Original Article

Estimation and Correlation of the Amount of Fluoride Output in Urine after the Application of Fluoride Gel and Fluoride Varnish in Children with Early Childhood Caries

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Abstract

Introduction: As all the topical fluorides contain a high concentration of fluorides and it is started at an early age when the swallowing reflexes are not well established, there is every chance the child may ingest a high amount of fluoride. As there are inconsistencies related to the usage of fluoride varnish in children, there is a need to study the toxicological aspects of fluoride varnish. **Objectives:** The objectives of this study were to estimate the amount of fluoride output through urine after the application of fluoride varnish and gel and to assess the safety levels of the same in early childhood caries (ECC) patients. **Methodology:** This study was conducted on twenty ECC-rehabilitated children of age group 4–6 years who were divided into two phases. In Phase I, the children were subjected to acidulated phosphate fluoride (APF) gel, and in Phase II, they were subjected to Fluor Protector varnish. In Phase I, the urine samples of each child were collected for the estimation of fluoride level which was considered as the baseline sample. After the application of APF gel, two urine samples were collected at 24 and 48 h for the estimation of fluoride levels. The same children were included in Phase II and were given placebo dentifrice for 7 days before commencing the procedure. The same experiment was repeated after the application of Fluor Protector varnish, and the collected urine samples were subjected for the estimation of fluoride level in laboratory with the use of fluoride ion-specific electrode and a miniature calomel reference electrode coupled to potentiometer. Values were recorded and the urinary fluoride concentration at different time intervals in each group was compared using the Friedman test followed by the Wilcoxon signed-rank test. **Results:** The mean urinary fluoride concentration in Group 1 at 24 h was 1.09 (standard deviation [SD] = 0.52) and at 48 h 0.74 (SD = 0.44). The mean urinary fluoride concentration in Group 2 at 24 h was 1.18 (SD = 0.65) and at 48 h 0.94 (SD = 0.59). There was a gradual increase in the 24th-h sample of both the groups, which also showed a decline of fluoride concentration at the 48th h. When both the groups were compared, Group 2 showed a marginal increase in fluoride concentration at different intervals of time, which was statistically nonsignificant. **Conclusions:** Fluor Protector varnish had an increased sustained release of fluoride ions when compared to APF gel, and there was a gradual decrease in the fluoride concentration which suggested that the fluoride concentration was approximating the baseline level (the safety level). The present study has provided a sound basis of recommendation for the safe and effective use of professionally applied fluoride products.

Keywords: Fluoride varnish, Fluor Protector, gel

Introduction

Dental caries is the most common chronic infectious disease of childhood and early childhood caries (ECC) is a serious public health problem of the present world in both developing and industrialized countries. Its consequences can affect the immediate- and long-term quality of life of the child and family and can have significant social and economic consequences beyond the immediate family as well.[1] Hence, a lot of emphasis in pediatric dentistry has been given for the prevention of caries so that the preventive measures will aid in overcoming the ECC. Out of the most accepted preventive measures, chemotherapeutic agents such as fluorides are successful in preventing and reversing caries. As most of the

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professionally applied topical fluorides enhance the duration of contact of fluoride with the continuously challenged tooth surface and the perpetual release of fluoride from them helps in the remineralization of the demineralized enamel, by acting as a catalyst during the critical pH.[2] It is justified by many *in vitro* and *in vivo* fluoride uptake studies, which clearly demonstrates the superiority of fluoride varnishes and acidulated phosphate fluoride (APF) gel with respect to the incorporation of fluoride in the outer surface of enamel and making it resistant to acid desolution.[3]

Fluoride has been considered an effective anticaries agent when delivered in many vehicles and concentrations, including a variety of professionally applied fluoride products.[1] Out of all topical agents, fluoride varnish and APF gel are the most commonly recommended professionally applied topical fluoride.[2]

