# The influence of caries preventive programs on the dynamic of cariogenic bacteria

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**Summary**

**This study was carried out in order to assess the clinical and microbiological efficacy of the two specific caries prevention programs (P1 and P2) as compared to the control group (C). Two supragingival plaque samples were collected and analysed qualitatively and quantitavitely by the "checkerboard" DNA-DNA hybridization method, for 10 different oral species. The results showed that the two caries prevention programs demonstrated their efficacy in the substantial reduction of DMFT scores and DMFS scores after six years of application. The mouthrinsing program combined with pits-and-fissure sealant (P2) was superior to fluoride mouthrinsing alone (P1) and almost prevented all caries. There was a significant statistical positive correlation between the levels of *S. mutans* and *S. sobrinus* and the prevalence of dental caries.**

**Summary**

## Introduction

The Department of Preventive Dentistry at the Faculty of Dentistry, University of Medicine and Pharmacy "Gr. T. Popa" of Iasi initiated two caries prevention programs at one primary school in Iasi. A total of two hundred 6-year old children of both sexes in grade 1 were randomly assigned to one or the other of the two caries prevention programs. One such program (P1) consisted of 1 min. mouth rinsing with 10 ml of a 0.2% NaF solution once a week. Participants in the second program (P2) used the same oral rinsing procedure as in P1 and, in addition, their first molars were treated prophylactically with a pit-and-fissure sealant. Children who did not wish to participate in the specific caries prevention programs served as a control group (C).

## Aims of the study

This study was carried out in order to assess the clinical and microbiological efficacy of the two specific caries prevention programs (P1 and P2) as compared to the control group (C).

## Material and method

***Study subjects***
Sixty-nine children from P1, 71 children from P2 and 60 children from C met the inclusion criteria of the study, which included complete clinical data and two pooled supragingival plaque samples (see below) from each child.

***Clinical monitoring***
All children were clinically examined at the school dental clinic by a dentist according to the WHO criteria [18]. No radiographs were taken. The following clinical parameters were recorded: DMFT index and DMFS index, gingivitis and plaque index.

***Supragingival plaque samples***
The three buccal and the three lingual plaque samples were pooled giving one pooled buccal surface sample and one pooled lingual surface sample from each child. The total of 400 polled plaque samples originated from children participating in P1 (n = 138), P2 (n = 142 and C (n = 120). All samples were shipped to the Laboratory of Oral Microbiology, University of Bergen, Norway and kept frozen at -20° C until analyzed

by "Checkerboard DNA-DNA Hybridization" [14].

***Checkerboard DNA-DNA Hybridization*** uses two main devices: one is a Minislot (Immunetisc, Cambridge MA), and the other one is a Miniblotter 45 (Immunetisc, Cambridge MA). A Minislot device *(figure 1)* allows lysates loaded in parallel channels to be aspirated through the membrane, depositing horizontal lanes on the membrane surface. Hybridization process is performed in vertical lanes of a Miniblotter 45 *(figure 2)* with digoxigenin-labeled whole genomic probes. The method permits the simultaneous determination of the presence of multiple bacterial species in a single or multiple dental plaque samples.

**Figure 1. Minislot**

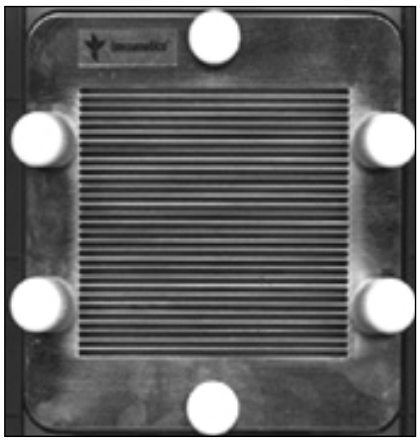


**Figure 2. Miniblotter**


***Detection and enumeration***

The X-ray films were evaluated visually by comparing test samples and standards. Readings were recorded based on DNA hybridization as: 0, not detected; 1, $< 10^5$ cells; 2 $\approx 10^5$; $10^5 < 3 < 10^6$; 4 $\approx 10^6$; 5 $> 10^6$ cells.

**Data analysis**

The SPSS 10.0 for Windows statistical software (SPSS Inc 2000) was used for data management and statistical tests.

## Results

***Basic caries vs. final caries***

Initially, there were no significant differences between the study groups with respect to mean DMFT and mean DMFS scores *(table 1).* Six years later *(table 1)* CC demonstrated a significant increase in these scores as compared to CP1 and CP2 (3.73 vs. 1.40 and 0.02 for DMFT and 4.14 vs. 1.65 and 0.69 for DMFS, respectively).

