Charles University in Prague
Faculty of Medicine in Hradec Králové
Department of Dentistry

# COMPARISON OF TWO TOPICAL FLUORIDE APPLICATIONS IN DECIDUOUS AND PERMANENT MOLARS FOR DENTAL CARIES PREVENTION

An outline of Ph. D. thesis in Dentistry

By

Ana Lucía Seminario Antúnez de Mayolo

Hradec Králové

2006

Charles University in Prague
Faculty of Medicine in Hradec Králové
Department of Dentistry

**Ana Lucía Seminario Antúnez de Mayolo**

# COMPARISON OF TWO TOPICAL FLUORIDE APPLICATIONS IN DECIDUOUS AND PERMANENT MOLARS FOR DENTAL CARIES PREVENTION

**An outline of Ph. D. thesis in Dentistry**

**Hradec Králové**

**2006**

This work was supported by grant MZ ČR 00179906

This dissertation work was done to obtain the doctoral degree at the Department of Dentistry, Faculty of Medicine in Hradec Králové, Charles University in Prague, Czech Republic.

**Author:** Ana Lucía Seminario Antúnez de Mayolo, DDS
Dept. of Dentistry
Faculty of Medicine in Hradec Králové
Charles University in Prague

**Supervisor:** Associate Prof. Věra Hubková, M.D., PhD.,
Head of Department of Dentistry
Faculty of Medicine in Hradec Králové
Charles University in Prague

**Opponents:**

1. **Doc. MUDr. Jan Handzel, CSc.,**
   **stomatologická klinika 1. LF UK a VFN, Praha**
2. **Doc. MUDr. Vlasta Merglová, CSc.,**
   **stomatologická klinika LF UK a FN, Plzeň**

The commentary has been elaborated by the Department of Dentistry, Faculty of Medicine in Hradec Králové, Charles University in Prague, Czech Republic.

Autoreferát byl rozeslán dne 29. 5. 2006

Obhajoba se koná před komisí pro obhajoby disertačních prací v doktorském studijním programu stomatologie

**v pondělí dne 26. června 2006 od 10.00 hodin,**

v Sazamově posluchárně stomatologické kliniky LF UK a FN, Hradec Králové (budova č. 1, areál FN).

It is possible to consult the original dissertation work at the Dean Office of the Charles University Faculty of Medicine, Šimkova 870, 500 38 Hradec Králové.

Assoc. Prof. Věra Hubková, M.D., Ph. D.
Chair of the commission for defence of dissertation works
of the doctoral study program in dentistry

CONTENTS

## I. Introduction

Despite great improvements in the oral health of populations across the world, dental caries still persists particularly among poor and disadvantaged groups in both developed and developing countries, being in children the most prevalent daily problem that dentist face. According to the World Oral Health Report 2003, dental caries remains a major public health problem in most industrialized countries, affecting 60–90% of schoolchildren and the vast majority of adults.

In Czech Republic, where the prevalence of dental caries in 12 years old children is 86%, preventive measures for keeping young children without tooth decay is necessary (1). The paediatric dental service at the Faculty of Medicine in Hradec Králové works intensively in the improvement of oral health in the children of the community and is interested in improve the its actual prevention programs.

The definition of dental caries has been changing in the last century according to new discoveries and modern tools of research. In the 1950s, Keyes introduced the concept of dental caries as "infectious and transmittable" after studies in rodents. Caries only was observed in animals when they were caged with or ate the faecal pellets of groups of caries-active rodents (2). Further investigations were focused in the study of specific bacteria as the cause of dental decay. These specific bacteria, identified as *Streptococci mutans* (SM), introduced the concept caries as a consequence of a SM specific infection and this definition has been sustained in caries microbiology over the last four decades (3).

The association of sugars in the development of dental caries has been widely studied since the first publication made by Gustafsson et al in 1954 with his Vipeholm

Study. He concluded that the more frequently sugar is consumed the greater the risk; sugar consumed between meals has much greater caries potential than when consumed during a meal (4). Continuing the study of sugars, Marthaler in 1967 concluded that foodstuffs containing simple sugars are far more cariogenic than common starchy foods and Newbrun two years later suggested the specific elimination of sucrose or sucrose-containing foods rather than restricting total carbohydrate consumption (5).

From the factors above founded the concept of dental caries changed as a multifactorial disease, being the principal components:

1. Microflora: acidogenic bacteria that colonize the tooth surface.
2. Host: includes factors as quality and shape of tooth, quantity and quality of saliva, etc.
3. Diet: intake of fermentable carbohydrates, especially sucrose, but also starch.
4. Time: total exposure time to organic acids produced by the bacteria of the dental plaque.

**The specific plaque hypothesis**

The presence of bacteria in the development of dental caries is a fact without controversies. During many years in the history of caries microbiology, lactobacilli were considered as the main microbiological agents in the decay process. Further studies in germfree rodents with inoculation of single bacteria strains demonstrated that not all bacteria were capable to develop decay lesions in rats fed diets containing high sucrose levels (6). Similar studies also demonstrated that a range of bacteria, which are not often isolated in large numbers of dental plaque as Streptococcus salivarius and Enterococci spp. would cause caries in rats. However, inside this range

of cariogenic bacteria there is a hierarchy leaded by SM, first isolated from dental lesions by Clarke in 1942 (7). These studies were the first to propose the hypothesis that SM are the most important bacteria related to dental decay. Later on, several studies were conducted in order to evaluate the association between number, concentration of SM in plaque and/or saliva and the dental caries status of populations, and to a lesser extent to the caries status of an individual.

