Caries Prevention by Use of Fluoride Varnish in Primary School Children in Minsk

Annerose Borutta[1], Maik Wagner[2], Natalia Shakovets[3], Tamara Tserekhova[4]

[1] Prof. Dr. Med. Habil. Dr.h.c. World Health Organization Collaborating Centre on Prevention of Oral Diseases, Centre for Dental, Oral and Maxillofacial Surgery, Friedrich Schiller-University, Jena, Germany. [2] Dipl. Math. Oec. Department of Business Statistics, Friedrich Schiller University, Jena, Germany. [3] Dr. Med. Dent. Department of Paediatric Dentistry, Belarussian State Medical University, Minsk, Belarus. [4] Prof. Dr. Med. Dent. Department of Paediatric Dentistry, Belarussian State Medical University, Minsk, Belarus.

Abstract

Aims: The aims of this two-year follow-up study were to evaluate the caries-preventive effect of Bifluorid12 (VOCO GmbH, Cuxhaven, Germany) on permanent molars in primary schoolchildren and to investigate whether or not there are differences in the caries static effect of Bifluorid12 on permanent molars among children with different caries experience in their primary molars. ***Methods:*** After a baseline examination to establish their caries status, 229 first-graders (mean age 6.2 years) at high caries risk from primary schools in Minsk, Belarus were randomly divided into a test group (A) and a control group (B). Each group was then divided into three subgroups (U). Subjects (dmft=0) in both the test (A) and control (B) groups were allocated to the three subgroups depending on their existing level of caries. Subgroup U3 received those with the highest caries prevalence and subgroup U1 those with the lowest. All children in the test group (A) were treated with Bifluorid12 twice a year for two years. Those in the control group (B) only received preventive advice. ***Results:*** After two years, the caries incidence of permanent molars in children in the test group (A) was significantly lower (0.22 DMFT/DMFS) than in the control group B (0.58 DMFT; 0.60 DMFS). The highest incidence was found in both U2 subgroups. The caries-incidence reduction in group A, based on DMFS, was 63.3%. Within group A, subgroup U1 showed a caries-incidence reduction of 87.9%, subgroup U2 of 85% and subgroup U3 of 39.7%. ***Conclusions:*** It can be concluded that Bifluorid12 showed a significant caries-incidence reduction on permanent molars among school children. The caries-preventive effect was different for the subgroups with regard to previous caries experience in primary molars. Children without caries experience in primary molars showed the highest caries-incidence reduction in their permanent molars.

Key Words: Fluoride Varnish, Caries Inhibition, First Permanent Molars, Caries Risk

Introduction

The oral health of children and adolescents in Germany improved significantly [1,2] between 1994 and 2004. A similar caries decline is possible in all industrial nations, although at differing levels. In Eastern European countries, up to now there has been an insignificant caries decline [3,4,5]. The caries decline always refers to the average of each age cohort. In addition, there are the so-called risk groups whose oral health status is characterised by a significantly higher caries prevalence than the average [6]. According to German guidelines for the use of fluorides for school children with elevated caries risk, fluoride varnishes are indicated [7]. Fluoride varnishes are able to inhibit caries by approximately 30-40% after applications two to four times per year.

Fluoride varnishes have been developed to prolong the contact time of the fluoride on tooth enamel. Richardson (1967) [8] showed an increasing fluoride intake in enamel after a prolonged contact time of fluoride on the enamel surface. Schmidt (1964) [9] developed a fluoride varnish with 2.23% sodium fluoride, which has been available since 1968 under the name Duraphat®. Subsequently, more fluoride varnishes were developed. Today commonly used fluoride varnishes include Duraphat (2.26% F as sodium fluoride), Fluor Protector (0.1 and 0.7% F as a silan fluoride) and Bifluorid12 (5.6% F as calcium fluoride and 5.6% F as sodium fluoride) [7].

Varnishes are characterised by the following benefits:

- High adhesion.
- Application without the need for total moisture control.