Fluoride varnish is a highly concentrated form of fluoride which is applied to the tooth's surface, by a dentist, dental hygienist, or other health-care professional, as a type of topical fluoride therapy.[4] It is not a permanent varnish, but due to its adherent nature, it is able to stay in contact with the tooth surface for several hours.[3] There are more than 30 fluoride-containing varnish products in the market today, and they have varying compositions and delivery systems. These compositional differences lead to a widely variable pharmacokinetics, the effects of which remain largely untested clinically. They are recognized by the Food and Drug Administration for use as desensitizing agents but currently not as an antidecay agent.[4] Both Canadian and European studies have reported that fluoride varnish is as effective in preventing tooth decay as professionally applied fluoride gel; however, it is not in widespread use for this purpose.[4,5]

Preventive programs utilizing topical fluorides need to begin early than late and even the age of 2 years is late for children with ECC.[6,7] However, because of the lack of swallowing reflex, there is a chance of ingestion of more fluoride released in oral cavity. In this context, a careful vigilance on toxicological aspect becomes mandatory to prevent fluorosis.

Concerns regarding fluoride toxicity have spurred recent researchers in re-evaluating the clinical efficacy of topical fluoride agents, and the Food and Drug Administration of the United States of America has still not given approval for the use of fluoride varnish so far.[8] Hence, the aim of this study is to estimate the fluoride excreted in urine after the application of varnish and APF gel and evaluate if it is in safety level and also by checking if the fluoride level in urine returns to baseline level in 24 h.

Table 1: Flluoride concentration at different time intervals after application

	n	Mean	Median (Q1-Q3)	*P*
Baseline				
APF	20	0.53 (0.37)	0.48 (0.29-0.68)	0.14 (NS)
Fluor Protector	20	0.58 (0.49)	0.42 (0.32-0.73)	
24 h				
APF	20	1.09 (0.52)	1.06 (0.62-1.52)	0.88 (NS)
Fluor Protector	20	1.18 (0.65)	1.00 (0.79-1.47)	
48 h				
APF	20	0.74 (0.44)	0.89 (0.29-1.04)	0.19 (NS)
Fluor Protector	20	0.94 (0.59)	0.79 (0.55-1.08)	

Wilcoxon signed-rank test.[9] *P<0.05 statistically significant. P>0.05 NS. NS: Nonsignificant, APF: Acidulated phosphate fluoride

Methodology

Twenty children of mean age 4–6 years who were diagnosed with ECC after complete rehabilitation were selected for the study and were divided into two phases.

- Phase 1: Twenty children were subjected to the application of APF gel using Styrofoam trays
- Phase 2: The same twenty children after 1 month will be applied with Fluor Protector varnish.

The following were the requisites formulated before the inclusion of the children in the study group.

Inclusion criteria

1. Children residing in Dakshina Kannada district. (The range of fluoride levels in water samples was 0.01–0.17 ppm.)
2. Age group: 4–6 years
3. Patients without any systemic illness
4. Children with ECC after oral rehabilitation.

Exclusion criteria

1. Children whose parents do not give consent for examination
2. Children who do not cooperate for examination
3. Children with a history of allergic reaction to fluoride products

Table 2: Pair - wise comparison

	n	Mean	Median (Q1-Q3)	Friedman test		Baseline versus 24 h		Baseline versus 48 h		24 h versus 48 h	
				χ^2 (df)	*P*	*Z*#	*P*	*Z*#	*P*	*Z*#	*P*
Baseline - APF	20	0.53 (0.37)	0.48 (0.29-0.68)	29.10	<0.001*	−3.77	<0.001*	−2.31	0.02*	−3.92	<0.001*
24 h - APF	20	1.09 (0.52)	1.06 (0.62-1.52)	(2)							
48 h - APF	20	0.74 (0.44)	0.89 (0.29-1.04)								
Baseline - Fluor Protector	20	0.58 (0.49)	0.42 (0.32-0.73)	30.10	<0.001*	−3.92	<0.001*	−3.17	0.002*	−3.21	0.001*
24 h - Fluor Protector	20	1.18 (0.65)	1.00 (0.79-1.47)	(2)							
48 h - Fluor Protector	20	0.94 (0.59)	0.79 (0.55-1.08)								

