the solution was adjusted to 5.0–5.5 by adding 5 M NaOH. Then, the aquadem was added to a 500 ml volume solution. The TISAB IV solution was added into the plastic jar and closed tightly.

One drop of SDF was taken and placed on a plastic dappen. The saliva residue was cleaned, and the tongue and cheek were isolated using a cotton roll on the buccal or labial and lingual side of the tooth that was to receive SDF. When closing the gingiva, petroleum jelly was applied for safety. The

subject's tooth surface was dried prior to SDF application. SDF was applied directly to the 14, 15, 44, and 45 teeth surfaces using a microbrush. The subjects were not allowed to rinse with water. The SDF was allowed to absorb into the tooth surface for one minute, and the excess was dried with a cotton roll. Full Concentration: A total of 20 ml of diluted urine sample was inserted into a measuring cup with a capacity of 100 ml. Then, 10 ml of TISAB IV solution were added to the measuring cup containing the urine sample. A total of 10 ml of aquadem solution was added to the urine sample to achieve a total volume of 40 ml. 50% Concentration: A total of 20 ml of full concentration urine sample was inserted into a measuring cup with a capacity of 100 ml. The sample was then diluted by adding 20 ml of aquadem solution, resulting in a total sample volume of 40 ml.

This study employed the 692 pH/Ion Meters to Measure Fluoride Ion Concentration (Metrohm). To turn on the ISE, an ion-specific electrode was attached to ISE 1. A reference electrode was then installed on ISE 1. The ISE could be turned on and off by pressing the on/off button on the tool. To operate the tool, the following procedures were followed: 1) the mode button was pressed until "c" was displayed; 2) "param" was pressed until the ion name "fluoride" appeared; 3) select was pressed until "parameter: measuring type" was displayed; 4) enter was pressed until "sample size volume total à 40 ml" appeared; 5) finally, enter was pressed until "concentration" appeared as "ppm 1 ppm." Calculating the Fluoride Ion Concentration Using ISE: An aquadem solution was prepared and used to clean and rinse the specific fluoride ion and reference electrodes after being used for measurement; it was then dried with a clean, dry tissue). The sample to be tested was measured to ensure it contained 20 ml urine, 10 ml of TISAB IV, and 10 ml of aquadem. The urine sample, TISAB IV, and aquadem were inserted with a urine ratio: TISAB IV: aquadem = 2: 1: 1 into a plastic beaker with a capacity of 100 ml. A fluoride ion-specific electrode and a reference electrode were placed into the plastic beaker containing the sample, ensuring there were no air bubbles, which could lead to inaccurate readings. A magnetic stirrer was added during the measurement of the fluoride ion concentration. The measurement result was recorded when the "drift" stopped and the fluoride ion concentration value appeared. Both electrodes were rinsed with aquadem and dried. Furthermore, a repeated measures anova test was conducted to determine whether there were significant differences at different times for each subject. A comparative analytic data analysis was employed.

3. Results and Discussion

3.1 Results

This study involved a clinical trial and laboratory experiment including young adult women 21 years of age who had no history of kidney disease or any other systemic diseases. The total number of subjects in this study was four. The study aimed to analyze the concentration of fluoride ions in the urine before and after the application of SDF in the subjects' dental enamel. Data analysis was done by comparing the fluoride ion concentration in the urine of the subjects before and after SDF application, with experiment 1 using a full concentration and experiment 2 using a full concentration with different volumes (Figure 1).

The analysis indicated that the data that were used were normal ($p > 0.05$), and there were no significant differences between the respondents at time 1, time 2, time 3, and time 4 ($p > 0.05$). Based on the repeated measures anova test, there was a significant difference between the times for the subjects ($p < 0.05$). The researcher compared whether there were differences in the urine test results at time 1 (before SDF application), time 2 (30 minutes after SDF application), time 3 (2 hours after SDF application), and time 4 (3 hours after SDF application) using a repeated measured anova post hoc Benferoni. The statistical analysis revealed no significant difference between time 1 and time 2, time 1 and time 3, time 1 and time 4, or between time 2 and time 3 ($p > 0.05$). However, there was a significant difference between time 2 and time 4 and between time 3 and time 4 ($p < 0.05$) (Table 1).

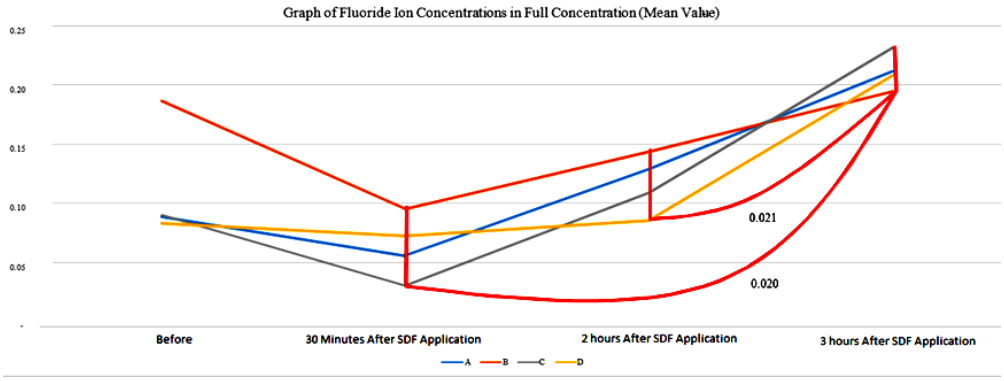

Figure 1. Graph of fluoride ion concentration in subjects urine with full concentration (mean value)