Corresponding author: Prof. Dr. med. habil. Dr. h. c. Annerose Borutta,WHO-Kollaborationszentrum “Prävention oraler Erkrankungen”, Bachstrasse 18, 07740 Jena, Germany; e-mail: Annerose.Borutta@med.uni-jena.de

• The hygienist can perform the applications.

• The use of applicators with cartridges allows an exact dose and better penetration of the varnish in the proximal surfaces.

• They are suitable for both the primary and secondary dentition.

• They can be used for individuals in a dental office/cabinet/practice as well as for groups taking part in preventive programmes.

• Application is simple, with no special equipment needed.

• They inhibit caries on smooth surfaces, in fissures and pits.

Aims

The aims of this two-year study were to evaluate the caries preventive effect of Bifluorid12 (VOCO GmbH, Cuxhaven, Germany) on recently erupted first permanent molars and to investigate whether there were differences in the caries-preventive effect of the varnish when the baseline caries levels of primary molars were taken into consideration.

Methods

Two hundred and twenty-nine randomly selected school children (average age 6.22 years) were recruited from four elementary schools of the city of Minsk, Belarus. A baseline oral examination was performed to assess the caries prevalence in primary and permanent teeth according DMFT/dmft and DMFS/dmfs Indices [10], then the subjects were randomly divided into two groups. Group A included 119 children and Group B 110 children. The two groups were then divided into three subgroups, according the baseline caries findings at primary molars. The first subgroup (U1) consisted of those children without caries in their primary molars (dmft=0), the second subgroup (U2) consisted of children with moderate caries, and the third subgroup (U3) included the 33.3% of children with the highest dmft values in their primary molars (SIC Index) [11]. Group A was the test group and group B the control group. Children from group A received F-varnish applications with Bifluorid12 and oral health education four times per year. The active ingredients of Bifluorid12 are NaF and CaF2, each at a concentration of 5.6%. In 1 ml of suspension there are 60 mg NaF (of which fluoride accounts for 22.6 mg) and 60 mg CaF2 (equivalent to 29.2 mg fluoride). Bifluorid12 is available in bottles, with 10 g of the active ingredients to 10 ml solvent, and Pele TIM foam pills. Bifluorid12 must be shaken well before administration as the solid particles must be well dispersed. The presence of two metal balls in the bottle makes the process easier. The fluoride varnish was applied twice a year at six-monthly intervals, in the afternoon, by an experienced nurse in the schools' medical offices. The applications were performed following the manufacturer's recommendations, with an applicator (cartridges) and a blunt cannula after control of saliva. Children from group B received only oral health education four times per year.

After the baseline oral examination, further examinations were repeated for all subjects after one and two years, using the DMFT/DMFS criteria as at the baseline examination. The study design was blind. The examiner did not know which group was the test and which group was the control group. The group membership was known only by the nurse. For the control and test group, as well as for the subgroups, caries prevalence in the first permanent molars from all three examinations as well as the caries incidence from baseline to the first year and from baseline to the second year of observation were calculated. Statistics were based on the t-test for independent samples at a significance level of 5%.

Permission to conduct the study was obtained from the institutional ethical committee. Parents gave written informed consent.

Results

1. Group A (Bifluorid12)

One hundred and fifteen of the 119 children in the test group (97%) completed the baseline, first- and second-year examinations and received four F-varnish applications. At baseline, 112 subjects had no caries at their permanent molars (DMFT=0). Two subjects had one DMF tooth and one subject had two DMF teeth. Of the 112 with no caries at their permanent molars at baseline, 28 were classified in U1 (dmft=0), 38 in U2 (0<dmft<5) and 46 in U3 (dmft=5). The subjects with one DMF tooth were classified in subgroup U3 and the one with two DMF teeth in the U2 *(Table 1).*

1.1 Caries prevalence

At the baseline examination, the whole group had a mean caries prevalence of 0.03 DMFT/DMFS, which resulted exclusively from the DT/DS. In subgroup U1, the DMFT was zero, in U2 it was 0.05, and in U3 it was 0.04.