**Biofilm induced disease hypothesis**

Being dental caries induced by oral bacteria a new question arrives: is it endogenous or exogenous specific bacteria that infect the individual? After evaluating the developed of dental plaque, resident flora always is found forming biofilms and these bacteria in the biofilm are always metabolically active, causing fluctuations in pH. These fluctuations may cause a loss of mineral from tooth when the pH is dropping or gain of mineral when the pH is increasing (8). The cumulative result of these de- and re-mineralization processes may be a net loss of mineral, leading to dissolution of the dental hard tissues and the formation of a caries lesion (9). This change in the concept of dental caries is reinforced by Fejerskov describing it as a "complex disease caused by an imbalance in physiologic equilibrium between tooth mineral and biofilm fluid" (10).

Oral bacteria do not exist as independent entities but rather function as a co-ordinated, spatially organized and metabolically integrated microbial community (11). The microbial community lifestyle grants benefits to each individual member, these are well explained by Marsh (12) as: a) a broader habitat range for growth, e.g. oxygen-consuming species create environmental conditions suitable for obligate anaerobes; b) a more efficient metabolism, e.g. complex host macromolecules can only be degraded by consortia of oral bacteria; c) increased resistance to stress and

antimicrobial agents, and d) enhanced virulence (pathogenic synergism). But dental caries involves also other relevant factors represented by Fejerskov and Manji (13), it includes individual as well as population level, many of these variables (oral hygiene, diet, etc.) will be highly influenced by the behavioural and socio-economic conditions prevailing.

**Fluorides**

The fluoride ion is the most electronegative of the elements and the major reason for the dramatically reduction of dental caries in the last 50 years. It was discovery when Dean et al compared the incidence of caries in individuals exposed to so-called high-fluoride water supplies with that in individuals exposed with lower levels (14). In the middle of the last century, research about fluoride action over dental caries concluded as the paradigm that, to exert its maximum cariostatic effect, fluoride had to become incorporated into dental enamel during development, and hence it was inevitable to have a certain prevalence and severity of fluorosis in a population to minimize the prevalence and severity of caries among children. Dental fluorosis was then regarded as an unfortunate side-effect of fluoride caries-protective benefit, and attempts to "play down" the possible toxic effect of fluoride on developing dental enamel often led the dental profession to present dental fluorosis as merely a cosmetic problem (15). In the evolution of medical research where now the science is "evidence-based medicine", the understanding of fluorides effect – systemic and topical - opened a new paradigm focused in the way of how this element affects mineralising and mineralised dental hard tissues.

## II. Aims of the investigation

The use of topical fluorides from health professionals has been extended and also demonstrated as efficient therapy against dental decay. We were interested in evaluation the efficacy of two topical fluorides, gels and varnishes, in the prevention of dental caries. We also studied oral bacteria, streptococci mutans (SM) and theirs close relationship to dental caries (DMF; D = decayed, M = missing, F = filled). The goals of our study are synthesized in the following objectives.

**General objective:**

To evaluate the use of two topical fluorides as a preventive method against dental caries in children from 3-6 years old.

**Specific objectives:**

1. To assess dmft and DMFT in children from 3-6y.
2. To assess SM levels in children from 3-6y.
3. To determine any relationship between SM levels and dental caries in children from 3-6y.
4. To assess exposition to carbohydrates in children from 3-6y.
5. To determine any relationship between exposition to carbohydrates and dental caries in children from 3-6y.
6. To determinate dental caries risk of children from 3-6y.

## III. Materials and methods

### 3.1. Type of study:

Cohort: prospective and longitudinal

### 3.2. Universe

All the children from 3-6 years old who received dental treatment at the Department of Dentistry, Charles University in Prague, Faculty of Medicine in Hradec Králové from March 2004 to March 2006.

### 3.3. Sample

Approximately 60 children, patients of the Department of Dentistry, Charles University in Prague, Faculty of Medicine in Hradec Králové were involved in the study based upon their parent informed consent.

### 3.4 Variables

**Independent variable:** TYPE OF FLUORIDE TOPICAL APPLICATION

1. AFP gel 1.23%
2. Fluoride Varnish 0.1%

**Dependent variables:**

1. DMFT, dmft, records.
2. SM levels.
3. Carbohydrate frequency ingestion.
4. Caries risk.

### 3.5. Description of the variables

#### 3.5.1. Independent variable: TYPE OF FLUORIDE TOPICAL APPLICATION

It is referred to the local fluoride that was used in our work. In this study the fluorides allocated were:

a) Acidulated Phosphate Fluoride gel 1.23%, it was applied with cotton sticks in the molars previously randomly selected. The gel stayed over the teeth for about 2 minutes, according to manufacturer's recommendations.

b) Fluoride Varnish 0.1%, it was applied with specifically sticks (provided by the same product) in the molars previously randomly selected. The varnish stayed over the teeth for about 1minute, according to manufacturer's recommendations.

