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THESIS

COMPARISSON OF TWO TOPICAL FLUORIDE APPLICATIONS IN DECIDUOUS AND PERMANENT MOLARS FOR DENTAL CARIES PREVENTION: TWO YEARS INCREMENTAL STUDY

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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 dentists 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. Although it appears that dental caries is less common and less severe in developing countries of Africa, it is anticipated that the incidence of caries will increase in several countries of that continent, due to changing living conditions and dietary habits, and inadequate exposure to fluorides. Research on the oral health effects of fluoride started around 100 years ago; the focus has been on the link between water and fluorides and dental caries and fluorosis, topical fluoride applications, fluoride toothpastes, and salt and milk fluoridation. Most recently, efforts have been made to summarize the extensive database through systematic reviews. Such reviews concluded that water fluoridation and use of fluoride toothpastes and mouthrinses significantly reduce the prevalence of dental caries. Water fluoridation, technically feasible and culturally acceptable, has substantial advantages in public health; alternatively, fluoridated salt and milk may be considered for prevention of dental caries.

The use of topical fluorides from health professionals has been extended and also demonstrated as efficient therapy against dental decay. We were interested in evaluate the efficacy of two topical fluorides, gels and varnishes, in the prevention of dental caries. We also studied oral bacteria, specifically streptococci mutans, and theirs close relationship to the development of dental decay.

Finally, we included in our study the frequency of carbohydrate ingestion – extrinsic sugars- and its relation to dental caries. Classification of caries risk in each child was also developed.

II. Theoretical background

2.1 Dental Caries

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 fecal pellets of groups of caries-active rodents (1). Further investigations in hamsters resulted in the isolation of certain streptococci from caries lesions, unlike other types of streptococci, caused rampant decay in previously caries-inactive animals (2). 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 Gustafson 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 multi factorial 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.

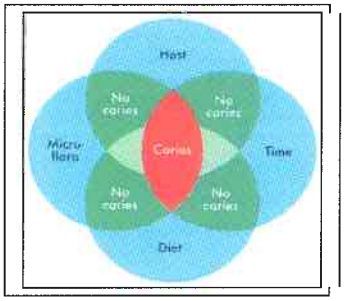

Fig1. Illustration of the interaction among etiological risk factors (microflora), external modifying risk factors (diet), internal modifying risk factors (host), and time in the time development of dental caries (Modified from Keyes, 1960).

2.2. Microflora – Dental Plaque

Since the first description of dental plaque – microbe-containing deposits on the teeth - made by Anthony van Leeuwenhoek in the 17th century, several definitions appeared, mainly due to the development of microscopes. According to Marsh, dental plaque can be defined as the diverse community of micro-organisms found in an extracellular matrix of polymers of host and microbial origin (6). The importance of the study of bacteria as community is because biofilms express properties not exhibit by the same organism growing in liquid (planktonic) culture, while bacteria are invariable found in nature as part of a consortium, the properties of which are more than the sum of the component species (6). In dental plaque, oral bacteria do not exist as independent entities; they function as co-ordinated, spatially organized and fully metabolically integrated microbial community.

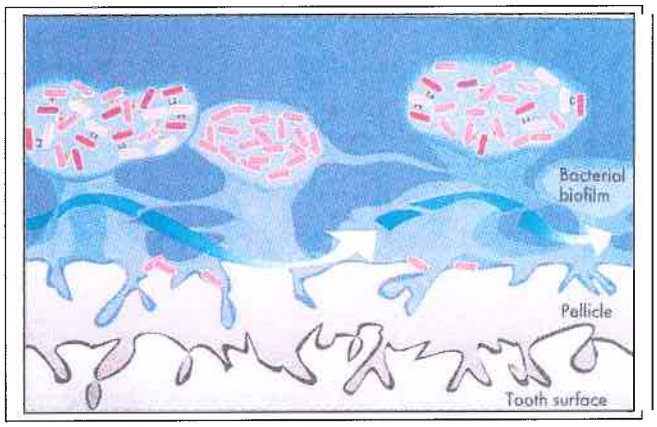

Fig 2. Schematic illustration of a plaque biofilm. The pellicle and the biofilm extracellular matrix are depicted as firmly embedded in each other, contributing to the well-known recalcitrance of dental plaque. The unique shapes of biofilms are believed to facilitate growth and symbiosis among the microbiota. The large *arrows* depict solvent flow that occurs through both large and small aqueous channels that are believed to carry nutrients and metabolic products to different members of the community. (From Darveau et al, 1997).

It is estimated that in 1 mm^3 of dental plaque - about 1mg of weight- contains more than 200 million of bacteria. These bacteria include streptococci, lactobacilli, as well as mycoplasma, "yeasts", and protozoa (in mature plaque); sticky polysaccharides and other products form the so-called *plaque matrix* and constitute 10% to 40% by volume of the supragingival plaque (7).

The development of the oral plaque has been divided in phases of formation:

Phase I, the development of dental plaque begins with a clean tooth surface covered by a conditioning film of salivary proteins and glycoproteins, called the *tooth pellicle*. Oral cavity contains more than 350 species of bacteria but only few can be able to colonize a cleaned tooth surface. This first step depends on the interaction of the surface molecules on the bacteria and the tooth pellicle. These molecules are easily vulnerable to alteration by chemical agents. Plaque adhesion is specially favored by high free energy between microorganisms and tooth (7).

Gibbons and Van Houte (8) studied the process of colonization of oral bacteria on human teeth. They called *pioneer colonizers* to the first bacteria over the tooth surface. Pioneer colonizers are characterized for succeed when competing with other microorganisms in the oral flora for a place over the tooth surface. These bacteria are principally streptococci strains S. oralis, S. mitior and S. sanguinis.

After initial deposition, pioneer-colonizing bacteria, especially *Streptococcus sanguinis*, begin to expand away from the tooth surface forming columns that move outwards on long chains of palisading bacteria. These parallel columns of bacteria are separated by uniform narrow spaces. Plaque growth continues with deposition of new species into these open spaces (8).

Di Renzo (9) in 1985 and Kolenbrander (10) in 1988 investigated the development of oral plaque. They concluded that after the deposition of pioneer bacteria new species start to attach to them in order to colonize the tooth surface. Plaque expansion is accomplished in a lateral direction, which causes the interbacterial spaces to merge. When these spaces are close enough, bacteria within start to secrete substances, signalling the surrounding bacteria to undergo a growth spurt. Within a short time, intermeshed bacteria cover the tooth surface adjacent to the gingiva. New bacteria derived from saliva or surrounding mucous membranes attach by bonding to bacteria already attached to the plaque. These associations, *intergeneric coaggregations,* are mediated by specific attachment proteins and occur between two partner cells. At this time, plaque is composed mainly of cocci and few rods.

Fig 3. Undisturbed pellicle in cross section (left). It is formed in different layers. Many non-attaching bacteria (right) are close to the outer surface of the pellicle. (From Saxton, 1975).

Fig 4. Formation of bacteria colonies. The largest bacterial colonies (arrows) are located close to the gingival crevicular fluid (magnification x4000). (From Saxton, 1975).

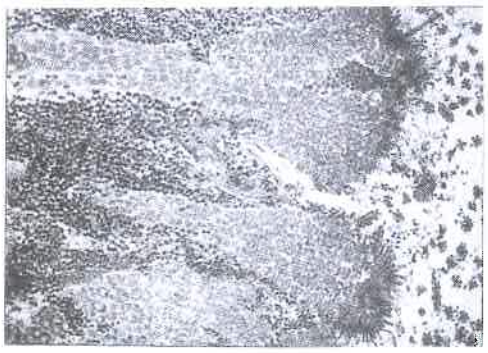

Fig 5. Cross section of columns of colonizing bacteria, separated by open spaces. (From Listgarten et al, 1975).

Phase II, there is an increasing of the levels of gram-positive rods, such as Actinomyces viscosus, and gram-negative cocci, including Neisseria and Veillonella species, occupy the remaining interstices. Tall rods cover the outer surface of the gingival plaque. At this time, there is an intensive increment of the plaque after 3 and 4 days compared to the first 2 days. This plaque is now mature and the so-called homoeostasis is established among the different microorganisms.