The frequencies of caries-free children were initially 32.51% (CP1), 35.42% (CP2) and 31.23% (CC) and finally, 33.29% (CP1), 43.59% (CP2) and 25.15% (CC). The increase in C and decline in P2 of caries-free children were statistically significant ($r = 0.148$, $p < 0.01$, $r = 0.256$, $p < 0.01$, respectively). So was also the difference in the end of the study between CP1 and CP2 ($r = 0.157$, $p < 0.05$).

There was a significant downward trend in DMFT scores from 1992 to 1998 for both P1 ($r = 0.248$, $p < 0.05$ - P1) and P2 ($r = 0.563$, $p < 0.01$ - P2). CP1 contributed with 45.64 % ($p < 0.001$) of the DMFT score decline and with 40.6 % ($p < 0.05$) of the reduction in DMFS scores.

**Table 1.** Basic and final clinical parameters and scores

| BASIC SCORES/FINAL SCORES | P1 | P2 | C |
|---|---|---|---|
| **n (% of total N)** | 69 (34.5)/ 69 (34.5) | 71 (35.5)/ 71 (35.5) | 60 (30.00)/ 60 (30.00) |
| **age (DS)** | 6 (1)/ 12 (1) | 6 (1)/ 12 (1) | 6 (1)/ 12 (1) |
| **DMFT (SD)** | 1.26 (1.20)/ 2.68 (1.48) | 1.71 (35.5)/ 1.73 (1.31) | 1.20 (1.19)/ 4.93 (6.63) |
| **DMFS (SD)** | 1.82 (2.019/3.47 (1.96) | 1.81 (1.98)/ 2.5 (1.83) | 1.71 (1.91)/ 5.85 (3.11) |
| **Gingivitis (SD)** | 0.37 (0.48)/ 0.24 (0.43) | 0.35 (0.48)/ 0.22 (0.42) | 0.26 (0.44)/ 0.30 (0.46) |
| **Pl I (SD)** | 1.30 (0.95)/ 0.68 (0.63) | 1.28 (0.95)/ 0.46 (0.50) | 1.50 (1.56)/ 1.78 (0.92) |

***Basic and final plaque and gingivitis***

There were no significant differences between the basic and final records for any of the study groups or between the groups with respects to Pl I and gingival inflammation *(table 1)*.

***Level and detection of frequency***

A total of 400 plaque samples were screened. Probe sensitivity in the checkerboard assay was evaluated by adjusting probe concentration to detect $10^4$ cells of the homologous species.

*Table 2* shows the 10 supragingival bacteria according to their mean detection levels in each of the three study groups. Most of the species were detected in all study groups, but in different amounts with an increase from P2 to P1 to C.

**Table 2.** Mean values (SD) for 10 bacteria analyzed by DNA-DNA hybridization

| BACTERIA | P1 Mean (SD) | P2 Mean (SD) | C Mean (SD) |
|---|---|---|---|
| ***S. mutans*** | 2.76 (1.72) | 1.80 (1.41) | 4.03 (2.10) |
| ***S. sobrinus*** | 2.39 (1.45) | 1.12 (0.75) | 3.75 (1.01) |
| ***S. gordonii*** | 2.02 (1.41) | 1.56 (1.15) | 2.56 (1.65) |
| ***S. sanguis*** | 1.05 (0.66) | 0.69 (0.68) | 1.25 (0.60) |
| ***S. mitis*** | 1.37 (0.66) | 0.83 (0.69) | 1.53 (0.56) |
| ***S. oralis*** | 1.33 (0.50) | 0.57 (0.62) | 1.80 (0.75) |
| ***L. acidophilus*** | 0.98 (0.79) | 0.25 (0.43) | 1.51 (1.14) |
| ***L. fermentum*** | 0.81 (0.79) | 0.49 (0.55) | 1.01 (0.81) |
| ***L. rhamnosus*** | 0.76 (0.75) | 0.38 (0.54) | 0.76 (0.72) |
| ***L. plantarium*** | 0.95 (1.34) | 0.46 (0.50) | 0.98 (0.96) |

***Streptococci species*** *(Figure 3)*

Of the six streptococci species analyzed, *S. mutans* and *S. sobrinus* were detected in significantly higher levels in CC (mean = 4.03) than in CP1 (mean = 2.76) and CP2 (mean = 1.80) ($p < 0.05$). The relation of streptococci species with the prevalence of dental caries was examined for each program. There was a positive correlation between the prevalence of *S. mutans* and *S. sobrinus* and the prevalence of dental caries for control group ($r = 0.523$, $p < 0.01$; $r = 0.349$, $p < 0.05$).