#### 3.5.2. Dependent variables:

**a) DMFT and dmft:** the evaluation of dental status was performed according to World Health Organisation (WHO) chart.

**b) SM level:** the evaluation of SM level was registered in two ways:

- ***Laboratory method***, this is the traditional method for counting bacteria. Saliva is collected, mixed with a proper transport medium and forwarded to a microbiologic laboratory. After incubation using a selective medium mitis-salivarius-bacitracin agar, approximately 100-150 colonies were observed as to their morphology. The percentages of colonies resembling S. mutans, S. salivarius, S. sanguinis and S. viridans were calculated.

- ***Chairside method***, or also called Strip Mutants test. It was described by Jensen and Bratthall in 1989 and is based on the ability of SM to grow on hard surfaces in a

selective broth (high sucrose concentration in combination with bacitracin). Because the bacitracin can be added to the broth just before use, the shelf live of the test can be prolonged considerably compared to that of agar plates. In proportion to their actual amount in saliva, MS in the specimens will adhere to the treated side of the strip and grow as small, dark or light blue colonies, 1mm in diameter or considerably less, when growth is very dense. The amount of MS per millilitre of saliva is estimated by comparing the colony density on the strip with the standard charts included in the instructions.

**c) Carbohydrate exposition**: for evaluation of diet, it was recorded the frequency of carbohydrate consumption during 4 continuous day (two weekend and two week days). Parents were asked to fill the type of aliments and time when they were ingested.

**d) Caries risk**: the evaluation of caries risk was obtained from the number of cavities, dental plaque score and frequency of carbohydrate ingestion. After tabulated, scale results were from 1 to 3 (low risk-severe risk).

**3.5.3: Criteria of inclusion:**

- Children from 3 to 6 years old, in general good health, cooperative behaviour and dental caries free.

**3.5.4: Criteria of exclusion:**

- Children with any kind of systemic disease.

- Parents without information acceptance document signed.

**3.6. Procedures**

1. Children previously selected, were divided into 3 cohorts with different preventive interventions:

**Cohort A**: instructions and supervisions of regular tooth brushing and topical applications of fluoride gel (each 6 months: 4 applications).

**Cohort B:** instructions and supervisions of regular tooth brushing and topical applications of fluoride varnish (each 6 months: 4 applications).

**Cohort C (control):** instructions and supervisions of regular tooth brushing (only).

2. Clinical and laboratory examinations recorded were:

- Dental status (WHO chart)
- Dental plaque status (OHI- DI)
- Salivary cariogenic streptococci (mitis-salivarius-bacitracin agar and Dentocult SM, Orion Diagnostica OY)
- Evaluation of carbohydrate frequency ingestion.
- Evaluation of caries risk.

3. Description of topical application procedures:

Deciduous and permanent molars were associated in caries free pairs (4 pairs per child in group A and 2 in group B). In each pair only one molar, randomly selected and previously cleaned by low speed toothbrush and hydrogen peroxide, received either fluoride gel NaF 1.23% (Mirafluor gel, Hager Werken) or fluoride varnish 0.1% (Fluor Protector, Vivadent). The other one rested without any fluoride application as control. Topical fluoride procedures were conducted according to manufacturer's instructions.

4. The study was performed according to the present chronogram

- Onset of study

i. Collection of children

ii. Informed consent

iii. Onset clinical and laboratory examination

iv. Distribution of children into cohorts

v. Tooth brushing instructions

vi. Topical applications of fluoride [Cohort A and B] **Jan – Mar 2004**

- 1st. recall

i. Clinical and laboratory examinations

ii. Tooth brushing re-instructions

iii. 2nd stage of topical applications [Cohorts A and B] **Sep 2004**

- 2nd recall [the same as in 1st. recall] **Mar 2005**

- 3rd recall [the same as in 1st. recall] **Sep 2005**

- 4th recall [the same as in 1st. recall] **Mar 2006**

- Final recollecting data

i. Calculations the data [dmft, DMFT, delta dt, delta ft, delta DT, delta FT, OHI-DI, S. mutans salivary levels]

ii. Statistical processing the data [differences among cohorts, differences among recalls]

iii. Evaluation of results

iv. Final report **Mar- Apr 2006**

**IV. Plan of statistical analysis**

The dental status, dental plaque and SM data from the onset examination and those from two years later were compared by paired Student's t-test at P=0.05, and correlation coefficients. Caries experience and caries increment only in deciduous molars (Group 1) or in permanent molars (Group 2) were also evaluated.