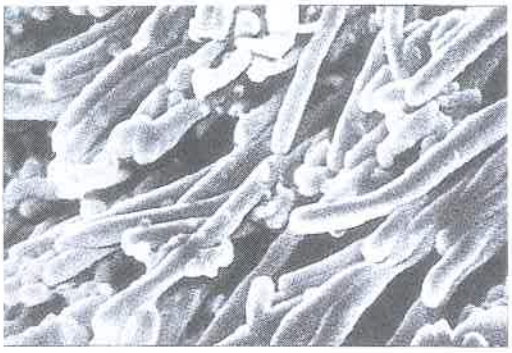

Fig 6. Outer surface of plaque in phase II of plaque development, covered by gram-positive tall rods and cocos (magnification x8000). (From Saxton,

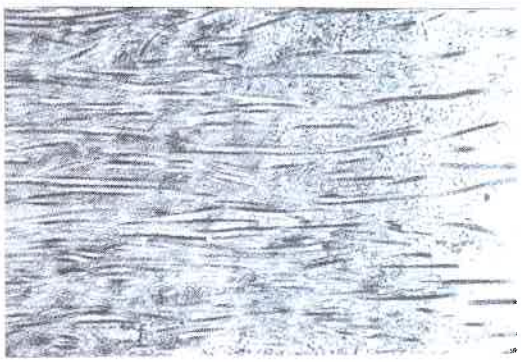

Fig 7. Filamentous bacteria on the surface of predominantly coccoid plaque during phase II of a plaque development, (magnification x1500). (From Listgarten, 1975).

Phase III, 5 to 7 days after initiation, plaque begins to migrate subgingivally and bacteria and their products permeate and circulate in the pocket.

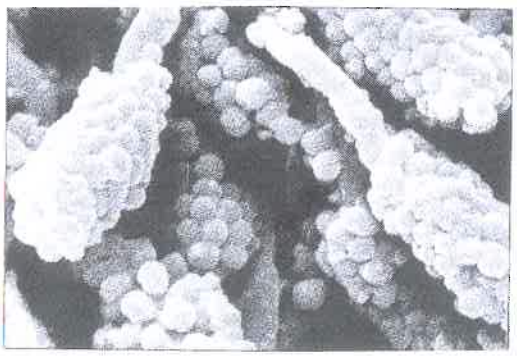

Fig 8. Corncob formation of coccoid and filamentous bacteria in phase III of plaque formation (magnification x8000). (From Saxton, 1973).

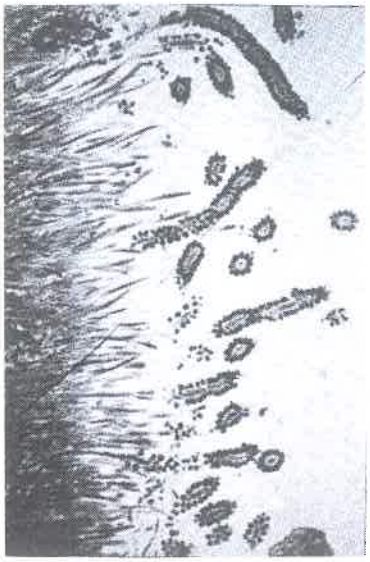

Fig 9. Cross section of the outer surface of gingival plaque, containing several corncob formations (magnification x1000). (From Listgarten, 1976).

Fig 10. Dense mat of filamentous bacteria oriented roughly perpendicular to the colonized surface, in phase III of plaque formation. (From Listgarten et al, 1975).

Phase IV, 7 to 11 days after initiation, the adversity of the flora increases to comprise motile bacteria, including spirochetes and vibrios as well as fusiforms. Attached gingiva plaque fills the gingiva sulcus, while spirochetes and vibrios move around in the outer and more apical regions of the sulcus (7).

Fig 11. Cross section of gingival plaque filling the gingival sulcus while spirochetes and vibrios move around in the outer and more apical regions of the sulcus during phase IV of plaque development (magnification x12000). (From Listgarten, 1976).

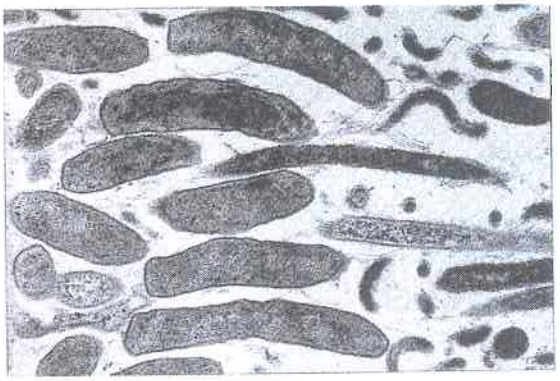

Fig 12. Accumulation of minerals in the deeper part of the plaque, resulting in calculus formation (magnification x4000). (From Listgarten, 1976).

2.2.1.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. In 1955, Hemmens et al. designed a carefully study clearly showing that these bacteria colonized lesions but they were not prevalent in plaque during the period of lesion formation. It was demonstrated that lactobacilli could not be regarded as the major etiological agent of tooth decay (11).

Further studies in germ free 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 (12). Similar studies also demonstrated that a range of

bacteria, which are not often isolated in large numbers of dental plaque as Streptococci salivarii 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 (13). Other bacteria like Actinomyces viscosus was particularly associated with root caries and some strains of streptococci oralis and streptococci milleri exhibited levels of caries induction in rats that approached those of SM, at that moment, there was no explanation for these results (14, 15).

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.

2.2.2. Caries-associated characteristics of streptococci mutans

In order to be cariogenic, bacteria should fill three conditions:

1. To be able to colonize a tooth surface.
2. To produce acids faster than the local neutralization of the plaque.
3. To be able to carry out the two items described above in a pH lower than the critical pH for enamel dissolution (16).

Streptococci mutans (SM) is acidogenic as well as aciduric and can adhere to tooth surfaces (17). These bacteria can produce intracellular and extracellular polysaccharides from sucrose. In periods of low nutrient supply, SM can degrade intracellular polysaccharides, indicating that these polysaccharides increase the virulence of some SM species (*S. mutans and S. sobrinus*) (18).

Studies in vitro have demonstrated that *S. sobrinus* is more acidogenic and aciduric than SM (19, 20). Polymerase chain reaction (PCR) detection methods indicate that *S. sobrinus* may be more prevalent than indicated by cultural studies, however, it is rarely present at the same level in plaque as SM (21). The reason for the inability of *S. sobrinus* to proliferate appears to be due to its inability to catabolize transported N-acetylglucosamine, an energy-requiring process, which depletes intracellular levels of phosphoenolpyruvate to the detriment of the organism. Only when external sources of fermentable carbohydrates are high, or the environment is very acidic as in bulimic subjects, this inhibitory effect becomes insignificant and *S. sobrinus* proliferates (22).

The species most commonly isolated from dental plaque in humans are SM (serotypes c, e and f) and S. sobrinus (serotypes d, g). They have been isolated from populations all over the world and have been related to human caries. The bacteria prevalence differs among populations and test values also differ, depending on the method of detection (23). About 10% to 30% of a population may have little or no SM, to 100, 000 CFUs/mL of saliva. The percentage of individuals with very high levels of SM (mayor 1 million CFUs/mL of saliva) in a population may vary considerably, depending on age, caries prevalence, dietary habits and so on (24).

Although several studies have shown the relationship among SM presence, plaque accumulation and dental caries lesions (25) they have failed in the prediction of caries based on SM levels in dental plaque or saliva (26). The only presence of SM in dental plaque is not enough for dental caries development. The fact that dental caries is a multi factorial disease make us consider other alternatives as the mechanism of SM colonization, the presence of different strains of SM, the host defence against SM infection, etc., in order to understand better and to prevent this disease.

One of these factors is the virulence of SM. SM cells possess a glucan-binding lecitin and at least three types of glucosyltransferases (GTF), one of them GTF-S catalyses the formation of a relatively water-soluble glucan composed entirely of ***ALFA*** (1,6) linkage and the others GTF-I and GTF-SI enzymes synthesize mainly water-insoluble glucans rich in ***ALFA*** (1,3) linkage but also with ***ALFA*** (1,6) linkage. MS synthesizes a single FTF, which catalyses the production of fructan, composed predominantly of ***BETA*** (2,1) linkages. Plaque fructans is rapidly accumulated in vivo after the ingestion of sucrose and then hydrolysed to fructose by fructan hydrolyses produced mainly by MS (27). These glucans facilitate the SM establishment in dental plaque by adhering bacteria to the enamel, and in rat studies SM with these genes inactivated and unable to produce these polymers are less able to colonize to dentition of rats. Consequently, they are less able to initiate carious lesions (28).