Table 1. *Distribution of DMF Teeth in Subjects Differentiated by Subgroup (U): Group A (Test Group), Baseline*

DMF teeth	U1	U2	U3	Total
	n (%)	n (%)	n (%)	n (%)
0	28 (100.0)	38 (97.4)	46 (95.8)	112 (97.4)
1		2 (4.2)	2 (1.7)	
2		1 (2.6)		1 (0.9)
Total	28 (100.0)	39 (100.0)	48 (100.0)	115 (100.0)

After one year, the DMFT increased to 0.11 (DT=0.02; FT=0.10; MT=0) and the DMFS to 0.12 (DS=0.02; FS=0.10; MS=0). In the U1 subgroup, the DMFT/DMFS remained at zero, whereas in the U2 subgroup it was 0.08 (DT/DS=0.03; FT/FS=0.05; MT/MS=0). In the U3 subgroup, the DMFT was 0.21 and the DMFS 0.23. The DT and DS values were identical (0.02), and were exceeded by the FT value (0.19) and FS value (0.21). In the U1 subgroup (28 subjects) and U2 subgroup (38 subjects), the DMFT was still zero. Only one child from the U2 subgroup with two DMF teeth at baseline now had three DMF teeth. However, in the U3 subgroup, there was a reduction of seven in the number of caries-free children. Of the nine children without caries (DMFT=0) at baseline, eight presented with one DMF tooth and one had two DMF teeth after one year of observation *(Table 2).*

After two years, the caries prevalence in the whole test group increased to 0.25 DMFT/DMFS, with each of 0.24 the values almost exclusively formed from the FT and FS values. The U3 subgroup showed a significant increase. The values of DMFT/DMFS were 0.48 (DT/DS=0.02; FT/FS= 0.46; MT/MS=0), whereas the children from the U1 subgroup had a DMFT/DMFS of 0.04 (DT/DS=0; FT/FS=0.04; MT/MS=0) and the children from the U2 subgroup had a DMFT/DMFS of 0.13 (DT/DS=0; FT/FS=0.13; MT/MS=0). In the U1 subgroup, only one subject presented with DMFT=1 and in the U2 subgroup, in addition to the child who originally presented with DMFT=3, two more children were found to have a DMFT=1. The largest increase was again found in the U3 subgroup. The number of children who had no caries on their first permanent molars at baseline had reduced by 12. Eight had DMFT=1, four had DMFT=2, one DMFT=3, and one DMFT=4 *(Table 3).*

1.2 Caries incidence

After one year, the mean caries incidence for the entire test group was 0.08 at DMFT level and 0.09 at DMFS level. Both resulted primarily from the FD/FS component. The U1 subgroup showed

Table 2. *Distribution of DMF Teeth in Subjects Differentiated by Subgroup (U): Group A, One-Year Results*

DMF teeth	U1	U2	U3	Total
	n (%)	n (%)	n (%)	n (%)
0	28 (100.0)	38 (97.4)	39 (81.3)	105 (91.3)
1			8 (16.7)	8 (7.0)
2			1 (2.1)	1 (0.9)
3		1 (2.6)		1 (0.9)
Total	28 (100.00)	39 (100.00)	48 (100.00)	115 (100.00)

Table 3. *Distribution of DMF Teeth in Subjects Differentiated by Subgroup (U): Group A, Two-Year Results*

DMF teeth	U1	U2	U3	Total
	n (%)	n (%)	n (%)	n (%)
0	27 (96.4)	36 (92.3)	34 (70.8)	97 (84.3)
1	1 (3.6)	2 (5.1)	8 (16.7)	11 (9.6)
2			4 (8.3)	4 (3.5)
3		1 (2.6)	1 (2.1)	2 (1.7)
4			1 (2.1)	1 (0.9)
Total	28 (100.0)	39 (100.0)	48 (100.0)	115 (100.0)

no increase; however, in the U2 subgroup, there was an increase of 0.03 DMFT/DMFS and in the U3 subgroup DMFT was 0.17 and DMFS was 0.19.