## V. Results and discussion

**TABLE 1. Data of group 1: permanent molars.**

| Stage of study | 0 | | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | mean | SE | mean | SE | mean | SE | mean | SE | mean | SE |
| Permanent teeth | 8.00 | 0.43 | 9.25 | 0.50 | 11.00 | 0.36 | 12.39 | 0.43 | 12.54 | 0.45 |
| D teeth | 0.07 | 0.07 | 0.14 | 0.08 | 0.32 | 0.10 | 0.11 | 0.06 | 0.11 | 0.06 |
| F teeth | 0.63 | 0.28 | 1.11 | 0.30 | 1.21 | 0.29 | 1.54 | 0.31 | 1.54 | 0.31 |
| M teeth | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| DMF teeth | 0.96 | 0.30 | 1.25 | 0.32 | 1.54 | 0.30 | 1.64 | 0.30 | 1.64 | 0.30 |
| RI | 95.00 | 5.00 | 88.46 | 7.81 | 66.18 | 10.27 | 84.21 | 8.59 | 84.21 | 8.59 |
| Dentocult | 1.71 | 0.23 | 2.89 | 0.08 | 1.79 | 0.21 | 1.96 | 0.25 | 1.96 | 0.25 |
| CFU | – | – | 8.14 | 1.40 | 6.55 | 0.91 | 4.18 | 0.73 | 4.44 | 0.84 |
| Diet | – | – | 3.19 | 0.15 | 3.13 | 0.12 | 3.23 | 0.17 | 3.21 | 0.17 |
| Risks | – | – | 1.50 | 0.10 | 1.79 | 0.09 | 1.54 | 0.11 | 1.50 | 0.11 |

**D: decay teeth, F: filling teeth, M: missing teeth, RI: restorative index, CFU: colony forming units**

**TABLE 2. Data of group 2: deciduous molars.**

| Stage of study | 0 | | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | mean | SE | mean | SE | mean | SE | mean | SE | mean | SE |
| d teeth | 0.20 | 0.14 | 1.00 | 0.32 | 1.13 | 0.35 | 0.93 | 0.38 | 0.93 | 0.38 |
| f teeth | 1.20 | 0.46 | 1.20 | 0.49 | 1.60 | 0.49 | 2.00 | 0.50 | 2.00 | 0.50 |
| m teeth | 0.33 | 0.27 | 0.33 | 0.27 | 0.47 | 0.27 | 0.47 | 0.27 | 0.47 | 0.27 |
| dmf teeth | 1.73 | 0.57 | 2.53 | 0.72 | 3.20 | 0.84 | 3.40 | 0.88 | 3.40 | 0.88 |
| Ri | 92.3 | 5.0 | 51.3 | 12.9 | 56.7 | 10.1 | 73.0 | 8.1 | 73.0 | 8.1 |
| Dentocult | 1.80 | 0.34 | 3.00 | 0.00 | 1.73 | 0.28 | 2.27 | 0.23 | 2.20 | 0.24 |
| CFU | – | – | 2.61 | 0.76 | 6.94 | 1.11 | 4.37 | 0.99 | 4.34 | 0.85 |
| Diet | – | – | 3.55 | 0.23 | 3.31 | 0.46 | 3.62 | 0.22 | 3.69 | 0.21 |
| Risks | – | – | 1.80 | 0.11 | 1.60 | 0.16 | 1.53 | 0.13 | 1.53 | 0.13 |

**d: decay teeth, f: filling teeth, m: missing teeth, ri: restorative index, CFU: colony forming units**

Oral health is indispensable for an acceptable quality of life. Despite great achievements in oral health of population globally, problems still remain in many communities all over the world - particularly among under-privileged groups in developed and developing countries. Dental caries and periodontal diseases have historically been considered the most important global oral health burdens. At present, the distribution and severity of oral diseases vary among different parts of the world and within the same country or region. Dental caries is still a major oral health problem in most industrialized countries, affecting 60-90% of schoolchildren and the vast majority of adults.

The use of topical fluorides has been demonstrated as necessary tool in the prevention of dental caries. Although there is a vast options of them we wanted to concentrate in two mostly used fluoride applications by professional cares, which are gels and varnish.

A brief summary of our work is condensate in the tables 1 and 2 (permanent and deciduous molars, respectively). There is a big jump and consequently a big difference between the first data evaluation (stage 0) and the stage 1 (after 6 months). It was because children, enthusiastic for being selected, brushed their teeth immediately before our examination. In the second control, all were warned to come without any special cleaning, just the daily usual hygiene that they use to do at home. Then, the real data was shown.

It is also important to note, that evaluation of CFU, carbohydrate ingest evaluation and caries risk was considered since the stage 1. When we started our study, we were afraid of the parent's cooperation. For that, we did not wanted to overload them with more time at the recall appointment and asking for a diet questionnaires. However, in the 96.6% of the parents (two of them decided to retire from our study) the response was evidently positive and even enthusiastic. We added these 3 new items in our research and results are showed since stage 1 in both tables.

In order to obtain each cohort stable during the two years of research, we selected patients in deciduous dentition and first phase mixed dentition. Nevertheless, some of them presented new teeth eruptions and the local environment must have changed if we compare with the initial stage of our work. For the next longitudinal studies, we do recommend to select children in the very initial phase of each category, as 3-4 years in deciduous dentition and 6-7 years old in first mixed permanent dentition. It will avoid changes in the number of permanent teeth along 2 years longitudinal study.

Dental decay in our study was present and increasing along the controls. In the group 1 (permanent molars) the increment was not significantly while in the second group (deciduous molars) this increment reached statistical significance. We found that the main incidence of new caries lesions was interproximal (specially incisors). Parents were not using dental floss as part of the daily dental hygiene of their children. Bellamy et al evaluated the impact of flossing the interdental space comparing it with manual toothbrush Thirty-nine subjects were sampled, over a three-week treatment period, in two balanced and equally sized treatment groups, with twice-daily manual brushing with or without daily flossing. The clinical study demonstrated that after three weeks, interdental plaque in floss users was significantly reduced versus baseline scores. Non-floss users showed no significant reduction. They concluded that daily flossing significantly reduced the amount of plaque found between the teeth compared to a manual toothbrushing regimen alone (16).