Table 1. Mean value of fluoride ion concentration in subject's urine at every application time

Subject	Time 1 (baseline)	Time 2 (30minutes)	Time 3 (2 hours)	Time 4 (3 hours)	p-value*
A	0.093	0.059	0.133	0.216	0.000
B	0.191	0.100	0.148	0.199	
C	0.093	0.034	0.113	0.235	
D	0.087	0.077	0.090	0.212	
p-values**	0.261	0.139	0.539	0.761	

*Repeated Measure Anova; **Kruskal-Wallis

3.2 Discussion

In this research, clinical and experimental laboratory experiments were conducted to analyze the difference in fluoride ion concentrations in the urine samples before and after the application of SDF (at several time points). A selective electrode ion device was used for the measurements, and the unit was parts per million (ppm). The ISE tool was adjusted according to the required measurements, such as the number of samples, the temperature, and the approximation of the concentration of the sample solution, all of which could affect the reading [16]. SDF is a colorless liquid solution containing silver ions, fluorine ions, and ammonia. It is one of the materials used to arrest the caries process and is able to reduce dentin hypersensitivity. The SDF used in this study was SDF 38% (saforide), which is known to have a high silver (24.4–28.8%; 249,000 ppm) and fluoride (5.0–5.9%; 55,800 ppm) content, with a pH of 10.4,6,14. If SDF is applied to several teeth in the oral cavity, it is assumed that the fluoride ion concentration in the body may increase [6,17]. However, accurate and quantitative measurements of fluoride ion concentrations in the urine using ISE methods before and after SDF application have not been made previously.

Increased concentrations of fluoride ions in the urine after SDF application can be caused by their absorption through the oral mucosa, which allows the ions to enter the circulatory system and then be excreted through the urine. SDF that is applied in the buccal or sublingual mucosa will be rapidly absorbed into the reticulated vein located beneath the oral mucosa and then carried through the internal jugular and braciocephalic veins until it flows into the systemic circulation and is excreted through the urine [15]. In addition to blood vessels, fluoride ions can enter the digestive system through the ingestion of fluoride contained in the saliva and ultimately be excreted through the urine. However, the process of fluoride absorption through the digestive system takes longer than through the blood vessels. At the time of urine sampling, the subjects were asked not to consume foods or beverages that contained high amounts of fluoride, especially black tea and seafood such as anchovies and sardines, which are consumed with the bones, as such consumption would affect the accuracy of the readings. The measurement of fluoride ion concentrations in solution, according to operational

standards, first required that the pure urine be diluted three times with aquadem (considered to be full concentration samples). All urine samples in experiment 1 and experiment 2 measured with ISE required the addition of TISAB solution or the Total Ionic Strength Adjustment Buffer and aquadem solution. This TISAB solution served to create uniformity of ionic strength in the solution and increase the accuracy of the concentration readings by causing the ion that was to be measured to become more dominant and readable by ISE [3]. This was done in an attempt to overcome any interference due to higher ionic strength compared to other ions in the sample, which might have affected the measurement results. The buffer solution used in this study was TISAB IV, which is a special solution that is used for the analysis of fluoride ions using selective electrodes.

From the results, the mean fluoride ion concentration in the urine samples was 0.1166 ppm before application, 0.0678 ppm 30 minutes after application, 0.1213 two hours after application, and 0.2160 ppm three hours after SDF application. Graph 1 shows that the fluoride ion concentration in the urine according to ISE decreased from baseline (urine before application) to after 30 minutes of application. The decrease in fluoride ion concentration at 30 minutes after SDF application was due to fluoride reabsorption in the tubules. In the reabsorption process, about 85% of the sodium chloride and water as well as glucose and amino acids in the glomerular filtrate are absorbed in the proximal convulted tubules. Thus, the remaining fluoride ions in the body that have gone through the reabsorption process will be excreted through the urine. This reabsorption process can affect the change in fluoride ion concentrations in urine. However, there was an increase in fluoride ion concentrations in the urine two hours after the application of SDF, which continued 3 hours after application. This is consistent with the theory that absorption in the body and excretion by the kidneys take place relatively rapidly, where the maximum fluoride excretion occurs 1.5 to 3 hours after exposure and usually fluoride disappears as a whole from the body after 12 hours [3]. Lockner *et al*., has found statistically significant increase in the 6 hours floride excretion after application of floride varnishes [18]. Omid *et al*. have ponder on that fluoride intake and excretion has a relatively low variability over time and suggesting that a single time-point collection may be adequate to monitor fluoride excretion [19].