The virulence of glucans is more in relation with the change of the plaque ecology than the accumulation of specific bacteria. For that, the synthesis of glucans (mainly mediated by sucrose) increases the porosity of plaque, allowing the deeper penetration of dietary sugar into the biofilm and increases the acid production close to the tooth surface (29, 30). The extracellular matrix material synthesized from sucrose by SM in plaque alters the diffusion properties of plaque, and thus prolongs the depression of plaque pH (31). In recent studies related to synthesis of water insoluble glucans by SM and caries incidence in young children, it has been suggested that the capacity of synthesis of these glucans may be more important than their levels in plaque (32, 33).

Sucrose is not the only source of these polysaccharides, isomers as palatinose, trehalulose, turanose, maltulose and leucrose have been studied and, in human dental plaque, there are significant numbers of bacteria that are able to ferment these sucrose

isomers (34). The most important question is if there are another bacteria able to produce acids and survive in such a low pH environment. Current investigations concluded that the increase of number of bacteria and its acidogenic and aciduric ability is also related with the quantity of fermentable carbohydrate consumption, which increase the proportion of polysaccharide-storing bacteria (31, 35). The investigators suggest a succession in the microflora resulting in dental caries caused by increased fermentable carbohydrate fermentation, in which many different bacterial taxa respond to the changing environment and the increased numbers exhibit a greater ability to produce and withstand exposure to acid. They found the emergence of SM in plaque was often preceded by an increase in the number of other types of acidogenic bacteria, which included not only the non-SM but also other members of the plaque flora that were not necessarily streptococci (32).

As result of these studies came up the fact that dental decay may happen in the absence of SM and individuals with high levels of SM do not necessarily have to harbor dental caries. Rather, the outgrowth of SM should be explained by a disturbance of the homeostasis in the dental biofilm (36). If the homeostasis of the oral microflora is lost, then an opportunistic infection can occur and an ecological plaque hypothesis seems more attractive (37).

2.2.3. 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 evaluation of dental plaque development, 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 (38). 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 (39). 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" (40).

Oral bacteria do not exist as independent entities but rather function as a coordinated, spatially organized and metabolically integrated microbial community (41). The microbial community lifestyle grants benefits to each individual member, these are well explained by Marsh (6) 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). This community protection can change individual characteristics, rendering a sensitive organism as apparently "resistant" to an antibiotic if neighboring, non-pathogenic cells produce a neutralizing or drug-degrading enzyme ("indirect pathogenicity"). Hol et al. demonstrated this in animal models where penicillin-sensitive pathogen (S. pyogenes) is protected by a ***BETA***-lactamase-producing commensal strain (Moraxella catarrhalis) and, as a result, is still capable of causing a lethal infection (42). In the mouth, gingival crevicular fluid can contain sufficient ***BETA***-lactamase to inactivate the concentrations of antibiotic delivered to the site (43).

Most of studies in microbiology where focus in specific bacteria in vitro where the phenotype of the microorganism develops significantly different than as a biofilm and in vivo. Confocal laser scanning microscopy of biofilms in general has revealed that biomass comprises "open" water channels through which nutrients and metabolic

waste products sieve (44). Dental lesions appear where biofilms are undisturbed mature and remains over the tooth surface for a long period of time. The most frequently areas are occlusal surfaces (especially at risk at long-lasting eruption), inter proximal surfaces (below contact points), along marginal gingiva, and –once exposed- enamel-cementum junction. Once the physiological equilibrium between tooth and biofilm is disturbed, a consequence net loss of mineral is observed (36). When a frank cavity is allowed to develop, a new ecological niche is observed where the composition of the biofilm gradually adapts to a declining pH environment. Metabolism and diffusion characteristic are significantly different from those of biofilms covering sound or inactive caries surfaces (45).

But dental caries involves also other relevant factors as graphically represented by Fejerskov and Manji (46), it includes individual as well as population level, many of these variables (oral hygiene, diet, etc.) will be highly influenced by the behavioral and socio-economic conditions prevailing.

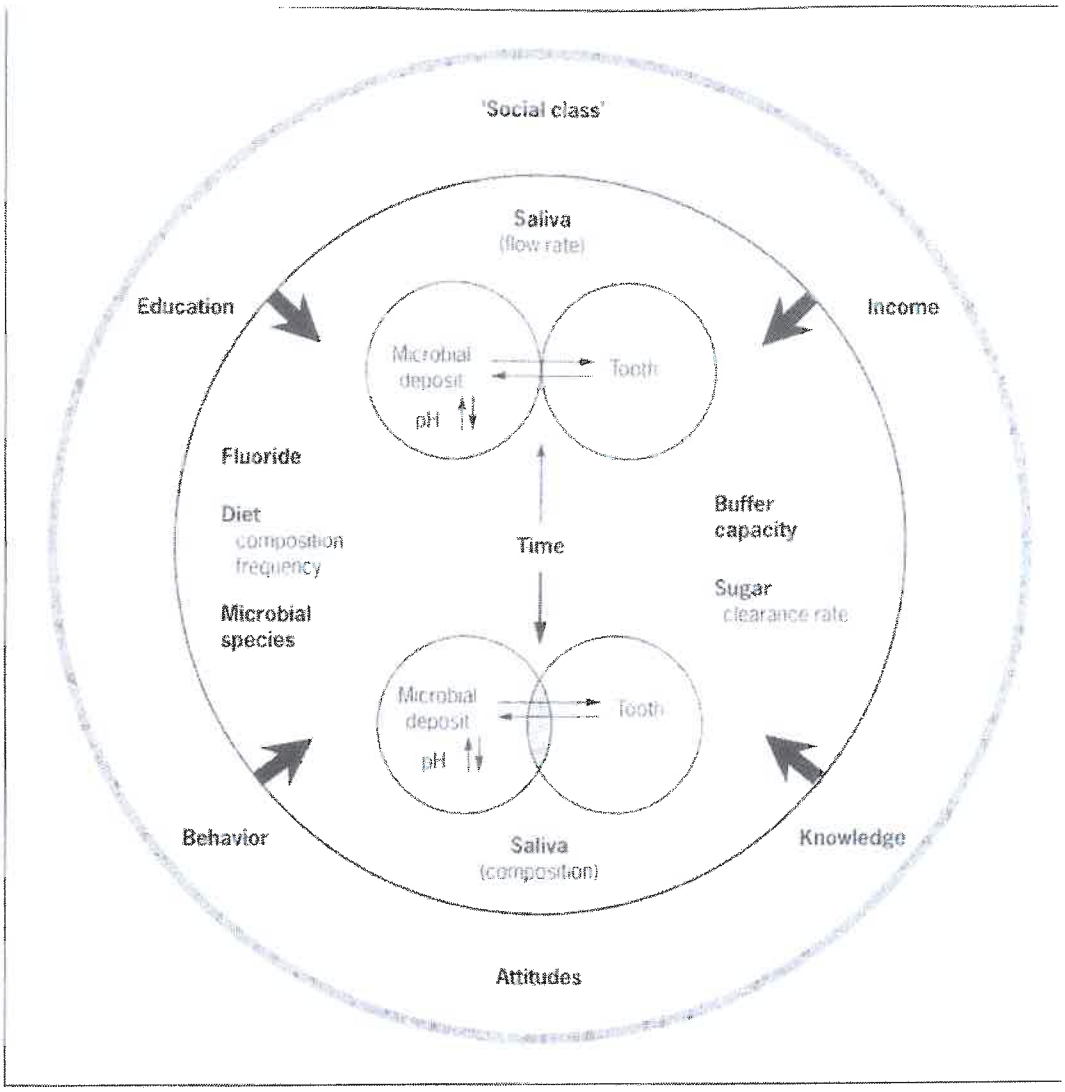

Fig 13. Schematic illustration of the relationship of dental caries aetiological factors: microbial deposit, tooth and biological determinants (inner circle). In the outer circle are listed various behavioural and socio-economic factors (or confounders) which influence the likelihood for lesion development at an individual and population level. Modified from Fejerskov and Manji, 1990.