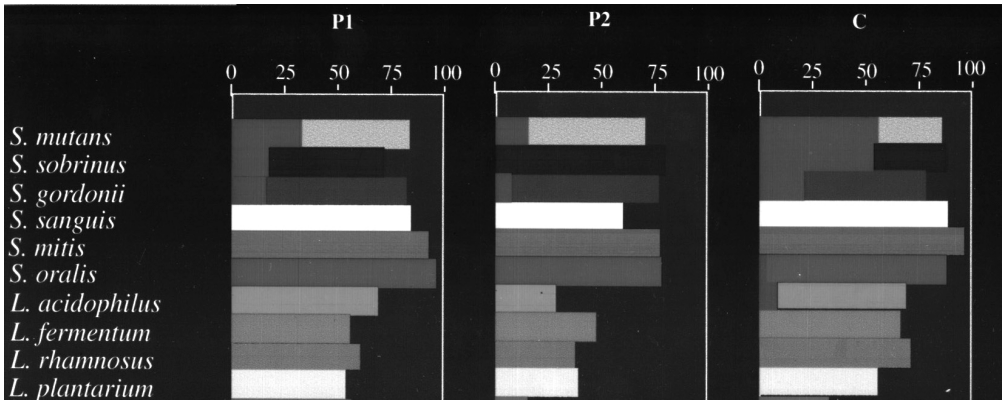
The other groups showed a non-significant positive correlation.

***Lactobacilli species*** *(Figure 3)*

Of the four examined species of lactobacilli, the most prevalent ones were *L. acidophilus* (73.6%), and *L. fermentum* (73.3%) in CC, *L. acidophilus* (72.4%) and *L. rhamnosus* (63.1%) in CP1, and *L. fermentum* (45.9%) and *L. plantarium* (46.2%) in CP2. For all three study groups, the levels of lactobacilli did not show a correlation with the prevalence of dental caries.

Apart from *L. acidophilus*, which was detected at high level ($\geq 10^6$) in proportions of 5.5% in CC and 5.4% in CP1, lactobacilli were detected at low level ($\leq 10^5$) in the three study groups.

**Figure 3.** Frequency distribution of streptococcus and lactobacillus species




## Discussion

***The Caries Preventive Programs***

Fluoride rinsing programs have been evaluated clinically under a variety of conditions and with different results. In their comprehensive review of the literature up to 1978, Birkeland and Torrel [1] concluded that a caries reduction of about 40% could be expected following a two to three year rinsing program. According to these authors, school-based fluoride rinsing is an effective public dental health procedure which, due to its simplicity and efficacy, has to be considered a valuable alternative to water fluoridation. In Romania there is currently no water fluoridation. After six years our results generally confirm previous estimates of efficacy for fluoride mouthrinsing in non-fluoridated areas [1, 9, 10,12, 13].

***DMFT and DMFS***

In our study the DMFT and DMFS scores decreased after six years by 45.64% and 40.60%, respectively, for P1 and with 64.90% and 57.26%, respectively, for P2.

Probably this decline cannot be attributed solely to the preventive programs. Thus, even if the community in Iasi is fluoride-deficient, the children may be exposed to fluorides unassociated with the mouthrinsing programs. The possibility exists that some factors that influence dental caries other than the intervention being studied may have contributed to the observed changes in caries prevalence. Changes in diet, access to dental care, secular improvement of caries, or other factors may also have had an effect on our data. However, the great decrease in DMFT and DMFS scores in P2 appears attributable mostly to the application of pits-and-fissure sealants. Similar studies by Sterrit [15, 16] resulted in the same conclusions. The reduction of DMFT and DMFS scores in P2 had the additional benefit of optimizing the introduction of sealants [12, 13]. It is not surprising that a survey of the reasons for the decline of dental caries during the past 25 years showed a wide variation in interpretation by the experts. There was, however, a high level of agreement on the "very important" and "important" factors. The survey clearly showed that there is a worldwide acceptance of merits of fluoride from toothpastes used in "homecare" situations. Virtually no one considered dietary factors as "very important" and only a few as "important". We must not forget that fluoride dentifrices nowadays are widely used everywhere.