Data of filling teeth was statistically significant in both groups and consequently, the DMFT and dmft had also statistically significant increment at the end of our study. Any missing tooth was recorded in the group 1 and the increment data from group 2 was too small to be considered statistically significant.

The evaluation of SM was performed by Dentocult SM and CFU variables. We wanted to compare the efficacy of chairside method and the traditional agar one. We did not

find statistical significant difference between the both methods. Our results are similar to the one performed by Davenport et al. They evaluated SM and lactobacilli levels by conventional and commercial dip-slide methods in three groups of young subjects, aged 5-6 years (93 subjects), 12-13 years (78 subjects) and 18-20 years (81 subjects). Using the same paraffin-stimulated saliva samples, ms and lactobacilli were estimated by conventional viable counts on modified mitis-salivarius agar (MSB) and Rogosa agar plates, and by inoculation of Dentocult SM and Dentocult LB dip-slides (Orion Diagnostica, Finland). They concluded that these dip-slide tests provided suitable and simple methods for screening salivary lactobacilli and SM levels which may have a useful role in the assessment of caries risk (17). In 1995, Pienihakkunen et al evaluated Dentocult SM test. The study assessed the practicability of this test in children, using dental floss to transfer the dental plaque to the strip. The subjects were children of 2-3 yr (n = 365) and 5-6 yr (n = 398). The mutans streptococci count on the strip was found to be a good indicator of infection and was surprisingly accurate in the prediction of the 3-yr caries increment (18).

Regarding Dentocult SM records and incidence of dental caries, our results did not demonstrate a conclusive statistical relation between them. In the literature on this topic, Newbrun et al compared two screening tests for SM and evaluated their suitability for mass screenings and private practice. Both tests use mitis salivarius medium with bacitracin (MSB) and are selective for SM. One test estimates colonies grown on agar (MSBA) and the other estimates colonies grown in broth that adhere to glass (MSBB). Both tests were very good in identifying children with low caries increments, but positive scores did not correlate well with high caries increments. They concluded that these tests are economical and suitable for mass screenings to identify low risk populations who do not require preventive treatment (19). Splieth and Bernhardt performed another interesting study in 1999. The aim of the study was to evaluate the validity of a site-specific chair-side mutans streptococci (MS) test for the

prediction of caries incidence in fissures. In 230 6- to 7-yr-old children, occlusal plaque samples of teeth 16 and 36 were cultured with Dentocult SM. Caries (DMFS), initial caries, sealants, and a plaque index (QHI) were recorded and oral hygiene habits were assessed. After 2 years, the status of the fissures was re-examined, and a fluoride history was recorded with a questionnaire filled out by the children's parents. The SM scores and caries incidence correlated significantly. Seventy-eight % of the caries progression in fissures was diagnosed correctly. Sensitivity was 50%, specificity 82%, positive predictive value 29%, and negative predictive value 92%. Children with caries progression tended to have lower fluoride scores. Low MS scores were most likely to be associated with low caries incidence, while high mutans streptococci scores seem to be partially compensated by other parameters (20).

In our work, we did not found statistical significant relation between Dentocult scores and dental caries. Since all our children started in our study without dental caries, and kept as low caries risk, the use of SM level as dental caries predictable factor was not suitable. After the new paradigm-shift about dental plaque working as a **biofilm** brings us the question: Is it still worthy to evaluate just **one type of bacteria** as the main agent related to dental caries? Since dental plaque and SM as part of it are present in the mouth as part of natural microflora, the efforts from us, as professionals, must be focused in maintain the harmony, in an adequate pH and accessible fluorides ions that keep the de- and remineralization process in balance. New areas of research with potential significantly impact on clinical practice include: a) preventive colonization of selected organisms; b) affecting biofilm architecture by the use of enzymes that can degrade the exopolymers that comprise the plaque matrix; c) the neutralization of parameters that select for the species that are implicated in disease; thus, strategies that reduce the pH response to dietary carbohydrates will help prevent the enrichment of acidogenic and aciduric bacteria; d) the identification of pathogenic clones could also improve diagnosis and might predict sites that are more susceptible to disease.

In the evaluation of carbohydrate ingestion, we did not find a statistical difference. Regarding the results, it was almost no change in the patrons of food. It was hard to make parents understand the importance of the quality and quantity of what their children eat. Children in the group 1 were able to buy their own snack at school and mainly it was a combination of starch and sucrose, which is the worst combination. More efforts on this topic are recommended.

In the final data about caries risk, group 1 kept almost the same value than in the beginning while group 2 showed a statistical significant decrease. It is probably a consequence that younger children are better controlled by the parents, hygiene and they directly guide diet.

Finally, we wanted to evaluate the efficacy of fluoride gel and varnish in caries free permanent and deciduous molars. In the group 1, we found statistical significant decrease of sound teeth in placebo and gel variable while the same molars had a statistical significant increase in filling and DMFT. The morphology of permanent molars, with more and narrow fissures and the increase of salivation – due to physiological changes in the mouth - might influence in the better efficacy of varnish. Since the attachment of this material is better than gel, it could play a key factor for the positive results.