2.3.Host factors: salivary proteins

Saliva is implicated in the maintenance of oral health and protection of teeth in several ways: a) Tooth surface is continuously protected against wear by a film of salivary mucins and proline-rich glycoproteins, b) the early pellicle proteins, proline-

rich proteins and statherin, promote remineralization of the enamel by attracting calcium ions, c) demineralization is retarded by pellicle proteins, in concert with calcium and phosphate ions in saliva and in the plaque fluid, d) several salivary (glyco)proteins prevent the adherence of oral microorganisms to the enamel pellicle and inhibit their growth, e) the salivary bicarbonate/carbonate buffer system is responsible for rapid neutralization of acids (47).

Saliva holds a large number of proteins related to dental protection as lysozyme, lactoferrin, lactoperoxidase, immunoglobulins, agglutinin, mucins and peptides -involve in bacteria killing activity- as histatins, defensins and the only human cathelicidin LL-37 (48). The vast types of salivary proteins are focused in the protection of the oral biofilm, avoiding any uncontrolled colonization. For that, each type of salivary gland secrets a characteristic spectrum of proteins, saliva contains the sum of contributions from different glands and as a result, the concentration of a single antimicrobial protein may change over the day according to the activity glandular source (47).

The saliva components are associated in systems protecting the teeth; as the carbonate/bicarbonate buffer system –fast speed neutralizing of acids- and specific proteins that form a protective coating on the enamel surface, serving as barrier to prevent free diffusion of acid. At this point, it is important to stress is that with the exception of immunoglobulins, antimicrobial components in saliva are not focused on elimination of specific (cariogenic) species, such as SM. They are involved in the preventive massive overgrowth of microorganisms, and govern the establishment and maintenance of a stable ecosystem in which harmless species outnumber potentially dangerous species, thus forming a protection in its own right (47).

Immunoglobulins have the property to inhibit bacterial adherence and colonization, blocking surface structures involved in binding. IgA (>85%) and less IgG

are about the 5-15% of total salivary proteins. The first one is synthesized by B-lymphocytes located in the vicinity of secretory epithelia while the second one manly derives from crevicular fluid leaked into the oral cavity.

Mucins constitute about 20-30% of the total proteins and constitute another important class of salivary glycoproteins. Characteristic of mucins is the abundance of carbohydrate side chain, which is covalently attached to polypeptide backbones, forcing the molecule into an extended conformation. Its combination with the presence of a hydrophilic sugar coat, are responsible for the characteristic viscoelastic character of saliva (47). Because of its hydrophilic properties, mucins containing pellicle lubricate dental surfaces, protecting them against mechanical wear. Mucins also have been implicated in the protection against viruses (49).

As part of minor salivary proteins with protective properties we can name lactoperoxidase and lysozyme. As antimicrobial enzymes, they hydrolyze cell wall polysaccharides, making bacteria more vulnerable to lysis due to e.g. hypo-osmotic conditions in saliva, or other antimicrobial components (47).

Finally, but no less important are histatins, these antimicrobial peptides are synthesized in the parotid and submandibular glands, meaning that under both stimulated and non stimulated saliva flow conditions, histatins will be secreted into saliva. The fungicidal, and to a lesser extent the bactericidal activity, of histatins is sensitive to ionic strength, diminishing with increasing salt concentrations (50).

The importance of the structure-function antimicrobial protein and peptide knowledge open a significant gate for designing small, biologically active peptides that can be used as natural antimicrobials, avoiding the construction of the whole polypeptide chain. This instrument may be used against multi resistant microorganisms,

or added in mouthrinses, restoring functionality in patients in whom the natural protection is compromised.

2.4. Diet

Stephan published the first clinical report evaluating the fast metabolism of sugars by oral bacteria in the forties. He demonstrated the production of organic acids in sufficient concentration to lower the pH of dental plaque (51). The direct linkage of the frequent exposure to sugar with dental caries was also established in the fifties by the Vipeholm Study and since then several studies have confirmed this connection (52).

Sugars are a form of fermentable carbohydrates that begins digestion in the oral cavity via salivary amylase. Sugars enter the diet in 2 forms: those found naturally in food – as fruits, honey- and those that are added to foods during processing to alter the flavor, taste, or texture of the food (53). The term sugars include all monosaccharides and disaccharides, being the most popular glucose, fructose, sucrose, maltose and lactose (54). Other disaccharides, particularly trehalose, trehalase and isomaltose, have a lower cariogenic risk than does sucrose.

Although it seems not to be any difference in the acidogenic potential or the ability to induce in situ demineralization among the common sugars as sucrose, maltose, glucose and fructose, the especial focus on sucrose is because of its involvement as the sole substrate in the synthesis of extracellular glucans.

The presence of any individual characteristics –as a low or high salivary pH, genetic predisposition, prior caries history, use of medications, incidence of systemic or local diseases that affect the immune system, and personal hygiene habits- also play a role in the associated caries risks of particular foods.

The form of the fermentable carbohydrate directly influences the duration of exposure and retention of the food on the teeth. Prolonged oral retention of cariogenic components of food may lead to extend periods of acid production and demineralization and to shortened periods of remineralization. Duration may also be influenced by the frequency and amount of fermentable carbohydrate consumed sugars (55), such as those found in beverages and milk drinks, pass through the oral cavity fairly quickly with limited contact time or adherence to tooth surfaces. Long-lasting sources of sugars, such as hard candies, breath mints, and lollipops, have extended exposure time in the oral cavity because the sugars are gradually released during composition (56).

The frequency of consumption seems to be a significant contributor to the cariogenicity of the diet. The importance of frequency is regarded as the outcome of the alternation of demineralization and remineralization. Higher frequency means more demineralization and less remineralization (57). Sugar-free gums can stimulate saliva, increasing the clearance of sugars and other fermentable carbohydrates from the teeth and the oral cavity and increasing buffer capacity, this includes sorbitol, xylitol, mannitol, erythritol, and isomalt. However, xylitol – a 5-carbon sugar that oral microflora can not metabolize-has additional anticariogenic effects attributable to antimicrobial action, stimulation of saliva resulting in increased buffer activity and an increase in pH, and enhanced remineralization (58). Sorbitol-sweetened gums stimulate saliva without causing a drop to the critical pH and have been shown to be equal to xylitol gum in terms of caries control (59).

2.5. Fluorides

2.5.1. Introduction

The fluoride ion is one of the most electronegative elements and the major reason for the dramatical reduction of dental caries in the last 50 years. It was discovered when Dean et al. compared the incidence of caries in individuals exposed to so-called high-fluoride water supplies with that in individuals exposed to lower levels (60). In the middle of the last century, research on fluoride action in dental caries concluded as the paradigm that, to exert its maximum cariostatic effect, fluoride became 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's caries-protective benefits, and attempts to "play down" the possible toxic effect of fluoride on developing dental enamel, often led the dental professionals to present dental fluorosis as merely a cosmetic problem (61). 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 on the way of how this element affects mineralizing and mineralized dental hard tissues.

2.5.2. Ways of administration

2.5.2.1. Systemic

Because of its extremely high electronegative charge and being small ionic diameter, fluoride has a great capacity to bind hydrogen forming a strong ionic. This property gives fluoride a big potential to interact both mineral phases and organic macromolecules, and also its small size acts as "structure former" in water. This can decrease the mobility of water molecules in solution and in hydration layers of proteins

and apatite surfaces with concomitant effects on ligand binding and exchange (62). During the development of teeth, the presence of fluoride in the maturation state produces more stable crystals. The electrostatic attraction between Ca^{2+} and the F- will be greater than between Ca^{2+} and OH^-, making fluoridated apatite lattice more crystalline and more stable. As a consequence it is less soluble in acid (63).

Once ingested, fluorides are fast and almost completely absorbed by the stomach and intestines. This absorption is passive and natural, does not depend on especial transport mechanisms. Almost 75% of blood fluorides are in plasma, the rest, in the blood cells. The maxim concentration of this element appears between 30 to 60 minutes after the ingestion, the half time of fluorides in blood is about 2 to 6 hours and elimination is after 24 hours. In children, the quantity of fluoride in blood is less than adults. Since children and adolescents are in growing phase, the ability of fluoride retention in hard tissues (bones and teeth) is higher than adults.

