Fluoride concentrations in saliva related to dental caries prevalence in primary teeth

K.J. TOUMBA, M.E.J. CURZON

Summary. Previous studies have indicated that there may be a relationship between salivary fluoride concentrations and dental caries. In these previous studies the emphasis was on dental caries in permanent teeth. Aim. The aim of this study was to evaluate a possible relationship between the prevalence of dental caries in the primary dentition and the fluoride concentration in saliva in 8-year-old children. Design. The study population consisted of 172 children aged 8 years living in the inner city area of Beeston of Leeds in the UK. Methods. Each child was examined for dental caries and unstimulated whole mixed saliva was collected for 2 minutes in the morning at least 2 hours post-prandial. Saliva samples were transported to the laboratory for immediate analysis or frozen for later analysis. All saliva samples were analysed for fluoride concentration using an ion-specific electrode after acid diffusion. Results. When the children were grouped according to their caries prevalence (dmfs 0, 1-5 and >5) and salivary fluoride levels (≤0.05, 0.06-0.10 and >0.10 mg/L fluoride) a statistically significant relationship was found ($\chi^2 = 22.88$, df = 4, $p<0.001$). Conclusion. For the primary dentition children with zero caries were shown to have significantly ($p<0.001$, Chi$^2$ analysis) higher salivary fluoride levels than caries prone children.

Key Words: Saliva, Fluoride, Dental caries, Primary teeth.

Introduction

Salivary fluoride levels vary from 0.01-0.10 mg/L depending on the water fluoride level and the fluoride usage and diet of the individual. Lagerlof and Oliveby [1994] stated that saliva influences caries attack mainly by its rate of flow and by its fluoride content. This group also reported that the salivary fluoride level was not influenced by the flow rate or by diurnal variation. Dawes et al. [1990] quote the normal concentration of fluoride in saliva as being about 1 mumol/L (0.019mg/L), and also confirmed that salivary fluoride levels were independent of flow rates, and that higher concentrations of fluoride in saliva led to the formation of calcium fluoride which had a longer clearance time.

Many researchers now believe that continuous low concentrations of fluoride in saliva, particularly at the plaque/saliva/enamel interface are necessary for caries prevention [ten Cate, 1997; Featherstone, 1999]. Leeverett et al. [1987] showed that caries-free subjects had higher salivary fluoride than high caries subjects. Shields et al. [1987] showed that subjects with no caries experience, from both fluoridated and non-fluoridated communities, had salivary fluoride levels of 0.04 mg/L or greater, whereas high caries subjects from both fluoridated and non-fluoridated communities had salivary fluoride levels of 0.02 mg/L or less. Bruun and Thylstrup [1984] studied the salivary fluoride levels of 13 year old Danish children residing in high (2.31 mg/L) and low (0.36 mg/L) water fluoride areas and their caries status. They reported that the children residing in the higher water fluoride area had higher salivary fluoride levels (0.047 mg/L) compared to the lower water area (0.026 mg/L) and correspondingly lower DMFS scores (7.0 versus 15.2). Duggal et al. [1991] also showed a consistent inverse relationship between salivary fluoride concentration and dental caries in 272 children living in rural areas in North India. Sjögren et al. [1993] reported that a caries-active group in Sweden had lower salivary fluoride levels than a caries-inactive group.

Toothbrushing with fluoride toothpastes leads to elevation of salivary fluoride levels. Duckworth et al. [1992] studied the oral bioavailability of...
fluoride from three different concentrations of fluoride toothpastes and found that salivary fluoride levels increased as the fluoride concentration in the pastes increased. Also there was an inverse relationship to the fluoride concentration of the pastes with caries increment data obtained from the clinical trial of Stephen et al. [1988] using the same pastes. These authors suggest that oral fluoride measurements are a valuable in vivo method for the evaluation of the potential anti-caries efficacy of fluoride-containing dental products. Afflitto et al. [1992] investigated salivary fluoride levels with differing fluoride toothpastes and a rat caries study, and supported the concept that fluoride in saliva following toothpaste use is important to anti-caries efficacy.

The clearance of fluoride from saliva following toothpaste use has been investigated in a number of studies. Duckworth et al. [1991] found that following a single brushing the salivary fluoride level decreased in two distinct phases. There was an initial rapid phase (40-80 mins) followed by a slow phase that lasted for several hours and was possibly due to release of fluoride from intra-oral reservoirs. Repeated regular brushing led to elevated baseline values and the fluoride stored in the oral reservoirs was postulated to maintain a prolonged caries-protective effect. Sjögren et al. [1994] found that salivary fluoride levels following toothbrushing decreased 1-2 times after a single rinsing, and 4-5 times after a double rinsing compared to no rinsing. Eating immediately after brushing led to a 12-15 times reduction in salivary fluoride levels.