The increase of caries from baseline to the end of the second year was DMFT/DMF=0.04 for subgroup U1, DMFT/DMFS=0.08 for subgroup U2, and DMFT/DMFS=0.44 for subgroup U3. Thus subgroup U3 showed the greatest caries increase during the control period.

2. Group B (control group)

One hundred and eight of the 110 children (98%) completed baseline, one- and two-year examinations. At baseline, caries experience in primary molars resulted in15 children being allocated to subgroup U1, 49 to subgroup U2, and 44 to subgroup U3 *(Table 4).*

2.1 Caries prevalence

The DMFT/DMFS for the entire group at baseline was 0.04 (DT/DS=0; FT/FS=0.04; MT/MS=0). In the U1 subgroup, DMFT/DMFS was zero, in the U2 subgroup it was 0.02 (DT/DS=0; FT/FS=0.2, MT/MS=0), and in the U3 subgroup it was 0.07 (DT/DS=0; FT/FS=0.7, MT/MS=0).

After one year, only 91 children were free of caries, whereas the others already had a DMFT of between 1 and 3. In subgroup U1, there was only one child with DMFT>0, whereas in subgroup U2 there were six children, and in subgroup U3 there were 10. This represents an increase of five and eight subjects with caries experience in the U2 and U3 subgroups, respectively *(Table 5).* After one year, the DMFT in the U1 subgroup was 0.07 (DT=0.07, FT=0, MT=0), in the U2 subgroup it was 0.18 (DT=0.02, FT=0.16, MT=0), and in the U3 subgroup it was 0.34 (DT=0.09, FT=0.25, MT=0). The DMFS values were identical with the DMFT values *(Table 5).*

After two years, the DMFT in the U1 subgroup increased to 0.62 (DT=0.13; FT=0.49; MT=0) and the DMFS to 0.64 (DS=0.13, FS=0.51, MS=0). In the U1 subgroup, there was a DMFT/DMFS of 0.33 (DT/DS=0.07; FT/FS=0.27; MT/MS=0), in the U2 subgroup it was 0.57 (DT=0.12, FT=0.45; MT/MS=0) and in the U3 subgroup it was 0.77

Table 4. *Distribution of DMF Teeth in Subjects Differentiated by Subgroup (U): Group B, Baseline*

DMF teeth	U1	U2	U3	Total
	n (%)	n (%)	n (%)	n (%)
0	15 (100.0)	48 (98.0)	42 (95.5)	105 (97.2)
1		1 (2.0)	1 (2.3)	2 (1.9)
2			1 (2.3)	1 (0.9)
Total	115 (100.0)	49 (100.0)	44 (100.0)	108 (100.0)

Table 5. *Distribution of DMF Teeth in Subjects Differentiated by Subgroup (U): Group B, One-Year Results*

DMF teeth	U1	U2	U3	Total
	n (%)	n (%)	n (%)	n (%)
0	14 (93.3)	43 (87.8)	34 (77.3)	91 (84.3)
1	1 (6.7)	4 (8.2)	5 (11.4)	10 (9.3)
2		1 (2.0)	5 (11.4)	6 (5.6)
3		1 (2.0)		1 (0.9)
Total	15 (100.0)	49 (100.0)	44 (100.0)	108 (100.0)

Table 6. *Distribution of DMF Teeth in Subjects Differentiated by Subgroup (U): Group B, Two-Year Results*

DMF teeth	U1	U2	U3	Total
	n (%)	n (%)	n (%)	n (%)
0	12 (80.0)	31 (63.3)	26 (59.1)	69 (63.9)
1	1 (6.7)	9 (18.4)	7 (15.9)	17(15.7)
2	2 (13.3)	8 (16.3)	6 (13.6)	16 (14.8)
3		1 (2.0)	5 (11.4)	6 (5.6)
Total	15 (100.0)	49 (100.0)	44 (100.0)	108 (100.0)

(DT=0.16, FT=0.61; MT/MS=0). In the U1 subgroup, there were three subjects with DMFT>0, and in the U2 and U3 subgroups there were 18 children *(Table 6)*.

