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# A comparison of two methods for the evaluation of the daily urinary fluoride excretion in Romanian pre-school children

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***Objective*** To compare two different methods of estimating daily fluoride urinary excretion in pre-school children under stable fluoride intake conditions. ***Design*** Thirty-five healthy kindergarten children, permanent residents of Targu-Mures, Romania, where the average drinking water fluoride concentration is 0.12 mg F/L, participated on two separate occasions, when they were aged 4-6 and 5-7 years, respectively in the collection of a) a mid-morning spot urine sample and b) a 16-h time-controlled urine sampling. In case a), the ratio of concentrations of fluoride and creatinine were measured, while in case b) the rates of fluoride excretion in two separate 8-hour periods were used to estimate the 24-hour fluoride urinary excretion. ***Results*** The estimated average daily fluoride urinary excretion values (S.D.) were 0.318 (0.182) mg F/day for method a) and 0.341 (0.193) mg F/day for method b). These values were not significantly different (Mann-Whitney U test; $p = 0.49$). The estimated daily fluoride doses were 0.040 (0.021) and 0.043 (0.022) mg F/kg body weight/day, respectively. The latter values were not significantly different (Mann Whitney U test; $p = 0.38$). ***Conclusions*** Results obtained suggest that under stable F-intake conditions the estimation of the daily fluoride urinary excretion by means of a mid-morning spot urine sample yields comparable results to those obtained with the more involved method of separate, two 8 h (16 h) time-controlled urine sampling recommended by the WHO. Use of spot urine sampling appears to be particularly useful for epidemiological studies.

*Key words: Fluoride/creatinine ratio, fluoride excretion, pre-school children, urine*

## Introduction

According to the World Health Organization (WHO) report, dental caries remains a major public health problem in most industrialized countries, affecting 60–90% of school children and the vast majority of adults (Petersen, 2003). The reduction of oral disease in the child population of most Western countries coincides with an increase of that disease in Eastern and Central Europe. In Romania, surveys of school children showed a relatively high level of dental caries, and untreated caries constituted most of the caries index (Petersen et al., 1994; Petersen and Tanase, 1997; Künzel, 2001).

Before introducing any fluoridation programme, it seemed necessary to estimate the total daily fluoride exposure of these children.

One of the accepted methods for monitoring fluoride intake is the measurement of urinary fluoride excretion (Villa, 1994 and 2004; Lennon et al., 1996; Marthaler, 1999; Villa et al., 2000). However, there are relatively few community-based studies monitoring 24-hour urinary fluoride excretion in young children exposed to different fluoride regimes (Rugg-Gunn et al., 1993; Zohouri and Rugg-Gunn, 2000; Ketley and Lennon, 2001; Ketley et al., 2002).

A pilot-study was conducted in Targu-Mures in 2001 to investigate the daily excretion of urinary fluoride of kindergarten children under their customary conditions of fluoride intake. The study comprised 92 children, aged three to seven years, attending two kindergartens. Individual urinary mid-morning spot samples were collected and the corresponding analyses were conducted immediately. The method based on spot samples used in this study was the fluoride/creatinine (Q) ratio determination (Kertész et al., 1989; Villa, 1994; Marthaler et al., 1995). The Q ratio can be used as an index of 24 hours urinary fluoride excretion and the daily fluoride dose for each subject can be estimated as well. The results of this study seemed to indicate that the fluoride intake of kindergarten children in Targu-Mures was slightly below the optimal recommended range (Székely et al., 2002).

In order to assess whether the results obtained in the previous study were comparable to one of the methods recommended by the WHO (Marthaler, 1999), another study was conducted in 2002. This study comprised 35 children, 5–7 year olds, who participated in the previous study as well. This baseline urinary excretion study followed the 16-hours time-controlled urine sampling method recommended by the WHO (Marthaler, 1999; Székely et al., 2004).

Thus, the current work was carried out aiming to i) compare the data obtained by using two different methods, the fluoride/creatinine (Q) ratio of urinary spot samples and the supervised 16-hours time-controlled urine sampling; and ii) assess the average 24-hour urinary fluoride excretion and daily fluoride intake of pre-school children of Targu-Mures by these methods.

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## Method

Prior to the commencement of both studies, the participating children's parents were informed through a leaflet regarding the aims and objectives of our investigations and a positive consent was requested. Approval of the Local Ethics Committee was obtained as well.