In group 2, we found a decrease of sound teeth in molars that received and fluoride varnish. The dmft also showed an increment statistically significant in the same molars. Younger children were very susceptible to the strong smell of varnishes; it was more difficult to follow manufacturer's recommendations. Probably the conditions of setting the varnish were not the perfect in all patients and it can be seen in the final data.

After two years of longitudinal study, we could demonstrate the efficacy of topical fluorides in comparison with placebo. Nevertheless, there are others elements to remind in the final evaluation of our study. The fact that each family can use or not fluoridated dental pastes

and fluoridated salt available in the market were excluded from our work. The cohorts previously defined were supposed to have almost the same conditions in order to have the best results. In one mouth, we had molars with gel, varnish and placebo. However, conditions always vary from one subject to another. Future studies might consider these inconveniences.

## VI. Conclusions

1. The use of topical fluorides in the way of gel and varnishes does prevent dental caries in permanent and deciduous molars in children from 3 to 6 years old.
2. The DMFT of children at 6 years old was 0.96, while the dmft of children at 3 years old was 1.73.
3. Although we found a high SM levels in children with highest records of dental caries, it was not statistical significant.
4. The mean of extrinsic sugars consumption per day by a 6 years old children was about 3.19, while in children of 3 years old it was 3.55.
5. We did not find any concluded relationship between extrinsic sugars and high presence of dental caries.
6. Caries risk in 6 years old years was 1.50, while in 3 years it was 1.80.

## VII. References


1. Krejsa O. et al. Caries Experience of 18-Year-Old Recruits in the Czech Republic in 1995. Caries Res, 1996; 30: 302.
2. Keyes P.H.: The infectious and transmissible nature of experimental dental caries: Findings and implications. Arch Oral Biol 1960 Mar; 1:304-20.
3. Loesche W.J.: Role of *Streptococcus mutans* in humans dental decay. Microbiol Rev 1986 Dec; 50(4): 353-80.
4. Gustafsson B.E.: Quensel C.E., Swenander Lanke L., Lundqvist C., Granhnén H., Bonow B.E., Krasse B.: The Vipeholm Dental Caries Study. Acta Odontol Scand 1954 Sep; 11(3-4): 207-31.
5. Marthaler T.M.: Epidemiological and clinical dental findings in relation to intake of carbohydrates. Caries Res 1967; 1(3): 222-38.
6. Orland F.J., Blaney J.: Experimental caries in germfree animals inoculated with enterococci. J Am Dent Assoc 1955 Mar; 50(3): 259-72.
7. Clarke J.K.: On the bacterial factor in the aetiology of dental caries. Br J Exp Pathol 1924; 5: 141-7.
8. Manji F., Fejerskov O., Nagelkerke N.J.D., Baelum V.: A random effects model for some epidemiological features of dental caries. Community Dent Oral Epidemiol 1991; Dec; 19(6):324-8.
9. Kidd E.A., Fejerskov O.: What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms. J Dent Res 2004; 83 Spec No C:C35-8.

10. Fejerskov O., Nyvad B.: Is dental caries an infectious disease? Diagnostic and treatment consequences for the practitioner; in Schou L (ed): Nordic Dentistry 2003 Yearbook. Copenhagen, Quintessence Publishing, pp: 141-151.

11. Marsh P.D., Bradshaw D.J.: Microbial community aspects of dental plaque; in Newman HN, Wilson M (eds): Dental Plaque Revisited: Oral Biofilms in Health and Disease. Cardiff, BioLine, 1999, pp: 273-253.

12. Marsh P.D.: Dental plaque as microbial biofilm. Caries Res 2004 May-Jun; 38(3): 204-11.

13. Fejerskov O., Manji F.: Risk assessment in dental caries; in Bader JD (ed): Risk Assessment in Dentistry. Chapel Hill University of North Carolina Dental Ecology, 1990, pp: 214-217.

14. Dean H.T., Arnold F.A.: Elvolve: Domestic water and dental caries: Additional studies of the relation of fluoride in domestic waters to dental caries in 4425 white children, age 12-14 years of 13 cities in 4 states. Public Health Rep 1942; 57: 1155-79.

15. Aoba T., Fejerskov O.: Dental Fluorosis: Chemistry and biology. Crit Rev Oral Biol Med 2002; 13(2): 155-70.

16. Bellamy P., Barlow A., Puri G., Wright K.I., Musset A., Zhou X.: A new in vivo interdental sampling method comparing a daily flossing regime versus a manual brush control. J Clin Dent 2004; 15(3): 59-65.

17. Davenport E.S., Day S., Hardie J.M., Smith J.M.: A comparison between commercial kits and conventional methods for enumeration of salivary mutans streptococci and lactobacilli. Community Dent Health 1992 Sep; 9(3): 261-71.

18. Pienihakkinen K., Jokela J.: A simple method for monitoring mutans streptococci in young children. Eur J Oral Sci 1995 Feb; 103(1): 61-2.

19. Newbrun E., Matsukubo T., Hoover C.I., Graves R.C., Brown A.T., Disney J.A., Bohannan H.M.: Comparison of two screening tests for Streptococcus mutans and evaluation of their suitability for mass screenings and private practice. Community Dent Oral Epidemiol 1984 Oct; 12(5): 325-31.