2.2 Caries incidence

In the first year, the DMFT incidence in the U1 subgroup was 0.07 (DT=0.07, FT=0, MT=0), in the U2 subgroup it was 0.16 (DT=0.02, FT=0, 14, MT=0), and in the U3 subgroup it was 0.27 (DT=0.09, FT=0.18, MT=0). Thus, the caries increase for the entire group had a DMFT value of 0.19.

The incidence of dental caries for the entire group after two years had a DMFT value of 0.58 (DT=0.13; FT=0.45, MT=0). For the U1 subgroup, an increase of 0.33 DMFT (DT=0.07, FT=0.27, MT=0) was observed, for the U2 subgroup it was 0.55 DMFT (DT=0.12; FT=0.43, MT=0), and for the U3 subgroup it was 0.7 (DT=0.16, FT=0.55, MT=0). Thus, after two years, there were significant differences ($P<0.05$) between the two groups and their subgroups, in favour of group A (the test group).

3. Caries-incidence reduction

Based on two years of clinically controlled surface-related caries incidence in the two groups, a caries-incidence reduction of 63.3% at the first permanent molars was found for children from group A (the test group) in comparison with those from group B (the control group). For the U1 subgroup, the caries-incidence reduction was 87.9%, for the U2 subgroup it was 86%, and for the U3 subgroup it was 39.7% *(Figure 1)*.

Discussion

The results of this study showed a different level of initial caries experience in primary molars in both groups. Therefore, it was possible to divide the children in both groups into three different subgroups, according to the caries level of their primary molars. At baseline, there were no significant differences in the DMFT/DMFS values between the two study groups and their subgroups. The baseline results characterise the variability of caries experience within a caries risk group. Such children (within a certain age cohort) from institutions with increased risk of caries by no means represent a homogenous group when it comes to caries experience. Furthermore, it may well be that the caries-free children in such groups are treated unnecessarily when they are involved in intensive caries-preventive programmes. This is not satisfactory, but should be tolerated for educational and psychological reasons, and no child of any group should be excluded from a prevention programme. Thus all children within a group should be involved in prevention programmes [12]. The caries-preventive effect of fluoride varnish Bifluorid12, in this study, was confirmed by measuring the caries-incidence reduction compared to the control group. The result of an over 60% reduction was better than the mean value from a Cochrane review of the preventive effect of fluoride varnishes in children and adolescents (Marinho et al. 2003) [13]. Perhaps this was unsurprising as the literature often quotes a range of results regarding the caries prevention effect of fluoride varnishes [14]. This was also confirmed in

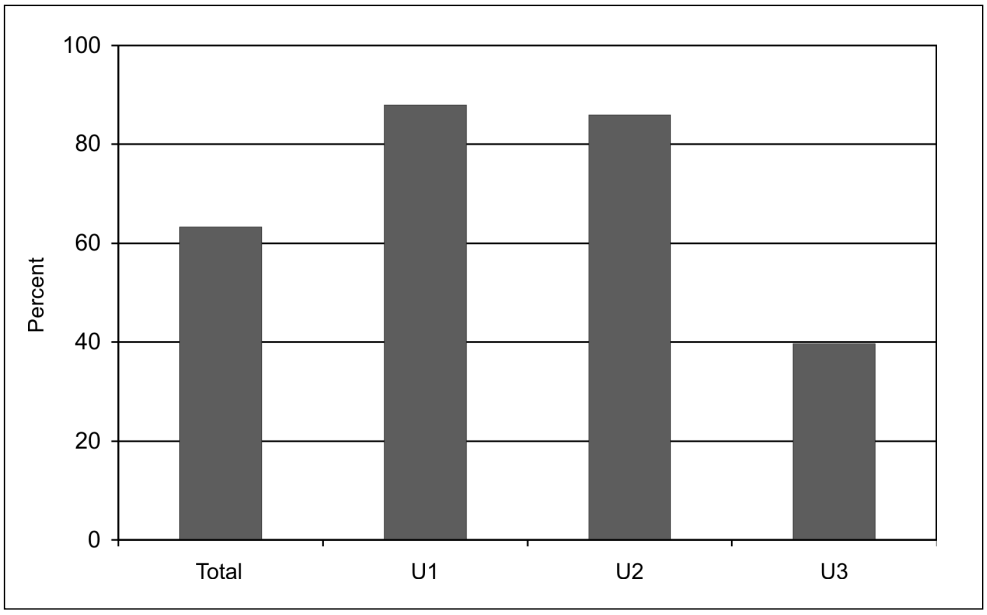

Figure 1. *Surface-related caries-incidence reduction on first molars by subgroup (U), after two years.*

