Renal fluoride excretion in children following topical application of fluoride varnish

**ABSTRACT**

**Aim** To demonstrate that the application of dental fluoride varnishes in children increases urinary fluoride excretion.

**Methods** From a randomly assembled group of 42 children aged between 5 and 8 years, residing in a community with non-fluoridated water, spot urinary samples were taken before the topical application of dental fluoride varnish and 2 hours afterwards. In an age-matched control group of 16 children from the same community, who received no treatment, samples were taken the same way. The urinary excretion of fluoride was analysed by determining fluoride ion (F-) level and fluoride/creatinine (F/Cr) ratio in the urine.

**Results** In the study group, the average pre- and post-treatment F/Cr ratios were 0.42 and 1.38 mg/g, respectively (p < .001). No significant changes were observed in the control group, which received no treatment. The average 2 hours afterward F/Cr ratios were 0.29 and 0.27 respectively (p = 0.426).

**Conclusion** The topical application of dental fluoride varnish leads to a significant increase in urine F-, which is attributable to the application of the product.

**Keywords** Fluoride; Fluoride/creatinine ratio; Fluoride varnish; Urinary excretion.

**Introduction**

The importance of fluoride in caries prevention and its role in promoting oral health has been widely accepted for over 60 years. Fluoride sources include water, diet, toothpaste, mouthwashes, supplements [Cagetti et al., 2002; Giuca et al., 2007] and professionally applied fluoride. It has been proven that the topical application of fluoride is a more effective and safer method of caries prevention than systemic administration [Limeback, 1999].

Nonetheless, excessive fluoride ingestion poses the risk of acute or chronic exposure and creates a metabolic overload without increasing protection against caries. Chronic exposure due to excessive and continued ingestion of fluoride causes dental fluorosis. Levy et al. in their study about associations between dental fluorosis and fluoride intake, observed that fluorosis is not very common and is usually mild [Levy et al., 2010]. One of the most frequent causes of fluorosis in children is the simultaneous administration of different fluoride sources; indeed, the use of several fluoride sources is recommended only in individuals at high risk of caries [Maupomé et al., 2003].

Between 75 to 90% of the fluoride consumed is absorbed through the stomach and the duodenum [Whitford, 1994], and the ion fluoride is primarily eliminated through the urine. In addition to the volume of urine excreted, the urine pH level is another determining factor in fluoride excretion [Ekstrand and Whitford, 1988]. Renal excretion occurs relatively quickly, with total elimination reached within 12 hours; maximum excretion is seen between 1.5 and 3 hours following ingestion [Bell et al., 1972].

The amount of fluoride found in the urine may be considered an accurate reflection of the volume ingested both in the form of food and from preventive treatment [Ramos, 1996]. Children and adolescents in the growth stage eliminate less fluoride through the urine due to fluoride accumulation in the bones and teeth [Mellberg and Ripa, 1983]. Therefore, by excluding fluoride ingested through food and water consumed over a given period of time, the difference between fluoride levels excreted through the urine before and after fluoride treatment can be attributed to an increase or decrease in fluoride administered orally as a preventive treatment. Indeed, a child’s developmental level of swallowing must be taken into account when assessing the undesirable ingestion of fluoride. The ability to swallow correctly influences the level of fluoride entering the system inadvertently by means of toothpastes [García-Camba de la Muela et al., 2009; Wong el al., 2010], mouthwashes [Zuanon and Aranha, 2005] and gel or varnishes [Ekstrand et al., 1980; Pessan et al., 2005; Olympio et al., 2009].

Fluoride varnish, one of the topical treatments for the prevention of caries, has been widely used since its
introduction in the 1960s and has been proven to be effective in a number of studies. Varnishes are primarily effective in permanent teeth [Pettersson et al., 2004; Xhemnica et al., 2008] and have produced variable degrees of effectiveness in primary teeth [Weintraub et al., 2006; Lawrence et al., 2008]. However, few studies have been published on the appropriateness of measuring fluoride eliminated through the urine following the topical application of dental varnish as an indication of systemic fluoride absorptions; small sample sizes and methodological differences have made it difficult to obtain conclusive data [Ekstrand et al., 1980; Pessan et al., 2005; Olympio et al., 2009].

The aim of the present study was to quantify the fluoride levels in urine, before and after topical application of dental fluoride varnish in children and demonstrate a subsequent increase in excretion attributable to the application of the product. The safety of the various sources of fluoride was not the objective of this work.

Material and methods

The study was carried out in a group of children of both sexes ranging from 5 to 8 years of age who attended a public school located in a municipality of the region of Madrid with non-fluoridated water (< 0.3 ppm). The study sample was randomly selected from a list of 300 students whose ages fell within the designated range and was made up of 42 children; a control group of 16 children of similar ages and genders were also included. The control group was smaller because some of the informed consents were not signed by the parents. A number was assigned to each child to anonymously identify all samples throughout the study.