20. Splieth C., Bernhardt O.: Prediction of caries development for molar fissures with semiquantitative mutans streptococci test. Eur J Oral Sci 1999 Jun; 107(3): 164-9.

## VIII. Curriculum vitae

### 8.1. Publications

**a) Original articles:**

1. **Seminario A.L., Broukal Z., Ivancaková R.:** Mutans Streptococci and the Development of Dental Plaque. Prague Medical Report 2005; 106(4): 349-58.

2. **Ivančaková R., Seminario A.L.:** Prevence zubního kazu v kojeneckém a batolecím věku. Pediatr. Pro Praxi 2004; 5(6): 17-9.

3. **SeminarioA.L., Hagel P.:** Evaluation of oral health and prevention of early childhood caries in children under 3y..
   Development of original questionnaire for children of immigrant families (in Spanish). Collaborative project with Winona State University, Olmsted County Public Health Services and Mayo Clinic Department of Paediatric and Department of Nursing, Rochester, USA, 2004.

**b) Review of literature:**

1. **Seminario A.L., Ivancakova R.:** Early Childhood Caries. ACTA MEDICA (Hradec Králové) 2003; 46(3): 91-4.

2. **Seminario A.L., Ivancakova R.:** Natal and neonatal teeth. ACTA MEDICA (Hradec Králové) 2004; 47(4): 229-33.

3. **Ivančaková R., Seminario A.L.:** Problematika natálních a neonatálních zubů. PEDIATRIE PRO PRAXI. 2004; 1: 20-1.

**c) Abstract:**

1. **Seminario A., Valdivieso M., Ivancakova R.:** Evaluation of vital functions in children (3-6 yrs) under midazolam and midazolam-nitrous oxide. (Abstract). European Journal of Paediatric Dentistry 2004; Vol. 5 Suppl., 3: 22.

2. **Ivancakova R., Seminario A., Jirousek Z.**: Submerging primary molar – An extraction in time! (Abstract). European Journal of Paediatric Dentistry 2004; Vol 5 Suppl., 3:53.

3. **Ivančaková R., Broukal Z., Seminario A.L., Lencova E.:** Longitudinal study of oral health in pre-school children – onset examination (Abstract). J Dent Res 2005; 84 Suppl., 1:13.

4. **Seminario A.L., Broukal Z., Ivančaková R.:** Oral Health Status in Pre- and Primary School Children (Abstract). J Dent Res 2005, 84 Suppl., 1:17.

### 8.2. Presentations

1. **Seminario A., Valdivieso M.:** "Sedation in paediatric patients", Paediatric Dentistry Congress, Peruvian University Cayetano Heredia, Lima, Perú, 2000.
2. **Hubková V., Ivančaková R., Seminario A.L., Bednář P.:** "Microbiology tests as dental caries prevention and the use of cariogram", Clinical seminar at the Dept. of Dentistry, Hradec Kralove, Czech Republic, 2004.
3. **Seminario A.L., Broukal Z., Ivancakova R.:** "The importance of oral bacteria assessment in the prevention of tooth decay", Pedostomatology days of Jindřichův Hradec. Jindřichův Hradec, Czech Republic, 2004.
4. **Ivančaková R., Seminario A.L.:** "Prevence zubního kazu v kojeneckém a batolecím věku", XXII. dny klinické a praktické pediatrie, Olomouc, Czech Republic, 2004
5. **Seminario A.L., Valdivieso M., Ivancakova R.:** "Evaluation of vital functions in children from 3 to 6 years old under conscious sedation with midazolam and midazolam plus oxide nitrous", 7th European Academy of Paediatric Dentistry, Barcelona, Spain, 2004.
6. **Ivančaková R., Tojnarová L., Seminario A.L.:** "Předčasné ztráty zubů u dětí předškolního věku ošetřovaných v celkové anestézii", Seminář ČSDS, Prague, Czech Republic, 2005.
7. **Ivančaková R., Hofman Z., Seminario A.L.:** "Následky předčasné ztráty druhého dočasného moláru v důsledku úplné reinkluze" Seminář ČSDS, Prague, Czech Republic, 2005.

8. **Seminario A.L., Broukal Z., Ivancakova R.:** "Streptococci mutans in preschool and primary school children after topical fluoride applications", 8th European Academy of Paediatric Dentistry, Amsterdam, Holland, 2006

### 8.3. Posters

1. **Ivančaková R., Seminario A.L, Jirousek Z.:** Submerging deciduous molar- An extraction in time! 7th Congress of the European Academy of Paediatric Dentistry, Barcelona, Spain, 2004.

2. **Ivančaková R., Broukal Z., Seminario A.L., Lencova E.:** Longitudinal study of oral health in pre-school children – onset examination. WCPD Congress, Liverpool, England, 2005.

3. **Seminario A.L., Broukal Z., Ivančaková R.:** Oral Health Status in Pre- and Primary School Children. WCPD Congress, Liverpool, England, 2005.

4. **Ivančaková R., Broukal Z., Seminario A.L.:** Caries experience and parental attitudes to oral health in pre-school children. 8th Congress of the European Academy of Paediatric Dentistry, Amsterdam, Holland, 2006.