The ways of systemic fluoride intake are water, salt and milk fluoridation.

Water fluoridation

According to WHO expert committee reviewed the use of fluorides for caries prevention in 1994 referred that first studies linking the fluoride content of drinking-water with reduced caries prevalence appeared in the 1930s, and more than a hundred studies have been reported from many different countries over the past 40 years. These studies are remarkably consistent in demonstrating substantial reductions in caries prevalence as a result of water fluoridation. Where caries prevalence was high, the modal percentage reduction in caries over a period of years was 40-49% in primary teeth and 50-59% in permanent teeth" (64).

Regarding **"Requirements for application"** of water fluoridation, the following points were made:

- A level of dental caries in the community that is high or moderate, or firm indications that the caries level is increasing.

- Attainment by the country (or area of a country) of a moderate level of economic and technological development.

- Availability of a municipal water supply reaching a large proportion of homes.

- Evidence that people drink water from the municipal supply rather than water from individual wells or rainwater tanks.

- Availability of the equipment needed in a treatment plant or pumping station.

- Availability of a reliable supply of fluoride chemical of acceptable quality.

- Availability of trained workers in the water treatment plant who are able to maintain the system and keep adequate records.

- Availability of sufficient funding for initial installation and running costs.

The WHO Expert Committee draws the following **conclusions** regarding water fluoridation:

1. Community water fluoridation is safe and cost-effective and should be introduced and maintained wherever socially acceptable and feasible.

2. The optimum fluoride concentration will normally be within the range 0.5-1.0 mg/l.

3. The technical operation of water-fluoridation systems should be monitored and recorded regularly.

4. Surveys of dental caries and dental fluorosis should be conducted periodically.

Fluoridated salt

The use of fluoridated salt for caries prevention was reviewed by a WHO expert committee in 1994 (64). The main advantages of salt as a vehicle for fluoride are that it does not require a community water supply and it permits individuals to accept or reject it; non-fluoridated salt, like non-iodized salt, can be made available to the population. Even where fluoridated salt is used in multiple products, as in parts of Costa Rica, Jamaica and Switzerland, salt fluoridation has been well accepted. So far, five countries have used salt as a vehicle for fluorides: Switzerland (since 1955), France (since 1986), Costa Rica (since 1987), Jamaica (since 1987), and Germany (since1991), and the introductory stages have been reached in Mexico and Spain.

The WHO Expert Committee draws the following **conclusions** regarding salt fluoridation:

1. Salt fluoridation should be considered where water fluoridation is not feasible for technical, financial or sociocultural reasons.

2. The optimum concentration must be determined on the basis of salt intake studies. A concentration of 200 mg F-/kg salt may be regarded as a minimum when several types of salt (domestic and salt for bakeries, restaurants and other large

kitchens) are fluoridated, but twice this concentration may be appropriate when only domestic salt is fluoridated.

3. The technical operations of salt fluoridation systems should be monitored and recorded regularly. In addition, the correct concentration and homogeneity should be periodically ascertained in the packages offered to the consumer.

4.The fluoride concentration should appear on all salt packages.

5. Surveys of dental caries and dental fluorosis should be conducted periodically.

Negative aspects on salt fluoridation are:

- salt consumption is lowest when fluorides are most needed i.e. in early years
- large individual variations in salt intake
- different concentrations would be needed to accommodate varying sub optimal levels that occur naturally in water supplies
- the use of fluoridated salt in processed food need to be controlled.

Fluoridated milk

The use of fluoridated milk for a WHO expert committee reviewed in caries prevention was recommended in 1994 (64). The **conclusions** drawn:

- Provided that a community has a well-developed milk distribution system, the technical procedures for producing fluoridated milk are straightforward.
- Encouraging results have been reported with milk fluoridation. WHO is currently preparing a report on the use of fluoridated milk, which will appear shortly.

2.5.2.2.Topical

Fluoride in the fluids surrounding the enamel crystals has been shown to have the potential to reduce the rate of demineralization. When present in the liquid phase of remineralization, fluoride will be incorporated into enamel crystals and the enamel will become more resistant to demineralization (24).

a) Gels

Fluoride gels have the advantage of being used in trays, so that all teeth in a mouth are treated at the same time. Gels are available as 1.23 % acidulated phosphate fluoride (APF) and neutral 2% sodium fluoride (NaF). The latter type has recently appeared on the market as a response to the concern that the APF gels may etch the glass filler particles of composite restorations. The anti-caries effectiveness of neutral NaF sodium fluoride gel has still not been adequately tested. The APF gels have shown an average caries reducing effectiveness of about 20%, when applied once or twice a year. Twice a year is recommended for patients with moderate to high caries risk (65). In order to reduce the risks for fluoride ingestion, the EAPD recommends fluoride gels should not be used for children below 4 years of age (66).

The European Association of Paediatric Dentistry recommends the following ructions for topical fluoride gel application:

- limit the amount of gel in the tray,
- seat the patient in an upright position,
- use suction throughout the application,
- instruct the patient to expectorate or use saliva ejector for 30 seconds after the application,
- never leave the patient unattended.

b) Varnish

Varnishes are professionally applied topical fluoride agents with high anti-caries properties, as has been shown from several clinical trials (67, 68). Varnishes may be applied to identify incipient lesions or tooth surfaces at risk, following the manufacturers' instructions.

When correctly used, the risk of ingestion of a high dose of fluoride is low (69, 70). When applied to teeth, varnishes dissolve slowly over several days, in contrast to fluoride gel application (70). This is probably a positive benefit as it has been indicated that the cumulative length of time during which the agent is in contact with the teeth, is important for any cariostatic result. Because it is easy to apply to the teeth, children with a strong gag reflex tolerate varnishes better than the gel-trays technique. However, it is important to realize that all of any varnish applied is eventually swallowed and that much of the fluoride from it will be ingested and absorbed. Therefore, the amount applied to the teeth of pre-school children should be the minimum necessary to cover the sites at risk (71).

c) Mouth rinses

Mouth rinses should not be used by children under the age of six because of the risk of swallowing, rather than expectorating the solution. They can be effectively used as part of a community prevention program (0.1% weekly or 0.02% daily sodium fluoride), although it is more costly than other methods and should be carefully monitored for effectiveness (67). Individual children at high caries risk, such as orthodontic patients with banded, bonded or removable appliances, and patients with reduced salivary flow, may use a daily rinse of 0.05% sodium fluoride at home (67).

Fluoride mouth rinses should be used at a time during the day that is different to any tooth brushing, in order to have an additive effect to fluoride toothpaste.

c) Toothpastes

The extensive use of fluoridated toothpastes has probably been one of the major reasons for the dramatic reduction in dental caries recorded over the past twenty years. Tooth brushing with fluoridated toothpaste is close to an ideal public health method, in that its use is convenient, inexpensive, culturally approved, and widespread. Nevertheless, one problem with young children's use of toothpaste is that they often swallow considerable amounts. Therefore, the use of standard concentration toothpastes (1000 ppm and more) may be associated with an increased risk for dental mottling (64). Even if the risks for severe dental mottling is almost negligible in populations without water fluoridation and if fluoride supplements are not used, parents should be strongly advised to:

- use low concentration fluoride for children below 6 years of age,
- use only a smear of toothpaste for very young children,
- supervise pre-scholars during tooth brushing.

The daily use of a fluoride toothpaste, in combination with oral hygiene instructions, is recommended as the basic part of a caries preventive program, even if other caries preventive methods, such as diet counseling, topical use of fluorides and fissure sealants, also are important (64). Where fluoride toothpaste is used in conjunction with other fluoride vehicles, the cumulative effect of fluoride must be taken into account for children less than 6 years of age. Care must be taken to ensure that a balance is maintained between the prevention of dental caries and minimizing the risk of dental mottling.

IV. Hypothesis

The use of topical fluorides does prevent dental caries in molars (deciduous and permanent) in children aged 3-6y.

V. Objectives

4.1. General objective:

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

4.2. 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 caries risk of children from 3-6y.