The inclusion criteria were an informed consent signed by parents and a sufficient amount of urine for analysis. Exclusion criteria were the existence of renal or other systemic illnesses. The project was reviewed and approved by the institutional review board of the Hospital Clínico San Carlos (Universidad Complutense de Madrid, Spain).

Fieldwork was performed in situ at the school in the morning. All children had eaten a breakfast consisting of chocolate milk and breakfast cookies or pastry that did not contain eggs or other animal- or vegetable-based foods. All of the subjects had brushed their teeth using their "normal toothpaste" without receiving any special instructions. All children were instructed to urinate before they left home in the morning.

In the morning, the 42 children in the study group and the 16 control subjects were gathered. After a brief explanation of the study procedure, each child from both groups was given a sterile polyethylene cup with a capacity of 100 ml and instructed to urinate in the cup. The collection procedure was supervised by members of the research team. The pre-treatment samples were identified by a numerical code assigned to each child.

At this time, children in the control group returned to their classrooms with instructions not to ingest water or any type of food and to abstain from urinating until they were called to provide the second urine sample, to show that in the non-fluoridated group there would be no change.

Plaque and saliva were removed from the children in the study group by means of cotton rolls, at which point 0.35 ml fluoride varnish (Duraphat®) was applied to all dental surfaces with the use of a brush. Immediately following the application of the varnish, children were instructed to spit out any residual saliva into a disposable cup.

Subsequently, the children in the study group returned to their school activities with instruction not to rinse, consume food or water, or urinate until they were called to provide the second urine sample. This second sample was collected approximately 2 hours after the first sample. For the second sample, the children from both groups were given a second sterile receptacle identified with their corresponding number and the classification "post-treatment sample.

The fluoride and creatinine concentration and pH were measured in all pre- and post-treatment urine samples. The pH measurements were carried out by means of the potentiometric method (the apparatus and equipment consisted of test tubes of 25 ml, Orion potentiometer 940_960, reference electrode and specific pH electrode), and the fluoride ion determination was performed using the potentiometric method with specific electron ions following the serial calibration technique (the apparatus and equipment consisted of: automatic pipette 2.5 ml, 25 ml test tubes, 940-960 Orion potentiometer, simple reference electrode junction and fluoride ion specific electrode model 94-09-00.). Creatinine concentrations were measured using high-performance liquid chromatography (devices and equipment: Granatario scale capable of weighing 0.01 g, precision scale capable of weighing 10 ug, automatic pipettes, volumetric flasks of 500 and 50 ml vials of 2 ml capacity auto-sampler, column 250 mm chromatographic Spherisorb stuffed length 10 microns, chromatograph high performance liquid comprising: high-pressure pump, auto-sampler with sample injection volume of 10 µl, UV detector at 235 nm, and integrator).

The F/Cr ratio was calculated automatically. Results for all variables were subjected to the appropriate quality controls (blind repetitions, repetition control, and precision control). All analyses were carried out in the clinical biochemistry laboratory of the Hospital Fundación Jiménez Díaz.

Normal distribution was tested through the Kolmogorov-Smirnoff test (or the Shapiro-Wilk test for
small sample sizes). Statistical analysis of the groups was performed using the paired t-test for normally distributed variables and the Wilcoxon signed-rank and Mann-Whitney U tests as non-parametric tests (software program SPSS, version 18.0; SPSS Inc., Chicago, IL).

Results

Control charts were constructed to validate the results obtained in each batch. With some of the fluorides samples, two paths aliquots certified urine samples of low and high level were processed, respectively, in order to check the accuracy of the determinations. For each of the variables analysed, 10% of the collected samples were processed, in order to perform an internal quality control on accuracy.

Table 1 shows the average urinary pH and creatinine levels before and after varnish application in the treatment and control groups. No significant changes were found in those measurements.

Table 2 shows the average F/Cr ratios for both urine samples (before and after varnish application) taken from the treatment and control groups. The difference of 0.96 mg/g found in the treatment group was statistically significant (p < 0.001). No significant differences were found between the F/Cr ratios from the first and second urine samples in the control group.

Discussion and conclusion

Similar to another investigation carried out by our group on the urinary elimination of fluoride [García-Camba de la Muela et al., 2009], we used one-time urine samples taken at the highest point of urine secretion, which is roughly 2 hours following the application of the varnish [Bell et al., 1972]. Although many authors use total fluoride excretion over 24 hours to evaluate
fluoride concentrations in urine, in this study, we chose to measure the fluoride/creatinine ratio (F/Cr). The F/Cr ratio is considered to be equivalent to total 24-hour fluoride excretion when the creatinine concentration is normal (between 0.5 and 3.0 g/l) [Kertesz et al., 1989; Zohouri et al., 2006; Székely et al., 2008]. However, while the concentration in urine over 24 hours allows an accurate reading of total fluoride excretion over this time period, such measurement does not accurately reflect the increase in excretion when a particular risk factor such as varnish is introduced, because 24-hour readings do not distinguish increases in secondary fluoride concentrations from fluoride introduced by other factors such as dentifrice [Garcia-Camba de la Muela et al., 2009] or diet.