Duckworth et al. [1994] studied the effect on salivary fluoride levels of delivering the same amount of fluoride per se in differing concentrations (i.e. in different volumes) and found that the fluoride concentration was more important. The saliva levels were elevated for up to three hours after a single use and for up to 18 hours following regular daily use. Duckworth and colleagues concluded that their study implied that a smaller volume at a higher fluoride concentration may increase efficacy. However, they do not comment on whether salivary fluoride levels can be elevated for prolonged periods with regular daily use of low concentrations of fluoride. The aim of this study was to examine the prevalence of dental caries in the primary dentition related to the salivary fluoride concentration in a group of 8-year-old children.

Materials and methods
Participants. Ethical approval was obtained from the Leeds Ethics and Research Committee. Children aged 8 years old and from seven schools all within the same postal area (LS11) of the Beeston area of Leeds participated in the study. Positive signed parental consent together with completed medical history forms were obtained for each child. A II children included in the study were examined by the investigator in their schools. This usually took place in either the medical room or a classroom.

Dental examination. The children underwent a dental examination with the child lying supine on a portable A dec (A-Dec UK Ltd, 75-76 Gravelly Industrial Park, Birmingham, UK) dental chair and illumination provided by a Daray lamp with a 60 watt bulb (Daray “Versatile” dental model SL 400/222 with G clamp, Daray Lighting Ltd, 7 Commerce Way, Stanbridge Road, Leighton Buzzard, Bedfordshire, UK) situated one metre away from the patient’s face. All of the dental examinations were performed by a single examiner who was a calibrated examiner for epidemiological surveys for the British Association for the Study of Community Dentistry. Dental examinations involved the charting of all tooth surfaces in order to obtain a decayed, missing and filled permanent teeth/surface (DMFT/S) or decayed, missing and filled primary teeth/surface (dmft/s) index score. The criteria of Palmer et al. [1984] was used for the diagnosis of caries at the cavitation level, i.e. involving dentine, and the data was recorded on a standard recording form. Visual rather than tactile criteria were used to diagnose caries to avoid any unwanted effects from injudicious probing of demineralised enamel surfaces [Ekstrand et al. 1987; Van Dompel et al. 1988]. The teeth were examined in the following order: upper right, upper left, lower left and lower right. The sequence for examining the surfaces of each tooth was distal, occlusal, mesial, buccal and lingual. The teeth were not brushed beforehand, any debris or moisture was removed with cotton wool rolls if visibility was obscured. A tooth was deemed to be present if any part of it was visible. Surfaces that could not be fully examined were excluded. The examinations were conducted with number 4 plane mouth mirrors and assisted with a number 54 sickle probe, the point of which had been ground flat to a diameter of 0.5 mm.

Reproducibility of dental examinations. The first
25 children (14.5% of the study group) were re-examined on the same day, but at least two hours after their initial examination in order to determine the investigator’s diagnostic reproducibility. All of the data was recorded by a dental nurse, and the children were examined in a random order to that of the earlier examination, in order to eliminate recognition of the children and bias. The results of the second examination were then compared with those of the initial examination to obtain Cohen’s [1960] kappa value of reproducibility for caries diagnosis.

Saliva collection. Samples of saliva were collected into plastic specimen containers as whole unstimulated saliva for a period of two minutes. The saliva samples were collected at least two hours post-prandial and were either analysed for fluoride immediately on return to the laboratory or stored frozen at -12°C until analysis. Salivary fluoride analysis. The method used was that of Taves [1968], in which fluoride was diffused from the samples using hydrochloric acid saturated with hexamethyldisiloxane (HMDS). Fluoride was collected in sodium hydroxide before its determination by a fluoride ion-specific electrode. From each sample of saliva 1.0 ml was pipetted into a 60 x 15 mm polystyrene petri dish (Falcon Code 1007, Fahrenheit Lab Supplies, Northfield Road, Rotherham, U K) and distilled water added to make a final volume of 3.0 ml. Polystyrene tube caps (Falcon Code 2051) with the rims reduced by a half were placed in the centre of each dish. To the centre of each cap well 0.1 ml of 1.65 M sodium hydroxide (NaOH) containing p-nitrophenol and phenolphthalein (0.1% solution in 95% ethanol) as an indicator to ensure that the trap remained alkaline and therefore was able to trap the fluoride. An alkaline trap remained yellow in colour while an acidified trap turned pink. The lids were then sealed on the petri dishes using petroleum jelly applied to the rims using a syringe. Finally, 1.0 ml of 6.0 M hydrochloric acid with HMDS (Sigma Chemicals, Poole, Dorset, U K) was added to each dish via a small hole previously made in the lid, and the hole was sealed immediately with petroleum jelly and with parafilm. The samples were placed on an IKA - Vibrax VXR rotary shaker (Sartorius Instruments Ltd, Belmont, Surrey, U K) at 200 rotations/minute and left to diffuse overnight, which was usually 16hrs. The following morning the lids were prised off and a note made of any of the dishes which had not formed a vacuum. Loss of the vacuum was an indicator that fluoride had been lost from the system. Each of the caps were removed and placed in an oven at 100°C until the NaOH had become crystalline. After drying the NaOH, the caps were placed on their test tubes (Falcon Code 2051) and shaken with 0.33 ml of 0.66 M acetic acid to dissolve the crystals and bring the pH to 5.2 for determination of fluoride by an Orion 920A Ionanalyzer and combination fluoride ion-specific electrode (Orion 960900, Orion Research Inc, Lewes Road, Forest Row, East Sussex, U K). Fluoride standards of 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0 and 5.0 mg/L (ppm F) were diffused in duplicate and used to construct a calibration curve. The fluoride concentrations in the unknown diffused samples were measured from this curve. Blanks consisting of water were run through in duplicate with each batch of analyses.