#Wilcoxon signed-rank test, *P<0.05 statistically significant. P>0.05 NS. NS: Nonsignificant, APF: Acidulated phosphate fluoride

Figure 1: Checking for pH of prepared TISAB solution

Figure 2: Procedure for creating a standard curve

Figure 3: Mixing known sample of fluoride solution with TISAB

Figure 4: Estimation of fluoride concentration in the samples

4. History of fluoride treatment in the past 6 months
5. Children with congenital/acquired kidney disease
6. Children using dentifrice with fluoride
7. Teeth with open cavitated lesion
8. Children on medication.

Methodology

This study was initiated after the approval of KVG Dental College and Hospital Ethical Committee. Children were selected based on the inclusion criteria, and consent of patients willing to participate in the study was obtained. A detailed medical and dental history was obtained from each patient.

The present study was divided into two different experimental periods, i.e., Phase 1 and Phase 2. The children were advised to use placebo dentifrice without fluoride for 7 days before the commencement of each phase. All the children in this study were asked to follow the same diet pattern for the consequent 3 days of the study in order to avoid the excess fluoride intake through diet.

Phase 1

The baseline urine samples of all the twenty children were collected in vessel 1 followed by immediate application of APF gel after proper isolation with cotton rolls and saliva ejector. All tooth surfaces will be subsequently covered as the APF gel is applied using Styrofoam trays. Children were asked to avoid solid food during the first 4 h and avoid brushing the next morning. After 24 h, the urine samples were collected on the following test day in vessel 2. The same children will be recalled for sample collection after 48 h in vessel 3. The fluoride levels of all the three samples of each child will be immediately determined in laboratory with the use of fluoride ion-specific electrode and a miniature calomel reference electrode coupled to potentiometer.

Phase 2

This began after 1 month of Phase 1 in the same children. The children were advised to use a placebo dentifrice for 7 days before the procedure. The same procedure was repeated as in Phase 1 but with the use of Fluor Protector varnish. The baseline urine samples of all the twenty children were collected in vessel 1 followed by immediate application of fluoride varnish after proper isolation with cotton rolls or gauze and saliva ejector. The Fluor Protector varnish of a 0.4-ml vial of single use was applied at 9:00 am using a small brush starting from interdental areas so that all the tooth surfaces will be subsequently covered. To facilitate the final setting of varnish, the teeth have to be carefully rinsed with water. Children will be asked to avoid solid food during the

first 4 h and avoid brushing the next morning. After 24 h, we collected the urine samples on the following test day in vessel 2. The same children were recalled for sample collection after 48 h in vessel 3. The fluoride levels of all the three samples of each child were immediately determined in laboratory with the use of fluoride ion-specific electrode and a miniature calomel reference electrode coupled to potentiometer.

Fluoride estimation

Fluoride ion measurement was performed under normal atmospheric conditions by fluoride ion-selective electrode (ISE) connected to an ISE meter (ORION 940900) with magnetic stirrer after calibration with five serial dilutions of standard fluoride solution.

To increase the accuracy and to reduce error, total ionic strength adjustment buffer solution was prepared (TISAB II). TISAB II was used to decomplex the fluoride ion, to provide a constant background ionic strength, and to hold the pH of water because between 5.0 and 5.5 as the fluoride electrode is sensitive to changes in pH [Figure 1].

Total ionic strength adjustment buffer preparation

In 500 ml of distilled water in a 1-L beaker, 57-ml glacial acetic acid, and 58-g NaCl, 4 g of trans-1,2-cyclohexylenediaminetertraacetic acid was added and was stirred to dissolve. Then, the beaker was placed in a cool water bath, and slowly, 6N NaOH (about 125 mL) was added with stirring until pH is between 5.3 and 5.5. Finally, it was transferred to a 1-L volumetric flask.