### *Subjects*

Data of the same 35 healthy kindergarten children, aged 4-6 years [mean age (S.D.) 4.95 (0.80)] at the time of the first study, where the Q ratio method was used, and aged 5−7 years [5.95 (0.80)] when the second study based on the supervised 16-hour time-controlled urine sampling method was used, were compared.

### *Method*

Subjects continued to receive their usual diet which included tea and mineral water drinking, and tooth brushing with fluoride toothpaste. Every parent completed a questionnaire regarding the use of fluoridated toothpaste and any other fluoridated product, e.g. rinses, tablets, tea and mineral water by their child.

In the first study individual urinary spot samples were collected from the children after breakfast between 9-10 a.m. and the fluoride and creatinine analyses of these samples were conducted immediately. The method based on spot samples used in this study was the fluoride/creatinine (Q) ratio determination (Kertész et al., 1989; Villa, 1994; Marthaler et al., 1999).

The urinary fluoride excretion analyses were conducted with an ORION 720A selective-ion meter with a 9609 BN fluoride-selective electrode from ORION Research Inc. (Beverly, MA, USA), using the blank-corrected direct calibration method (Villa, 1988). Fluoride concentration of drinking water was assessed using the direct calibration method, as well. The calibration plot was built using a 1:1 mixture of calibration standards and TISAB II. Blank correction was carried out following a previously reported technique (Villa, 1988). The pH of the urine samples was not measured.

Urinary creatinine concentration was determined by the kinetics method using a Specol spectrophotometer with zv amplifier, FR optical diffusion system and photocells.

The second study was based on the supervised time-controlled urine sampling during 16-hours per day in order to measure the fluoride excreted per unit time during two approximately 8-hours periods (Marthaler, 1999).

The urinary samples were collected at the kindergarten where children spend eight hours every day, excepting weekend and holidays and the access to toilet can be controlled and supervised. The daytime urine samples were collected during approximately eight hours under the supervision of the kindergarten personnel. The first 8-hour period started at about 08:00 when children emptied their urinary bladders upon arrival to the kindergarten and lasted until 16:00 when they returned to their homes. The night urine samples were collected in a separate plastic container under parental supervision. This latter period lasted approximately eight hours. Details of each individual subject: number, name, age, gender, body weight and the urine volume for each specific period were recorded in tabular form and on the labels of the plastic containers. Fluoride analyses were conducted immediately. The basic information available was: a) time point of initial voiding of the bladder; b) time point of last urine collection into the collecting flask; c) total volume of urine collected between the initial and final time point.

Both studies were carried out on Wednesdays and were performed on the same month of successive years (March). Fluoride concentrations of drinking water at the kindergarten, which is served by the same waterworks of the community under study, were measured (triplicate) on the study days.

The questionnaires on dietary and tooth brushing habits were applied to the children's parents previous to the urinary samples collections.

### *Data management*

Data processing included calculating parameters of urine and fluoride excretion and fluoride intake. When using the Q ratio method (urinary spot samples) the individual daily fluoride urinary excretion was estimated using a previously proposed equation (Villa, 1994) which represented the best fit between the Q ratio of a mid-morning urine spot sample and the experimentally measured 24-hour fluoride excretion from 42 pre-school children under stable fluoride intake conditions. The corresponding numerical relationship was: Daily F-excretion (mg F/day) = [Q (mg F/g creatinine) + 0.13] / 3.59; $r = 0.94$, $p < 0.001$. Estimation of the total daily F-intake was obtained dividing the daily F-excretion by 0.35, which is the average value of the fractional urinary fluoride excretion (FUFE) obtained in several previous studies (Villa et al., 1999, 2000; Franco et al., 2005). This latter value is further analyzed in the Discussion section.

In the case of the 16-hour time-supervised urinary collection, the data obtained in each period of urine collection allowed the calculation of the following parameters: a) duration of the period (h); b) urinary flow rate (ml/h); c) fluoride excretion rate (µg/h).

From data of the two 8-hour periods the following parameters were assessed: a) integral daily urinary fluoride excretion (mg) using the extrapolation model previously proposed (Marthaler, 1999); b) daily fluoride intake (mg F/24-h) assuming a constant value (0.35) of the fractional urinary fluoride excretion (FUFE); c) estimated daily fluoride dose (mg/day/kg body weight), dividing the individual daily fluoride intake by each of the individuals' body weight.

Data were entered into a database for computational analyses and processing. Results obtained using the two different methods of estimation of the daily fluoride intake were compared using the Mann-Whitney U test since the frequency distribution of the data was skewed. The level of significance was set at $\alpha = 0.05$.