***Fluoride and Sealants***

Fluorides are highly effective in preventing caries lesions occurring on the smooth surfaces of the teeth. Unfortunately, fluorides are not equally effective in protecting the occlusal pits and fissures where 95% of all caries lesions occur [17]. Thus, sealants are used to protect the occlusal surfaces. A major effort should be made to incorporate the use of sealants along with other primary preventive measures such as plaque control, fluoride therapy and sugar-intake control.

Ripa et al. [12] completed a 2-year study of children in the second and third grades assessing the efficacy of a 0.2% fluoride mouthrinsing used alone compared with such a rinse plus sealants. Twenty-four occlusal caries lesions developed in the 51 rinsing subjects and only three in the 84 children who rinsed and received sealants. By comparing the decline in caries in CP1 and CP2,

it is obvious that the main reduction in DMFT and DMFS scores can be attributed to sealant application with some additional contribution by the fluoride mouthrinsing. After six years of application, 58.34% of sealants were present *in situ* among children in P2. We then decided to seal also the pits and fissures of all second permanent molars of children at the age of 12 years. Because of the preponderance of pit-and-fissure caries, the use of sealants for children who participate in a fluoride mouthrinse program seems to be particularly appropriate [11]. The use of sealant in combination with weekly fluoride mouthrinsing should result in an almost complete elimination of caries in primary school children.
During six years of application of the two preventive programs, no statistically significant changes could be found in prevalence of gingivitis and plaque index scores. This can be explained by good oral health rather than by fluoride mouthrinsing.

***The Microbiological Examination***
The purpose of the bacterial investigation was to evaluate the composition of supragingival dental plaque samples taken from subjects with different clinical characteristics, in order to evaluate the role played by selected supragingival species in the caries process. Our results demonstrated some differences in the supragingival microbiota of subjects in the three study groups. However, the prevalence and levels of 10 supragingival species did not differ significantly between P1 and P2.
*S. mutans* and *S. sobrinus* were detected at the highest levels in the control group in which there was a strong positive correlation between high levels of *S. mutans* and *S. sobrinus* and prevalence of caries. This is in agreement with the general view that these species are the predominant caries initiating bacteria in humans [9, 13, 15, 16].
We found a significant correlation between dental caries and *S. mutans* and *S. sobrinus* as well as with low level of lactobacilli. This can be explained by the fact that the plaque samples from Iasi were not mature plaque. Also, lactobacilli are mainly correlated with white spot lesions and carious dentine.
The literature showed higher concentration of *S. mutans* in areas of individual teeth with incipient caries as compared with adjacent caries-free areas. Microbiological examinations of dental plaque showed that lactobacilli species increased in numbers later than *S. mutans.*
*S. mutans* and lactobacilli species multiply in numbers in the plaque concomitantly with development of incipient caries and that their numbers may decline when the environment favours enamel remineralization. Presumably, changes in the environment, which result in increases in pH, encourage remineralization, promote succession of other bacteria and reduce the numbers and proportions of muttons streptococci and lactobacilli. The low prevalence of caries in CP1 and, particularly in CP2 explains why we did not detect higher levels of cariogenic bacteria species as part of the human dental plaque flora.

***Interaction of Fluorides with Bacteria***
Fluorides at prophylactic levels do not influence the composition of dental plaque *in vitro* [2]. There is little evidence that fluorides cause significant quantitative changes in the plaque microbiota. A major effect of fluoride on oral bacteria both *in vitro* and *in vivo* is the modification of the microbial metabolism. Fluoride will reduce the ability of plaque bacteria to produce acid [5] and interfere with the carbohydrate metabolism [5]. The ability of fluorides to inhibit the glycolysis by blocking the enzyme enolase, has long been known. In addition, fluorides may react with the underlying layer of dissolving enamel promoting its remineralization as flourhydroxyapatite. The end result of this process is a "physiologic" restoration of the initial lesion by remineralization of enamel and the formation of a more resistant enamel surface.

## Conclusions

1. The two caries prevention programs demonstrated their efficacy in the substantial reduction of DMFT scores and DMFS scores after six years of application.
2. The mouthrinsing program combined with pits-and-fissure sealant (P2) was superior to fluoride mouthrinsing alone (P1) and almost prevented all caries.
3. There was a significant statistical positive correlation between the levels of *S. mutans* and *S. sobrinus* and the prevalence of dental caries.
4. The DNA probe hybridization checkerboard assay is a useful tool for bacterial monitoring of caries prevention programs.

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