Creating a fluoride standard curve

Before sample analysis, serial dilutions of a fluoride standard were made to produce five samples of known fluoride concentrations. Each sample was mixed with 1:1 (v/v) with total ionic strength adjustment buffer. This was then transferred to a plastic vial into which a magnetic stirrer was placed [Figure 2].

The mV readings were obtained using the fluoride-specific probe. The mV was then used to create a fluoride standard curve where the determination of the fluoride concentration of the unknown urine samples could be done. We got a slope of −60, which indicated that the TISAB II solution and the electrode are in a position to give reading for the unknown urine samples [Figure 3].

After the calibration of fluoride-selective electrode with five serial dilutions of standard solutions, fluoride estimation of urine samples was performed [Figure 4].

Fluoride ISE (ORION) which was a potentiometric technique using an ISE was used to obtain the fluoride readings in the urine samples. In this method, a lanthanum fluoride plate doped with europium++ is used at the base of the probe to quantify fluoride activity in a solution. For each sample, the value was recorded and chat was prepared. Statistical analysis using Student's paired *t*-test and ANOVA with Bonferroni *t*-test was done.

Results

Statistical method

Data were analyzed using IBM SPSS (Statistical Package for the Social Sciences) Version 22 IBM cooperation,India. The urinary fluoride concentration at different time intervals in each group was compared using repeated-measures ANOVA followed by Bonferroni *post hoc* test. At each time, interval fluoride concentration in urine between the two groups was compared using an independent sample *t*-test. $P < 0.05$ was considered to be statistically significant.

In both the groups, 50% of the children were male and 50% were female. There was no significant difference in the gender distribution between the two groups.

In both the groups, the age of the children was the same. Hence, there was no significant difference in the mean age between the two groups.

The mean urinary fluoride concentration in Group 1 at baseline was found to be 0.53 (standard deviation [SD] = 0.37), at 24 h 1.09 (SD = 0.52), and at 48 h 0.74 (SD = 0.44). There was a statistically significant variation in the urinary fluoride concentration at different time intervals (F [1.05, 19.94] = 280.29, $P < 0.001$) [Table 1].

On pairwise comparison from baseline, the maximum fluoride concentration was observed at 24 h; subsequently, there was a gradual reduction in the urinary fluoride levels. However, the fluoride levels at each time interval were observed to be significantly different from baseline ($P < 0.001$) [Table 2].

The mean salivary fluoride concentration for Group 2 at baseline was found to be 0.58 (SD = 0.49), at 24 h 1.18 (SD = 0.65), and at 48 h 0.94 (SD = 0.59). There was a significant variation in the urinary fluoride concentration at different time intervals (F [1.09, 20.72] = 327.44, $P < 0.001$). On pairwise comparison from baseline, the maximum fluoride concentration was observed at 24 h; subsequently, there was a gradual reduction in the salivary fluoride levels. The fluoride levels at each time interval were observed to be significantly different from baseline ($P < 0.001$).

On comparing Group 1 and Group 2 at different time intervals, a significant difference in the mean urinary fluoride concentration is observed at baseline ($P = 0.001$). At the interval of 24 h, the mean difference of 0.02 between the two groups is statistically significant ($P < 0.001$); the difference of means at 48-h interval, i.e., −3.21, was significant ($P < 0.001$).

Discussion

As all the topical fluorides contain a high concentration of fluorides and it is started at an early age when the swallowing reflexes are not well established, there is every chance the child may ingest a high amount of fluoride. As there are inconsistencies related to the usage of fluoride varnish in

children, there is a need to study the toxicological aspects of fluoride varnish. Taking into account the toxicological aspect, regular swallowing of low doses of fluoride from several sources by small children has been associated with the development of mild dental fluorosis.[8] In this context, a careful watchfulness on new fluoride products is necessary. Among the commercially available products, Fluor Protector, a fluoride varnish, has been used in the present study, and through this study, we are trying to rule out if the amount of fluoride ingested crosses the safety level and for this urine analysis is done.