the present study by the variations in results from the subgroups and between test and control groups. For example, in the two-year control period, in subgroup 1, caries levels were virtually unchanged. As the children in this subgroup had caries-free primary and permanent molars at baseline, they did not need the application of fluoride varnish to their teeth. Only one child from subgroup 1 developed caries in permanent molars after two years. Among the children who had a baseline dmft value of up to five in the primary molars (subgroup U2), the initial caries prevalence in the permanent molars remained the same after the first year. During the second year, only two children developed caries in their permanent molars. The weakest caries-preventive effect occurred in the U3 subgroup, in which after the first year a significant reduction in caries-free children was observed. After two years, 12 children in this group developed caries, with an increase in DMFT of from one to four. In all other subgroups, the majority of children had a DMFT increase of only one.

The original design of the study, dividing the children according to their caries risk by caries experience in their primary molars and assessing the different effect of fluoride varnish in the subgroups, showed that individual health behaviour as well as the effect of fluoride has to be taken into consideration. It can be assumed that children from subgroups 1 and 2 had better oral health behaviour than children from subgroup 3, insofar as the effect of fluoride can be enhanced by adequate oral health behaviour. The development of caries in the control group (B) showed significant differences compared to group A. The initial number of caries-free children from control group B during the two-year period was reduced by 33.3%, as opposed to only 13.1% in the test (fluoride) group. The results from the various subgroups of group B showed similar differences to the test group, although the differences were strongly in favour of the test group. Thus the findings confirm earlier statements [15], that a high level of caries in the primary teeth is an indicator of an increased risk of caries in the permanent dentition. Furthermore, as has previously been found [16], the results showed that the caries-prevention effect of the fluoride varnish in children with low caries risk (subgroups U1, U2) was more pronounced than in children with increased caries risk (subgroup U3). In the literature, it is noted that fluoride varnishes are especially indicated for children with high caries risk and the effect depends on the frequency of application. Intervals of three to six months are recommended and are considered to be toxicologically safe [17].

It has not been clearly demonstrated to what extent the combination of NaF and CaF2 and its higher fluoride concentration in Bifluorid12 is able to build a larger fluoride reservoir of CaF2 on the enamel surface, which is important for the control of de-and remineralisation and for efficient caries-preventive effect [18]. However, in vivo studies have demonstrated larger fluoride deposits and a deeper penetration of fluoride in the enamel when samples of bovine enamel with initial caries lesions were exposed to Bifluorid12 [18,19]. Higher fluoride concentrations and prolonged exposure time lead to an increasing size of the calcium fluoride globules and have a positive influence on the formation of a CaF2-like layer on the enamel surface [20]. Thus, in this study, it may be that high caries inhibition may be related to the combination of fluoride compounds in Bifluorid12.

Conclusion

It can be concluded that Bifluorid12 showed a significant caries-incidence reduction on permanent molars among school children. The F-varnish can be recommended for preventive programmes. In this study, its effectiveness depended on caries experience in primary molars. The caries-preventive effect of Bifluorid12 was different for the subgroups with regard to previous caries experience in primary molars. Children without caries experience in primary molars showed the highest caries-incidence reduction in their permanent molars.

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References

1. Micheelis W, Schiffner U. *Vierte Deutsche Mundgesundheitsstudie (DMS IV)* [Fourth German Oral Health Study] [German]. Cologne, Germany: Deutscher Zahnärzte Verlag, 2006.