V. Materials and methods

5.1. Type of study:

Cohort: prospective and longitudinal

5.2. 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.

5.3. Sample

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

5.4 Variables

Independent variable: TYPE OF TOPICAL FLUORIDE 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.

5.5. Description of the variables

5.5.1. **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 instructions.

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 instructions.

5.5.2. Dependent variables:

a) DMFT and dmft: the evaluation of dental status was performed according to WHO chart (ATTACHMENT 1).

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 microbiological 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. viridians 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, SM 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 SM per milliliter of

saliva is estimated by comparing the colony density on the strip with the standard charts included in the instructions.

c) Carbohydrate exposition: for diet evaluation the frequency of carbohydrate consumption during 4 continuous days (two weekend and two-week days) has been recorded. Parents were asked to fill the type of aliments and time when they were ingested (ATTACHMENT 2).

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) (ATTACHMENT 3).

5.5.3: Criteria of inclusion:

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

5.5.4: Criteria of exclusion:

- Children with any kind of systemic disease.
- Parents without information acceptance document signed (ATTACHMENT 4).

5.6. Procedures (ATTACHMENTS 5, 6).

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 chronology

- 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 of the data [differences among cohorts, differences among recalls]
  iii. Evaluation of results
  iv. Final report **Mar- Apr 2006**

VI. 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.

VIII. Results

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
DMFT	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
Permanent teeth	0.00	0.00	0.00	0.00	0.00	0.00	0.60	0.29	0.60	0.29
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
dmft	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.
Permanent teeth: during the 2years of study, eruption of permanent teeth was observed.

TABLE 3. INCREMENT in comparison with stage 0
Group 1: permanent molars.

Stage of study	1		2		3		4	
	mean	SE	mean	SE	mean	SE	mean	SE
Permanent teeth	* 1.25	0.28	* 3.00	0.35	* 4.39	0.36	* 4.54	0.41
D teeth	0.07	0.11	0.25	0.13	0.04	0.10	0.04	0.10
F teeth	0.21	0.18	0.32	0.23	* 0.64	0.19	* 0.64	0.19
M teeth	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
DMFT	0.29	0.17	* 0.57	0.20	* 0.68	0.19	* 0.68	0.19
RI	0.00	6.45	-8.33	10.21	5.56	5.56	5.56	5.56
Dentocult	* 1.18	0.24	0.07	0.25	0.25	0.26	0.25	0.26
CFU	–	–	-1.59	1.27	* -3.96	1.09	* -3.70	1.33
Diet	–	–	-0.05	0.18	0.05	0.16	0.03	0.16
Risks	–	–	* 0.29	0.12	0.04	0.16	0.00	0.15

* significant increment (P < 0.05)

TABLE 4. INCREMENT in comparison with stage 0
Group 2: deciduous molars.

Stage of study	1		2		3		4	
	mean	SE	mean	SE	mean	SE	mean	SE
Permanent teeth	0.00	0.00	0.00	0.00	0.60	0.29	0.60	0.29
d teeth	* 0.80	0.26	* 0.93	0.33	* 0.73	0.30	* 0.73	0.30
f teeth	0.00	0.22	0.40	0.32	* 0.80	0.35	* 0.80	0.35
m teeth	0.00	0.00	0.13	0.09	0.13	0.09	0.13	0.09
dmft	* 0.80	0.26	* 1.47	0.41	* 1.67	0.44	* 1.67	0.44
RI	*-40.6	13.2	*-35.1	12.4	*-23.7	9.8	*-23.7	9.8
Dentocult	* 1.20	0.34	-0.07	0.36	0.47	0.34	0.40	0.34
CFU	–	–	* 4.33	1.46	1.77	1.32	1.73	1.25
Diet	–	–	-0.24	0.51	0.07	0.24	0.14	0.27
Risks	–	–	-0.20	0.11	*-0.27	0.12	*-0.27	0.12

* significant increment (P < 0.05)

TABLE 5. Relation of final increments of dental status values to mean risks throughout examinations
Group 1: permanent molars.

	Dentocult < 2		Dentocult ≥2		CFU < 5		CFU ≥ 5	
No. of children	11		17		14		14	
Permanent teeth	5.27	0.51	4.06	0.58	▲5.57	0.54	3.50	0.50
D teeth	0.00	0.00	0.06	0.16	0.07	0.07	0.00	0.18
F teeth	▲1.18	0.38	0.29	0.17	0.86	0.33	0.43	0.20
DMFT	1.18	0.38	0.35	0.15	0.93	0.32	0.43	0.17

▲ significant increment ($P < 0.05$)

TABLE 6. Relation of final increments of dental status values to mean risks throughout examinations
Group 1: permanent molars

	Diet < 3		Diet < 3		Risks < 2		Risks <2	
No. of children	10		18		20		8	
Permanent teeth	4.90	0.57	4.33	0.57	4.95	0.46	3.50	0.80
D teeth	-0.10	0.23	0.11	0.08	0.05	0.14	0.00	0.00
F teeth	0.70	0.24	0.50	0.33	0.50	0.27	0.72	0.27
DMFT	0.40	0.22	0.83	0.26	0.75	0.23	0.50	0.33

▲ significant increment ($P < 0.05$)

TABLE 7. Relation of final increments of dental status values to mean risks throughout examinations
Group 2: deciduous molars.

	Dentocult < 2-3		Dentocult ≥ 2-3		CFU <4		CFU ≥ 4	
No. of children	8		7		9		6	
d teeth	0.63	0.42	0.86	0.46	0.44	0.34	1.17	0.54
e teeth	0.88	0.58	0.71	0.42	0.89	0.51	0.67	0.49
f teeth	0.00	0.00	0.29	0.18	0.00	0.00	0.33	0.21
dmft	1.50	0.60	1.86	0.70	1.33	0.55	2.17	0.75

TABLE 8. Relation of final increments of dental status values to mean risks throughout examinations
Group 2: deciduous molars.

	Diet < 3		Diet > 3		Risks < 2		Risks >2	
No. of children	5		10		7		8	
d teeth	0.60	0.60	0.80	0.36	0.00	0.00	1.38	0.46
e teeth	0.60	0.60	0.90	0.46	1.00	0.65	0.63	0.38
f teeth	0.00	0.00	0.20	0.13	0.00	0.00	0.25	0.16
dmft	1.20	0.73	1.90	0.57	1.00	0.65	2.25	0.56

- significant increment ($P < 0.05$)

TABLE 9. Relation of placebo, gel and varnish and DMFT throughout examinations

Group 1: permanent molars

	Sound teeth		D teeth		F teeth		DMFT	
	%	SE	%	SE	%	SE	%	SE
0th stage of study								
Placebo	75.0	7.5	1.8	1.8	23.2	7.0	25.0	7.5
Gel	78.6	7.9	0.0	0.0	21.4	7.9	21.4	7.9
Varnish	67.9	9.0	3.6	3.6	28.6	8.7	32.1	9.0
1st stage of study								
Placebo	67.9	7.8	3.6	2.5	28.6	7.5	32.1	7.8
Gel	71.4	8.7	0.0	0.0	28.6	8.7	28.6	8.7
Varnish	60.7	9.4	7.1	5.0	32.1	9.0	39.3	9.4
2nd stage of study								
Placebo	∪62.5	8.0	8.9	3.7	28.6	7.9	∩37.5	8.0
Gel	∪60.7	9.4	7.1	5.0	32.1	9.0	∩39.3	9.4
Varnish	64.3	9.2	7.1	5.0	28.6	8.7	35.7	9.2
3rd stage of study								
Placebo	∪60.7	7.9	3.6	2.5	∩35.7	8.1	∩39.3	7.9
Gel	∪60.7	9.4	0.0	0.0	∩39.3	9.4	∩39.3	9.4
Varnish	57.1	9.5	3.6	3.6	39.3	9.4	42.9	9.5
4th stage of study								
Placebo	∪60.3	7.8	3.5	2.5	∩35.6	8.0	∩39.2	7.8
Gel	∪60.8	9.5	0.0	0.0	∩39.3	9.4	∩39.4	9.5
Varnish	56.1	9.6	3.5	3.6	39.2	9.4	43.0	9.6