Of all the available fluoride vehicles, fluoride varnish and 1.23% APF gel are the most commonly used professionally applied topical fluoride agents till date.[10] As both APF gel and fluoride varnishes are used very lavishly for the prevention of ECC in children, our study aims at evaluating which is safer so that if an added advantage of safety along with prolonged retention in varnish can be achieved which is an advantage for people prone to high caries attack like early childhood caries (ECC) Type III patients.

In the present study, a comparison between APF gel and Fluor Protector varnish has been conducted in order to correlate the safety levels of both by the amount of fluoride excreted through urine after application in ECC patients. Twenty-four-hour urinary fluoride excretion was chosen as the response variable to evaluate the bioavailability of fluoride from the products tested. Most of the fluoride ingested will be metabolized in kidneys and excreted through urine; hence, the urine samples of children were collected for fluoride ion estimation after the application of both the fluoride vehicles. Pessan *et al*.[11] have considered fingernails and urine as the biomarkers of fluoride through dentifrice and varnish in 4–7-year-old children. They concluded that urine was an effective biomarker when compared to fingernails.

In the mean urinary fluoride concentration, there was a gradual increase in the 24th-h sample of both the groups, which also showed a decline of fluoride concentration at the 48th h. When both the groups were compared, Group 2 showed a marginal increase in fluoride concentration at different intervals of time, which was statistically nonsignificant. Despite this transient increase, Fluor Protector varnish and APF gel can be regarded as safe because the baseline values were rapidly reestablished both for the mean and individual values. Hence, from the obtained result, we can conclude that Fluor Protector varnish had an increased sustained release of fluoride ions when compared to APF gel. Similar results were also seen in the studies conducted by Szekely et al., *et al*.[12] and Baez et al.,[13]

When the baseline APF gel value was compared with 24-h APF gel value, it was found to be highly significant. This is because of the application of APF gel which led to the increased fluoride release in the 24th-h time interval. When the 24th-h values were compared with 48th-h values, there was a gradual decrease in the fluoride concentration, which suggested that the fluoride concentration was approximating the baseline level, which was the safety level. Similar results were also seen in the second group that comprised Fluor Protector varnish. Omprakash Yadav *et al*.[14] estimated the fluoride concentration in urine and saliva using sodium fluoride tablet, where they found that the fluoride concentration increased at the first time interval of 6 h and almost similar to the baseline values at the 12th-h time interval.

From these above results, we can conclude that on application of either of the fluoride vehicles, there is a gradual reduction in the mean fluoride concentration over a period of time which approximates the baseline or safety level. Hence, both of these products can be used without any hesitation by the clinician in pediatric patients. However, further investigation to support this assumption is required.

Conclusions

On comparison of the fluoride level in urine samples of children after the application of APF gel and Fluor Protector varnish, the peak fluoride level was seen at the 24th h and a gradual reduction in the fluoride level at the 48th h which approximates the baseline or the safety level.

- At different time intervals, the Fluor Protector varnish has shown a marginal increase in fluoride output compared with APF gel. This suggests that Fluor Protector varnish has a marginally higher sustained release of fluoride compared with APF gel
- Hence, the concerns regarding the toxicological aspects in application of fluoride varnish or gel should not be a hindrance in its regular clinical usage in pediatric patients with ECC
- Professionally applied topical fluorides have consistently been proved excellent in their ability to reduce dental caries and should continue to be strongly recommended for clinical use
- However, the purpose of professional fluoride treatments is to benefit the tooth surface by a topical effect, and care should be taken to limit ingestion of professionally applied products
- The present study has provided a basis for sound recommendation for the safe and effective use of professionally applied fluoride products.

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Conflicts of interest

There are no conflicts of interest.

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