2. Pieper K. *Epidemiologische Begleituntersuchungen zur Gruppenprophylaxe 2004* [Accompanying epidemiological survey of group prevention programmes of 2004] [German]. Bonn: Deutsche Arbeitsgemeinschaft für Jugendzahnpfleg [German Academy of Paediatric Dentistry], 2005.

3. Kuzmina E, Kuzmina I, Smirnova T. Oral health and related risk factors among children and adolescents in Russia [abstract]. 25th anniversary symposium of WHO Collaborating

Centre Prevention of Oral Diseases: “Inequalities in Oral Health - Challenge to Public Health”. 2008 May 23-24; University of Jena, Germany.

4. Kuzmina E, Smirnova T, Vasina S, Shanduntz A, Zimina V. Dental caries prevalence in 12-year-old children living in various districts of Moscow region [abstract no. 110]. *Caries Research* 1994; **28:** 211-212.

5. Melnitschenko EM, Melnitschenkowa EI. Oral epidemiological situation among children in cities of Belorussia. 1st announcement: Changes in caries prevalence under condition of a preventive programme. *Public Health* 2000; **5**: 21-24.

6. Borutta A, Kneist S. Oral health of preschool children: a challenge for intensive prevention. *Quintessence* 2006; **57:** 1189-1197.

7. Gülzow HJ, Hellwig E, Hetzer G. *Fluoridation Measures*. Guideline Update. Cologne, Germany: ZZQ Agency for Quality in Dentistry. 2005 July 25. Available via [“E-Schwerpunkte”/”Clinical Guidelines”]: http:www3kzbv.de/zzqpubl.nsf

8. Richardson B. Fixation of topically applied fluoride in enamel. *Journal of Dental Research* 1967; **46**: 87-91.

9. Schmidt HF. Ein neues Tauchierungsmittel mit besonders lang anhaltendem intensivem Fluoridierungseffekt [A new application agent with especial long lasting effect of fluoridation] [German]. *Stoma* 1964; **17**: 14-20.

10. World Health Organization (WHO), Fluoride varnish for community-based caries prevention in children. Geneva: WHO, 1997.

11. Bratthall D. Introducting the Significant Caries Index together with a proposal for a new global oral health goal for 12-year-olds. *International Dental Journal* 2000; **50**: 378-384.

12. Menghini G, Steiner M. Oral health in Switzerland [working document 26]. Neuchâtel, Switzerland: Swiss Health Observatory, 2007 Aug.

13. Marinho VC, Higgins JP, Logan S, Sheiham A. Fluoride varnishes for preventing dental caries in children and adolescents. *Cochrane Database of Systematic Reviews* 2003; **(3):** CD002284.

14. Petersen LG, Magnusson K, Andersson H, Almquist B, Twetman S. Effect of quarterly treatments with a chlorhexidine and a fluoride varnish on approximal caries in caries-susceptible teenagers: A 3-year-clinical study. *Caries Research* 2004; **34**: 140-143.

15. Seppä L, Hausen H. Identification of caries risk patients. *Oralprophylaxe* 1988; **10**: 96-107.

16. Bruun C, Bille J, Hansen KT, Kann J, Quist V, Thylstrup A. Three-year caries increments after fluoride rinses or topical application with a fluoride varnish. *Community Dental Oral Epidemiology* 1985 ;**13**: 299-303.

17. Petersson LG. Fluoride mouthrinses and fluoride varnishes. *Caries Research* 1993; **27:** 35-42.

18. Attin T, Hartmann O, Hilgers RD, Hellwig E. Fluoride retention of incipient enamel lesions after treatment with a calcium Fluoride varnish *in vivo*. *Archives of Oral Biology* 1995; **40**: 169-174.

19. Attin T, Schneider K, Buchalla W: Stability of the KOH-soluble fluoride fraction formed on enamel after application of various fluoridation regimes. *Deutsche Zahnärtliche Zeitung* 2001;**12**: 706-711.

20. Lindemann G. Adsorption of selected organic compounds onto calcium fluoride. *Scandinavian Journal of Dental Research* 1985; **93**: 381.