∩ ∪significant increase or decrease respectively in comparison with 0th stage

TABLE 10. Relation of placebo, gel and varnish and dmft throughout examinations

Group 2: deciduous molars

	Sound teeth		d teeth		e teeth		f teeth		Dmft	
	%	SE	%	SE	%	SE	%	SE	%	SE
0th stage of study										
Placebo	85.7	7.5	0.0	0.0	14.3	6.8	0.0	0.0	14.3	6.8
Gel	85.7	7.0	0.0	0.0	14.3	6.3	0.0	0.0	14.3	6.3
Varnish	89.3	6.7	0.0	0.0	10.7	5.7	0.0	0.0	10.7	5.7
1st stage of study										
Placebo	82.1	7.6	5.4	2.9	12.5	6.3	0.0	0.0	17.9	7.2
Gel	82.1	8.9	10.7	5.7	7.1	4.9	0.0	0.0	17.9	6.7
Varnish	82.1	8.9	7.1	4.9	10.7	7.8	0.0	0.0	17.9	8.5
2nd stage of study										
Placebo	76.9	10.4	5.8	3.1	15.4	6.8	1.9	1.9	23.1	8.1
Gel	76.9	12.2	3.8	3.8	19.2	7.3	0.0	0.0	23.1	9.4
Varnish	∪ 69.2	12.8	7.7	5.2	19.2	9.2	3.8	3.8	∩ 30.8	9.5
3rd stage of study										
Placebo	∪ 75.0	9.1	5.4	3.9	17.9	6.2	1.8	1.8	∩ 25.0	8.5
Gel	75.0	10.2	0.0	0.0	25.0	8.9	0.0	0.0	25.0	8.9
Varnish	∪ 70.4	12.7	3.7	3.7	22.2	7.3	3.7	3.7	∩ 29.6	9.2
4th stage of study										
Placebo	∪ 74.0	9.0	5.3	3.9	17.6	6.2	1.8	1.9	∩ 25.4	8.7
Gel	75.1	10.2	0.0	0.0	25.0	8.9	0.0	0.0	25.0	8.9
Varnish	∪ 70.2	12.6	3.7	3.7	22.1	7.2	3.7	3.7	∩ 29.1	9.1

∩ ∪significant increase or decrease respectively in comparison with 0th stage

TABLE 11. Relation of CFU to Dentocult score

	Group 1			Group 2			Total		
Dentocult score	No. of children	Mean CFU	SE	No. of children	Mean CFU	SE	No. of children	Mean CFU	SE
0	18	3.18	0.75	4	4.40	0.95	22	3.40	0.64
1	15	6.93	1.25	8	4.78	2.09	23	6.18	1.09
2	11	7.42	1.76	14	3.64	0.79	25	5.30	0.95
3	68	5.71	0.73	34	6.63	0.91	102	6.02	0.57
Regression coefficient	112	0.49	0.45	60	0.99	0.66	172	0.61	0.37

No significant relation

TABLE 12. Relation of number of decayed teeth to Dentocult scores

	D + d teeth			d teeth only		
Dentocult score	Number of children	Mean	SE	Number of children	Mean	SE
0	26	0.92	0.30	26	0.77	0.28
1	17	0.53	0.27	17	0.53	0.27
2	19	0.68	0.20	19	0.42	0.16
3	78	1.03	0.17	78	0.87	0.15
Regression coefficient	140	0.078	0.099	140	0.061	0.089

No significant relation

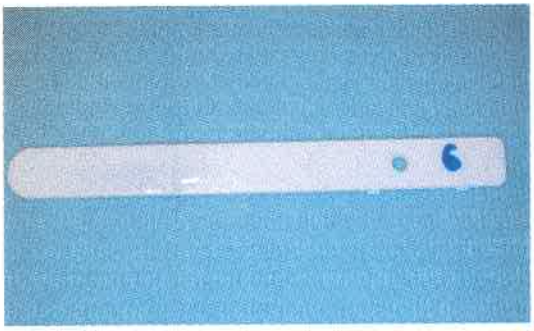

Fig 14. Dentocult SM score 0

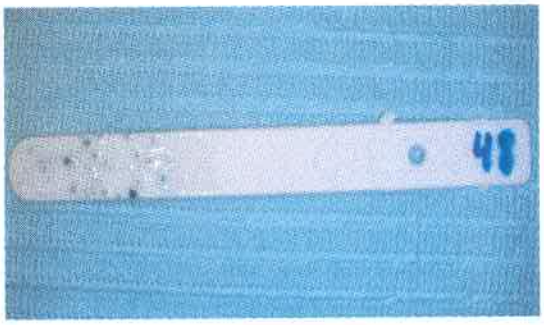

Fig 15. Dentocult SM score 1

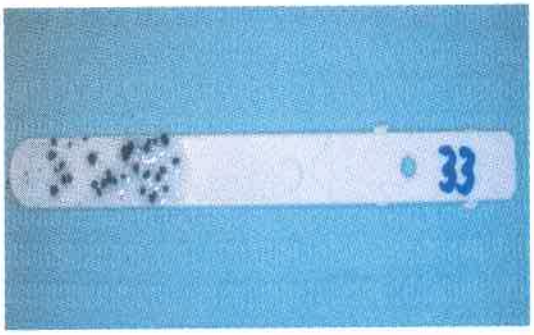

Fig 16. Dentocult SM score 2

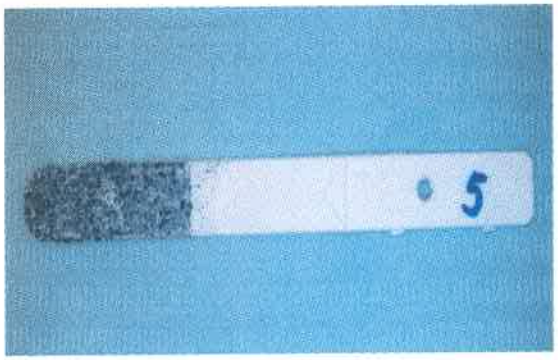

Fig 17. Dentocult SM score 3

IX. Discussion

Oral health is indispensable for an acceptable quality of life. Despite great achievements in oral health of populations 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 professionally applied fluorides, which are gels and fluoride varnishes

A brief summary of our work is summarized in 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 who were warned to come without any special cleaning, just the daily routine hygiene that they use to do at home. Then, the real data was showed.

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. As it can be seen in table 3, there is a statistical significant difference in the number of permanent teeth along the 2 years of our 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 toothbrushing. 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, inter dental 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 (72).

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 (tables 3-4). 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 (tables 5-8). We wanted to compare the efficacy of chair side method and the traditional agar one. As it is

shown in table 11, there is no 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 (73). 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 (74).

Regarding Dentocult SM records and incidence of dental caries (tables 11-12), our results did not demonstrate a conclusive statistical relation between them. In the literature about 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 S. mutans. 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 (75). 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 prognosed 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 (76).

In our work, we did not found statistical significant relation between Dentocult scores and dental caries (tables 5, 7, 11, 12). 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 on 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 uses 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 (tables 3, 4, 6, 8). 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 are eating. Children in the group 1 were able to buy their own snack at the school and mainly it was a combination of starch and sucrose, which is the worst combination. More efforts on this topic are recommended.

Final data of caries risk assessment (tables 3-4) showed that 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 diet is directly guided by them.

Finally, we wanted to evaluate the efficacy of fluoride gel and varnish in caries free permanent and deciduous molars. In the group 1 (table 9), 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 increased salivation – due to physiological changes in the mouth- might play a role 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 (table 10), we found a decrease of sound teeth in molars that received 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 harder to followed manufacturer's recommendations. Probably the conditions of setting the varnish were not 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 fluoride application in comparison with placebo. Nevertheless, there are other 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 on 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.

X. 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.

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XII. ATTACHMENTS

ATTACHMENT 1. Caries Prevalence: DMFT and DMFS – WHO CHART-

DMFT and DMFS describe the amount - the prevalence - of dental caries in an individual. DMFT and DMFS are means to numerically express the caries prevalence and are obtained by calculating the number of

- Decayed (D)
- Missing (M)
- Filled (F)
- Teeth (T)
- Surfaces (S).