Data collection and statistical analyses. All the data collected at each examination was recorded on a form and together with the results of the analyses of salivary fluoride were entered onto an Elonex-486-PC using the Survey Plus1 package (version 4.35, Providence Software Services, 64 Berkley Road, Westbury Park, Bristol BS6 7PL). Chi square analyses were performed for the salivary F levels in relation to the caries status. Cohen’s (1960) kappa value of reproducibility for the researcher for caries diagnosis was performed.

Results

Data was gathered for 172 children (mean age 8.8 years) who participated in the study. Table 1 gives the dental caries data prevalence as dmfs (±SD ) for the child population, stratified by caries level. Table 2 shows the fluoride (F) concentrations in saliva related to the dental caries prevalence of the primary teeth together with the Chi² statistical analysis. Salivary F levels were found to be higher in caries-free individuals (mean, 0.13 mg/L F) compared with caries-prone individuals (mean, 0.05 mg/L F) for the primary dentition. Chi² analysis of the three dmfs bands with three bands of salivary F levels (<0.05, 0.06-0.10, and >0.10 mg/L F) was performed on the data and salivary F levels were found to be significantly (p<0.001) related to the dmfs.

The reproducibility for the caries diagnosis was good. Cohen’s Kappa value of reproducibility for caries diagnosis was found to be 1.00 for diagnosing caries at the dentine level.
Discussion

Even if the present study was based on fluoride measurements from only one single sample of saliva per subject, it seems that the inverse relationship of salivary fluoride concentration to dental caries prevalence holds true for the primary dentition also. The time of saliva sampling and factors that increase fluoride clearance (e.g. eating and drinking) will have an important effect on the salivary fluoride levels. The children in the present study all provided saliva samples early in the morning by 10.00h and had nothing to eat for at least two hours. Further, the children all resided within the same postcode and were of similar socio-economic status. Duckworth et al. [1994] found that following toothbrushing with fluoridated pastes the saliva levels were elevated for up to three hours after a single use and for up to 18 hours following regular daily use.

A possible explanation for the differences in the salivary fluoride levels of the children is related to differences in toothbrushing habits among the children. Most children claim to brush twice daily whether in reality they do or not. Ten Cate [1997] states that only low levels of fluoride are necessary as long as the fluoride is deposited and released slowly. This is achieved by the formation of calcium fluoride (or like) deposits intra-orally which slowly re-release the fluoride. Regular toothbrushing with fluoridated pastes will maintain the salivary fluoride level. Thus salivary fluoride concentration could be used as a predictor of caries risk. This has been demonstrated by means of linear discriminant analyses to correctly predict which children would develop caries within six to 12 months in 82.8% of cases [Leverett et al. 1993a and 1993b]. The more frequently children brush their teeth with fluoridated toothpastes the more elevated the level of salivary fluoride will be depending on the concentration of fluoride used. The level of salivary fluoride will be sustained for longer periods in comparison to non-toothbrushers and impart greater caries prevention.

Bruun et al. [1982] showed that salivary fluoride levels varied widely reflecting the different dosages of the fluoride topical agents applied (dentifrice, tablets, mouthrinse and topical solution), whereas the caries reduction data irrespective of type of treatment appeared very similar (with a magnitude of about 30%). The authors were able to explain their observations on the basis of the salivary fluoride levels in combination with the recent understanding of enamel-fluoride kinetics. Thus it is the activity of the fluoride ion in the oral environment that is now considered to be of the utmost importance for caries prevention rather than having very high levels of fluoride in surface enamel [Fejerskov et al. 1981].

Conclusions

Salivary F levels were found to be higher (p<0.001, Chi² analysis) in caries-free individuals (mean, 0.13 mg/L F) compared with caries-prone individuals (mean, 0.05 mg/L F) for the primary dentition of 8-year-old children.
References


Lagerlof F, Olieveye A. Caries-protective factors in saliva. A dvances in Dental Research 1994; 8: 229-238.