## Results

Parents' answers to the questionnaires applied for each study revealed that there were no relevant differences between each child's behaviour in relation to tooth brushing and dietary habits in the two separate occasions. Mouth rinses and dietary supplements were not used. Average tea consumption was 0.8 and 1.0 cups per day when the two separate studies were performed. The average fluoride concentration of the different teas consumed by the participating children was 1.3 mg F/L (range 0.8-2.2 mg F/L). Children did not consume either mineral water or tea when attending the kindergarten. The average daily frequency of tooth brushing when both studies were conducted was 1.8/day and the average F-concentration in tooth pastes used by the studied children was 1,080 mg F/kg.

The average water F-concentrations (S.D.) on both occasions were 0.118 (0.008) mg F/L and 0.131 (0.004), respectively. There was no significant difference between these values (t-test; $p = 0.09$)

Using the Q ratio method, the mean value (± S.D.) of the daily urinary fluoride excretion was 0.318 ± 0.182 mg F/day and the estimated average daily F-dose, using a constant fractional urinary fluoride excretion (FUFE) value of 0.35 was 0.040 ± 0.021 mg F/kg/day (Table 1).

The corresponding results obtained by the supervised 16-hour time-controlled urine sampling method were 0.341 ± 0.193 mg F/day and the estimated average daily F-dose, using a constant fractional urinary fluoride excretion (FUFE) value of 0.35 was 0.043 ± 0.022 mg F/kg/day (Table 1).

The estimated daily urinary fluoride excretion and daily fluoride dose were not significantly different in our studies (Mann-Whitney U test; $p = 0.49$ and $p = 0.38$, respectively).

## Discussion

In recent years, interest in assessing the total daily fluoride intake (TDFI) has increased, especially in young children that are at risk of developing enamel fluorosis of their anterior teeth (Zohouri and Rugg-Gunn, 2000; Villa et al., 2000; Haftenberger et al., 2001).

A generally accepted way for estimating TDFI is based on the fluoride excreted in 24-h urine (Rugg-Gunn et al., 1993; Marthaler et al., 1995, 2000; Villa et al., 2000; Haftenberger et al., 2001). However, estimating TDFI and the daily F-dose requires knowledge of the value of the fractional urinary fluoride excretion (FUFE) under customary F-intake conditions and by age group (Villa et al., 2000).

Previous studies (Kertesz et. al., 1989; Villa, 1994) indicated that the ratio "Q" = fluoride concentration (mg/l)/creatinine concentration (g/l) of a mid-morning urinary spot sample could be used as a reasonable estimator (on a community basis) of the 24-hour fluoride urinary excretion of pre-school children, provided that there are no "peak" F ingestion in some particular time of the day as with dietary supplements or fluoridated milk taken once a day. Total daily urinary creatinine excretion is relatively constant and the variations from day to day and within any 24-hour period are small (Marthaler, 1999; Harms and Scharfe, 1997). The Q ratio can be used as an index of 24 hours urinary fluoride excretion and the daily fluoride intake could also be estimated taking a constant FUFE value for this age group.

Recent guidelines for monitoring of renal fluoride excretion in community preventive programmes on oral health were published by the WHO (Marthaler, 1999). The different methods that are proposed in this publication are mainly related to the practical difficulties found for reliable 24-hour urine collection in epidemiological studies. Thus, different extrapolation models are proposed for estimating daily fluoride urinary excretion from partial 8- or 16-hour urine collection (Marthaler, 1999). However, these guidelines emphasise that these methods are particularly suited when the community preventive programmes on oral health that are applied in the communities under study involve "peak" F-intakes throughout the day as is the case of fluoridated milk or fluoridated salt programmes.

When no "peak" F-intake occurs such as when the baseline daily F-intake average value has to be assessed in a non-fluoridated community, i.e. with low fluoride concentration in its drinking water, or when epidemiological surveillance of a preventive programme of artificially adjusted fluoridated water has to be performed, the rather limited previous information available (Kertesz et. al., 1989; Villa, 1994) suggested that a simple, economic and effort saving method based on the determination of the fluoride / creatinine (Q) ratio can be used on a community basis.