It is thus used to get an estimation illustrating how much the dentition until the day of examination has become affected by dental caries. It is either calculated for 28 (permanent) teeth, excluding 18, 28, 38 and 48 (the "wisdom" teeth) or for 32 teeth (The Third edition of "Oral Health Surveys - Basic methods", Geneva 1987, recommends 32 teeth). Thus:

- How many teeth have caries lesions (incipient caries not included)?
- How many teeth have been extracted?
- How many teeth have fillings or crowns?

The sum of the three figures forms the DMFT-value. For example: DMFT of 4-3-9=16 means that 4 teeth are decayed, 3 teeth are missing and 9 teeth have fillings. It also means that 12 teeth are intact.

Note: If a tooth has both a caries lesion and a filling it is calculated as D only. A DMFT of 28 (or 32, if "wisdom" teeth included) is maximum, meaning that all teeth are affected.

A more detailed index is DMF calculated **per tooth surface, DMFS**. Molars and premolars are considered having 5 surfaces, front teeth 4 surfaces. Again, a surface with both caries and filling is scored as D. Maximum value for DMFS comes to 128 for 28 teeth.

For the **primary dentition**, consisting of maximum 20 teeth, the corresponding designations are "deft" or "defs", where "e" indicates, "extracted tooth".

In Tables presenting caries data for **adults**, the following designations are used:

DMFT: Mean number of decayed, missing or filled teeth			
%DMFT:	Percentage of population affected with dental caries	**MT:**	Mean number of missing teeth
%D:	Percentage with untreated decayed teeth	**MNT:**	Mean number of teeth
DT:	Mean number of decayed teeth	**%Ed:**	Percentage edentulous

WORLD HEALTH ORGANIZATION, methods and indexes. Geneva, 2002.

ATTACHMENT 2. Diet records

HODNOCENÍ STRAVOVACÍCH NÁVYKŮ

JMÉNO:..... **KÓD:.....**

DATUM NAROZENÍ:.....

DATUM VYŠETŘENÍ:.....

	ČAS	PÁTEK	SOBOTA	NEDĚLE	PONDĚLI
SNÍDANĚ					
MEZI					
OBĚD					
MEZI					
VEČEŘE					
PO					

ATTACHMENT 3. Caries risk evaluation

I. CARIES RISK ASSESMENT

For evaluating the caries risk we considered these 4 basic etiological factors.

A) The guest's susceptibility is measured by caries experience, which means the number of caries lesions at the moment of examination.

B) The microflora is evaluated by erythrosine B in the 4 upper incisives (for its easy accessibility). The surface was divided in 3 thirds, and the record was from

0: no dental plaque

1: gingival third with dental plaque.

2: two thirds of dental plaque.

3: the whole surface cover by dental plaque.

After the 4 teeth record a simple average from all was obtained recorded.

C) Fermentable carbohydrates were counted and average from 4-day dietary (two week and two weekend days) was registered.

I. CLASSIFICATION OF CARIES RISK

The caries risk (CR) is classified as:

1. Low CR.
2. Moderate CR.
3. High CR.

1. LOW CARIES RISK

- Caries experience: no more than 2 occlusal caries lesions.
- Baseline Plaque Index: low or equal 1.
- Frequency of consumption of dietary carbohydrates: 3 times a day or less.

2. MODERATE CARIES RISK

- Caries experience: from 3 to 6 occlusal caries lesions.
- Baseline Plaque Index: more than 1 and less or equal 2.
- Frequency of consumption of dietary carbohydrates: more than 3 to 4 times a day.

3. HIGH CARIES RISK

- Caries experience: more than 6 occlusal caries lesions or at least 1 smooth* surface lesion.
- Baseline Plaque Index: more than 2.
- Diary frequency of extrinsic sugars ingests: more than 4 times a day.

* smooth lesion: vestibular, lingual or proximal surface.

ATTACHMENT 4. Parent agreement information

Identifikační číslo:

Informovaný souhlas s účastí v projektu
(rodiče)

Název projektu: **Porovnáni účinku lokální aplikace fluoridového gelu a fluoridového laku v prevenci kazu dočasných a stálých molarů**

Výzkumní pracovníci: **Dr. Ana Lucía Seminario, Dr Zdeněk Broukal, Dr. Romana Ivančaková.**

1. Potvrzuji, že jsem četl (a) a porozuměl (a) informaci o tříleté studii orálního zdraví předškolních dětí.

2. Je mi známo, že naše účast ve studii je dobrovolná, že můžeme ze studie kdykoli vystoupit bez udání důvodu a že účast ve studii neomezí stomatologickou péči ani jiná práva našeho dítěte.

3. Souhlasím, že naše dítě ..... (uveďte jméno) a my, jeho rodiče, se studie zúčastníme.

Jméno rodiče	Datum	Podpis
Jméno informující osoby	Datum	Podpis
Jméno výzkumného pracovníka	Datum	Podpis

ATTACHMENT 5. RECORDS 1: PERMANENT MOLARS

A. Personal data Name of child:.....

Child No.	A1	**Birth date (YYMMDD)**	A2	**Sex (G/B)**	A3
Date of examination	A4	**Date of fluoride application**	A5	**Stage of study (0, 1, 2, 3, 4, ...)**	A6

B. Dental record

Teeth	17	16	15 55	14 54	13 53	12 52	11 51		21 61	22 62	23 63	24 64	25 65	26	27
codes	B1	B2	B3	B4	B5	B6	B7		B8	B9	B10	B11	B12	B13	B14
upper jaw															
lower jaw															
codes	B15	B16	B17	B18	B19	B20	B21		B22	B23	B24	B25	B26	B27	B28
Teeth	47	46	45 85	44 84	43 83	42 82	41 81		31 71	32 72	33 73	34 74	35 75	36	37

dental status coding	**permanent**	**deciduous**
caries free	0	A
dental caries	1	B
dental caries + filling	2	C
filling without caries	3	D
missing due to caries	4	E
missing from other reasons	5	X
tooth with crown	6	F
tooth with abutment crown	7	G
not erupted	8	X
missing due to orthodontic extraction	9	X

C. Plaque evaluation

Tooth				
plaque score				
Code	C1	C2	C3	C4

D. Fluor application: tooth/fluor type

Tooth				
application (Gelly, Varnish, Placebo)	G	P	V	P
Code	D1	D2	D3	D4

E. Level of S. mutans

Dentocult SM (0-3)	
Code	E1

CFU	
Code	E2

F. HODNOCENÍ STRAVOVACÍCH NÁVYKŮ

Code	F1

G. RIZIKA

Code	G1

OBSERVATIONS

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ATTACHMENT 6. RECORDS 2: DECIDUOUS MOLARS

A. Personal data Name of child:.....

Child No.	A1	**Birth date (YYMMDD)**	A2	**Sex (G/B)**	A3
Date of examination	A4	**Date of fluoride application**	A5	**Stage of study (0, 1, 2, 3, 4, ...)**	A6

B. Dental record

teeth	17	16	15 55	14 54	13 53	12 52	11 51		21 61	22 62	23 63	24 64	25 65	26	27
codes	B1	B2	B3	B4	B5	B6	B7		B8	B9	B10	B11	B12	B13	B14
upper jaw															
lower jaw															
codes	B15	B16	B17	B18	B19	B20	B21		B22	B23	B24	B25	B26	B27	B28
teeth	47	46	45 85	44 84	43 83	42 82	41 81		31 71	32 72	33 73	34 74	35 75	36	37

dental status coding	**permanent**	**deciduous**
caries free	0	A
dental caries	1	B
dental caries + filling	2	C
filling without caries	3	D
missing due to caries	4	E
missing from other reasons	5	X
tooth with crown	6	F
tooth with abutment crown	7	G
not erupted	8	X
missing due to orthodontic extraction	9	X

C. Plaque evaluation

tooth				
plaque score				
code	C1	C2	C3	C4

D. Fluor application: tooth/fluor type

tooth								
application(Gelly,Varnish,Placebo)	V	P	P	G	V	P	P	G
code	D1	D2	D3	D4	D5	D6	D7	D8

E. Level of S. mutans

Dentocult SM (0-3)	

code	E1
CFU	
code	E2

F. HODNOCENÍ STRAVOVACÍCH NÁVYKŮ

code	F1

G. RIZIKA

code	G1

OBSERVATIONS

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