It must be pointed out here that we did not find any existing studies on the absorption of fluoride applied topically in the form of varnish, which require spot urinary samples for determination of the F/Cr ratio. One of the reasons for using this method was that the F/Cr ratio is a particularly reliable indicator when studying groups of individuals [Székely et al., 2008].

In our study, we used 0.35 ml Duraphat® varnish, which contains 5% sodium fluoride with 22,600 ppm F-. The first baseline urinary F/Cr ratios of the study and control groups were 0.42 mg/g (SD=0.65) and 0.29 mg/g (SD=0.24) respectively, and we have not found an explanation for this finding. As expected, the difference between both measurements did not reach statistical significance. These baseline values were lower than those reported by other authors, a finding that may be explained by the fact that this study was carried out in an area with non-fluoridated water. In their assessment of urinary fluoride elimination in a group of 7 children aged 16-36 months who resided in an area in northeastern England that had fluoridated water, Zohouri et al. obtained an F/Cr ratio of 1.49 mg/g. [Zohouri et al., 2006]. Kertesz et al, in their study on 326 children aged 8-13 years that also had fluoridated water, reported an average ratio of 1.51 mg/g. [Kertesz et al., 1989].

In their study on 700 children whose ages ranged from 2 months to 14 years who lived near an aluminum foundry, Declercq et al. found a mean F/Cr ratio of 0.52 mg/g following spot urinary sampling. The authors also evaluated urinary fluoride excretion in two other groups of children living in the same community. The first group, which drank fluoride-rich (2 mg/l) mineral water, revealed an F/Cr ratio of 0.69 mg/g. The second group of subjects, to whom a fluoride tablet containing an unspecified concentration of fluoride ion was administered, had a ratio of 0.82 mg/g [Declercq et al., 1995]. The results of this study are consistent with the absence of significant effects of these wastes on the urinary excretion of fluoride in children. A study carried out by Seixas et al. using adults who also worked in aluminium foundries reported even higher baseline F/Cr ratios ranging between 1.3 and 3.0 mg/g [Seixas et al., 2000].

In our study, the second urine samples were collected 2 hours after the application of the varnish. The changes in the F/Cr ratios found in the study group were particularly significant (p <0.001), and no modifications were observed in the control group.

In their attempts to demonstrate systemic fluoride absorption following the topical application of dental varnish, few authors have examined the urinary elimination of fluoride after this preventive treatment. Ekstrand et al. administered doses of 3 to 5 mg Duraphat® to 4 children aged 4, 5, 12, and 14 based on their age. Before applying the varnish, the mean concentration of fluoride in urine over 12 hours was 0.1 mg F in the younger children and 0.2 mg F in the older children. Following the application of the varnish, the urine concentrations increased to 0.5 mg F over 12 hours in the two younger children and 1.1 mg F in the older children. The plasma fluoride levels were well below those considered to be toxic [Ekstrand et al., 1980].

Pessan et al. observed a substantial increase in fluoride levels in urine following the application of approximately 4.52 mg fluoride in the form of varnish (Duraphat®) and a return to baseline conditions within 24 hours in a sample of 11 children between the ages of 4 and 7 years who lived in an area with fluoridated water and who used fluoride toothpaste. Hence, the authors concluded that fluoride varnish is a safe method of the topical application of fluoride even in children living in areas with fluoridated water and who use fluoride toothpaste on a regular basis [Pessan et al., 2005].

Lastly, in the previously mentioned study by Olympio et al. a comparison was made between two types of fluoride varnish: Duofluorid XII™, which contains 6% calcium fluoride, 6% sodium fluoride, and 5.63% of F-; and Duraphat®, containing 5% sodium fluoride and 2.26% F-. The two products were administered to a sample of 7 children aged 5 years who lived in an area with fluoridated water and brushed their teeth with a placebo toothpaste for 7 days. The quantity of varnish used was 0.2 ml per child. Urine was collected 24 hours prior to and 48 hours following the application of the varnish. The study results showed that the concentration of fluoride increased slightly following the application of Duofluorid XII™, although the increase was not significant with respect to baseline levels; the application of Duraphat® did lead to a significant increase in urinary fluoride excretion, although the concentration returned to baseline levels at 48 hours [Olympio et al., 2009].

This research shows that following the topical application of dental fluoride varnish in children, the fluoride/creatinine ratio in urine increases significantly...
compared to concentrations revealed in pre-treatment tests. More studies are necessary to investigate the relationship between professionally applied fluoride and systemic absorption.

References