**Table 1**. Summary of estimated mean daily F-excretion and daily F-dose from 35 children using the fluoride/creatinine (Q) ratio and 16-hour time controlled methods.

| | *Fluoride / Creatinine (Q) ratio* | | *16-hour-time controlled method* | |
|---|---|---|---|---|
| *Parameters* | *Estimated total daily F-excretion (mg F/day)* | *Estimated daily F dose* (mg F/kg/day)* | *Estimated total daily F-excretion (mg F/day)* | *Estimated daily F dose* (mg F/kg/day)* |
| N | 35 | 35 | 35 | 35 |
| Mean | 0.318 | 0.040 | 0.341 | 0.043 |
| S.D. | 0.182 | 0.021 | 0.193 | 0.022 |
| Median | 0.290 | 0.036 | 0.298 | 0.040 |
| Minimum | 0.131 | 0.015 | 0.130 | 0.012 |
| Maximum | 1.04 | 0.121 | 1.055 | 0.122 |

* Assuming a constant fractional urinary fluoride excretion (FUFE) value of 0.35.

This study compared the above simple method with the more involved method of time-supervised (16-hour) urine collection proposed in the WHO guidelines (Marthaler, 1999). Although the fact that the results obtained by the two methods that are being compared do not differ statistically does not necessarily mean that they are the same, they clearly suggest that, on a community basis, they yield comparable results. This finding provides additional support for using the urine spot sampling method when no "peak" fluoride ingestion occurs in the community under study.

The influence of the urinary pH on the proportion of ingested fluoride that is excreted with the urine has been thoroughly discussed by Whitford et al. (1976, 1988, and 1991). In the current study, the urinary pH of the samples were not measured since the eventual individual differences in fluoride urinary excretion between participating children were not as important as the average values of the amount of fluoride excreted using two different methods of urinary sampling.

Our results show that these two methods provide a similar estimation of the average daily fluoride excretion values for the same children under customary fluoride intake conditions. Thus, these results could be directly compared with the proposed "typical" daily fluoride excretion range of values for this age group in non-fluoridated and optimally fluoridated areas presented in Table 5 of the WHO guidelines (Marthaler, 1999). The average values of daily F-excretion from both studies is approximately 0.32 mg F/day, which is a consistent value with either the upper limit for a low F-intake community or the lower limit of an optimally fluoridated location, proposed by Marthaler (1999) for this age group.

In addition, once the individual daily fluoride excretion values are evaluated it should be possible to estimate the individual daily fluoride intake (TDFI) (and the daily fluoride dose), dividing the daily fluoride urinary excretion by the average fractional urinary fluoride excretion (FUFE) and the body weight of each participant child. In this study we estimated the TDFI using a constant FUFE value of 0.35, which is an approximate average value obtained in our (Villa et al., 1999, 2000; Franco et al., 2005) and other researchers' previous studies (Ketley and Lennon, 2000, 2001). However, different FUFE values for preschool children have also been reported. Haftenberger et al., (2001) determined an average FUFE value of 0.52 in German preschool children and a value of 0.80 was reported for Iranian children aged four years (Zohouri and Rugg-Gunn, 2000). It can be seen that a precise FUFE value for this age group is still uncertain and further studies on this issue could help to arrive at a commonly accepted average value. Nevertheless, the value of 0.35 used in the current study is the lowest value hitherto reported in the literature and consequently its use would provide the highest estimated daily fluoride intake and the highest daily fluoride dose, i.e. it would yield the most conservative values for these variables.

It might be argued that the comparison of the two methods for estimating daily fluoride intake is not strictly valid since both studies were carried out a year apart. However, on a community basis, the current results appear to indicate that the simpler "Q-method" yields comparable results to the more involved "time-supervised" method. This finding may have useful applications in epidemiological studies where the communities under study present "stable" F-intake conditions.

As discussed previously an average value of 0.041 mg F/kg/day for the estimated daily fluoride dose is obtained in the two separate studies performed. This value appears to be rather high for an area with only 0.12 mg F/L in the drinking water. A plausible explanation for this finding is that additional sources of F-intake, such as tea consumption and ingestion of fluoridated toothpastes might be contributing to the total F-intake of the population under study as described in the first paragraph of the 'Results' section of this paper. In a recently published paper (Maguire et al, 2007) it was concluded that fluoride toothpaste contributes a significant proportion of total ingested fluoride in children, particularly in low-fluoride areas.

On the other hand, the daily fluoride dose estimated for this non-fluoridated community (average fluoride drinking water concentration = 0.12 mg F/L) strongly suggests that the introduction of community based systemic fluoride programme in the studied community would unduly increase the risk of enamel fluorosis. This preliminary conclusion shows once again that presently, F-concentration of drinking water is not a good indicator of the safety of the introduction of systemic fluoride preventive programs, and in addition, highlights the importance of performing fluoride urinary excretion studies in preschool children in order to propose safe and efficient caries preventive programs.

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