Urinary fluoride excretion is influenced by several factors, such as pH and gastrointestinal absorbtion ability [20]. pH plays an important role in the absorption, distribution, and excretion of fluoride. Fluoride ions are rapidly absorbed into calcified tissues such as bones and teeth and are well absorbed in the gastrointestinal tract (as much as 70–90%). After the absorption process occurs, fluoride ions will be transported into the bloodstream and distributed through the organs. The absorption of fluoride ions in the body may decrease if there is an increase in pH in the digestive tract and increased concentrations of calcium, magnesium, and aluminum. Fluid ions that are not absorbed by the intestines will be found in the feces. The higher the fluoride retention during bone growth, the lower the amount of fluoride excretion in the urine [20]. Plasma is the main compartment in which fluoride should transit for the subsequent distribution of hard and soft tissue and excretion. In this study, the mean fluoride ion concentrations in the subjects' urine over time ranged between 0.034 and 0.235 ppm, which according to the WHO are still within normal limits. This is because the mean values are below the standard fluoride ion concentration in the urine, which is 0.7 to 1.2 ppm in the maximum dose [3].

Based on the results of the Kruskal–Wallis test, there were no significant differences between fluoride ion concentrations before and 30 minutes after the application, before and two hours after the application, or before and three hours after the application of SDF ($p > 0.05$). However, the fluoride ion concentrations between 30 minutes after and three hours after application as well as between two hours after and three hours after SDF application were found to differ significantly ($p < 0.05$). This is consistent with several studies suggesting that the concentration of fluoride ions in blood plasma peaks at 30 minutes while the maximum excretion time occurs 1.5 to three hours after the absorption of fluorine ions [3]. This study provided clinical implications for enamel remineralization in the oral cavity. This is consistent with previous research, which stated that products that can increase the concentration of fluoride ions can also increase the remineralization potential of enamel lesions and result in the increased bioavailibility of fluoride ion concentrations in saliva. The application of

fluoride ions will lead to the formation of a CaF2 coating on enamel. This CaF2 layer will release the bioavailibility of fluoride ions.20 The average amount of SDF applications that was applied to four teeth was ± 8.53 mg, with a fluoride content of ± 0.44 mg F. This amount is well below the toxic dose of fluoride in the body (35–70 mg F) [15].

In this study, SDF was administered to healthy tooth enamel, where no enamel or dentinal caries were exposed. This may be one of the limits of the study, since SDF is indicated for extreme caries risk, patients unable to tolerate standard medical and psychological treatments, difficult to treat carious lesions, patients with no access to dental care, and patients with dentine hypersensitivity. SDF will work maximally and effectively if given according to its indication. The mechanism of action of SDF is to release both silver and fluoride ions. The silver ions act as antimicrobials while the fluoride ions play a role in the remineralization process. Fluid ions can also help in balancing the demineralization and remineralization processes, as after the absorption process in the body, fluoride ions are re-secreted through saliva, which has a topical effect on the inhibition of caries formation. High silver ion concentrations within the SDF can form a protective layer and reduce dentine hypersensitivity. The direct application of SDF to tooth enamel can cause silver and fluoride ions to penetrate into the tubules, which can increase the mineral density and strength of the enamel. The fluoride and silver ions contained in the SDF interact synergistically to form fluorapatite. The first step is the formation of calcium fluoride and silver phosphate in the basic environment, followed by the separation of calcium and fluoride. The final step is the formation of fluorapatite, which is believed to be capable of assisting in the process of enamel remineralization [2].

From the results of this study, it can be concluded that SDF is safe and non-toxic if it is used to arrest the process of dental caries and reduce dentine hypersensitivity. This is supported by the low fluoride ion concentrations found in the urine of the subjects. Previous studies have also found no evidence that long-term SDF application causes fluorosis [2]. In addition, the subjects made no complaints of discomfort after topical SDF treatment. No leukoplakia was found in the gingiva or mucosa, and no inflammation, pigmentation, or ulceration occurred 24 hours after SDF application [15]. Further studies testing the concentrations of fluoride ions in the urine before and after SDF application should include urine sampling more than three hours after application, to determine specifically when the concentration of fluoride ions in the urine reverts back to the baseline. Additional research is needed on the accuracy of measurements of fluoride ion concentrations in urine with a larger number of subjects and experiments including different fluoride ion concentrations in samples with selective electrode ion devices.

4. Conclusion

There was a decrease in the fluoride ion concentrations from the baseline to 30 minutes after the application of SDF. This was due to the reabsorption of fluoride ion residue prior to the application of SDF in the tubules, which might have affected the fluoride ion concentrations in the urine. An increase in fluoride ion concentrations from the baseline appeared two and three hours after the application of SDF. Based on the literature, there could still be an increase in fluoride ion concentrations three hours after application.

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