## 8.4. Other activities

a) **Course certifications**

1. Specialisation in Paediatric Dentistry, Peruvian University Cayetano Heredia, Lima, Perú. 1998-2000.
2. Master in Stomatology, Peruvian University Cayetano Heredia, Lima, Perú, 2000-2002.
3. Master in Public Health, Institute of Public Health, Prague, Czech Republic, 2004-actually.
4. 2002: Alliance Francaise: Certification of French Language.
5. 2003: Charles University in Prague: Certification of Czech Language.

b) **Fellowships**

1. Dept. of Dentistry, Charles University in Prague, Faculty of Medicine in Hradec Králové, Czech Republic, August-December, 2002.
2. Dept. of Preventive Medicine and Public Health Department, Mayo Clinic, Rochester, USA, October-November, 2004.
3. Oral Health Programme, World Health Organization, Geneva, Switzerland, October-November, 2005.

**SUMMARY**

Despite great improvements in the oral health of populations across the world, dental caries still persists particularly among poor and disadvantaged groups in both developed and developing countries, being in children the most prevalent daily problem that dentist face. According to the World Health Organisation (WHO)-Oral Health Report 2003-, dental caries remains a major public health problem in most industrialized countries, affecting 60–90% of schoolchildren and the vast majority of adults. The use of fluorides in the prevention of dental caries has demonstrated a dramatically drop of dental decay levels around the world.

OBJECTIVE: The aim of our work was to evaluate the use of two topical fluorides in the prevention of dental caries.

METHODS: Approximately 60 children in good general health, divided in two groups, 1st phase mixed dentition (group 1, mean age: 7.21) and deciduous dentition (group 2, mean age: 3.84) were selected for this study. Deciduous and permanent molars were divided in pairs and randomly received fluoride gel (1.23%), fluoride varnish (0.1) or placebo each six months during two years. The DMFT and dmft were registered and applications of topical fluoride each six months were supplied during two years. Evaluation of dental plaque, streptococci mutans levels, carbohydrates consumption and instruction of dental hygiene were also included since the first step and in each control. Onset data and those after two years were compared by paired Student's t-test at $P=0.05$ and correlation coefficients were calculated.

RESULTS: onset data: group 1: DMFT 0.96, group 2: dmft 1.73. Data after two years; group 1: DMFT 1.62 ($P<0.05$), group 2: dmft 3.40 ($P<0.05$). We also found statistical significant reduction of sound teeth in molars and an increased of DMFT and dmft scores in the group of molars that received placebo ($P<0.05$).

CONCLUSIONS: After two years of topical fluoride applications, parental instructions and following oral health status, we found that topical fluorides –in the way of gel and varnishes- reduced dental caries levels in deciduous and permanent molars. The use of topical fluorides as regular preventive therapy against dental caries in children is recommended.

**Souhrn**

Navzdory zlepšení stavu orální zdraví populace v posledním desetiletí, zůstává problematika zubního kazu stále problémem u chudých a sociálně slabých obyvatel jak ve vyspělých, tak v rozvojových zemích. Podle údajů Světové zdravotnické organizace (Zpráva o stavu orálního zdraví, 2003) trpí zubním kazem 60-90% dětí školního věku a většina dospělých. K významnému poklesu výskytu kazu došlo používáním fluoridů v prevenci zubního kazu.

Cíl studie: Cílem studie bylo hodnocení účinnosti dvou forem lokální fluoridové prevence.

Metody studie: Do studie bylo zařazeno celkem 60 dětí, celkově zdravých, rozdělených do dvou skupin. První skupinu tvořily děti se smíšených chrupem v první fázi fyziologické výměny (průměrný věk: 7,21). Druhou skupinu tvořily děti s dočasným chrupem (průměrný věk: 3,84). Dočasné druhé moláry a první stálé moláry byly rozděleny po dvojicích a na okluzní plošky byly náhodně aplikovány fluoridový gel (1,23% F), fluoridový lak nebo placebo. Ošetření se opakovalo v šesti měsíčních intervalech během dvou let. Na začátku a na konci studie byly zjišťována a hodnoceny následující parametry: přítomnost zubního kamene, počet kariogenních streptokoků ve slině, výživové zvyklosti (především konzumace cukrů) a úroveň hygieny dutiny ústní. Výsledky byly statisticky zpracovány pomocí dvojitého Studentova testu ($P=0,05$) a spočítány koeficienty korelace.

Výsledky studie: Na počátku studie byly hodnoty DMFT u první skupiny 0,96, na konci studie (po 2 letech) to bylo 1,62 ($P<0,05$). U druhé skupiny byly hodnoty dmft na počátku 1,73 a na konci 3,40 ($P<0,05$). Statisticky významně poklesl počet zdravých molárů a došlo ke zvýšení hodnoty DMFT a dmft u molárů, kde nebyl aplikován žádný fluoridový prostředek (placebo), ($P<0,05$).

Závěr studie: Po dvouleté lokální aplikaci fluoridů (gel a lak)a opakované instruktáži hygieny dutiny ústní se domníváme, že lokální fluoridová prevence vedla k poklesu výskytu zubního kazu na dočasných a stálých molárech. Pravidelnou lokální aplikaci fluoridů proto doporučujeme v prevenci zubního kazu u dětí.