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Tooth-Surface-Specific Effects of MI Varnish™: A 3-Year Randomized Clinical Trial

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Abstract: Aim: Our previous three-year randomized control trial showed that the application of MI Varnish™ (5% NaF/CPP-ACP) every 3 months reduced further caries development in 6- and 12-year-olds over a 3-year period. The purpose of this secondary analysis was to investigate whether MI Varnish™ had a differential effect on cumulative caries increment on different tooth surfaces. Methods: Group 1 (n = 48) (6-year-old children) and Group 3 (n = 47) (12-year-old children) received quarterly varnish applications, while Group 2 (n = 48) (6-year-old children) and Group 4 (n = 37) (12-year-old children) did not receive varnish applications. ICDAS caries scoring classified lesions as non-cavitated (n/c) lesions (ICDAS 1 and 2), cavitated (c) lesions (ICDAS II 3–6), non-cavitated lesions around restorations (CARn/c), and cavitated lesions around restorations (CARc). Thus, 'decayed' in DFS was calculated as (ICDAS 1–6 + CARn/c + CARc). The Chi-square test, Welch test (paired-t test), risk ratio test, and Pearson correlation coefficient were used for statistical analysis (α = 0.05). Results: After comparing baseline and 36-month data, in group 1, there was a significant ($p < 0.01$) reduction in caries in occlusal (23.11%) and proximal (21.35%) surfaces and a non-significant reduction in buccal/lingual surfaces (5.28%). In group 2, caries reduction was significant ($p < 0.01$) in occlusal surfaces (38.52%) but non-significant in proximal (7.78%) and buccal/lingual (7.12%) surfaces. In groups 3 and 4, significant ($p < 0.001$) increases in caries were observed in proximal (36.03% (group 3)/54.30% (group 4)) and buccal/lingual surfaces (51.02% (group 3)/45.98% (group 4)), and a non-significant increase was observed in occlusal surfaces (11.49% (group 3)/22.01% (group 4)). The relative risk had increased by 4% only on proximal surfaces in 6-year-olds. Conclusions: the application of MI Varnish™ every 3 months demonstrated a caries reduction effect on interproximal and occlusal surfaces among 6- and 12-year-old children. (Trial registration ISRCTN10584414).

Keywords: caries increment; MI Varnish™; hygiene habits; tooth surface effect

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1. Introduction

Dental caries is the most prevalent type of oral disease worldwide, and it causes the destruction of the hard tissue of a tooth via the interaction of bacteria and fermentable carbohydrates [1,2]. Nowadays, the dental caries rate is increasing, and it has become a major public health challenge worldwide, affecting the majority of children and adult populations, leading to pain and discomfort [2]. Unfortunately, a similar situation exists in Riga, the capital of Latvia [3–6]. Notwithstanding the leading role of fluoride as a preventive measure for dental caries, there is neither water fluoridation nor any preventive programs in Riga, but at the same time, there is a high consumption of carbohydrates among children, leading to an increased caries prevalence in Latvia [3–6]. At the same time, the consumption of carbonated soft drinks and sweetened snacks, as well as the prevalence of habits such as drinking four medium-sized cups of tea with sugar each day, remains high in Latvia [3–6].

Fluoride varnishes, with their high fluoride concentration, are known to be a more effective means of decreasing further caries development due to their long contact periods with tooth surfaces, resulting in high fluoride uptake and the formation of CaF_2 deposits that act as fluoride reservoirs on tooth surfaces [7,8]. The addition of Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) has been shown to provide an additional anti-cariogenic effect through the formation of the CPP-stabilized amorphous calcium fluoride phosphate (CPP-ACFP) phase [8].

Thus, many believe that CPP-ACFP (in varnish form), can exhibit a greater preventive effect against caries compared to standard fluoride toothpaste. This was investigated in our previous 3-year randomized controlled trial, which showed that the application of MI Varnish™ every 3 months could reduce the risk of further caries development in both of the age groups studied in Riga, Latvia [6]. In another study, the caries increment varied between the tooth surfaces, with the highest caries rate being on the proximal surfaces of the teeth in similar age groups in Riga [4]. Therefore, it is conceivable that MI Varnish™ may have different caries-preventive effects on various tooth surfaces.

The aim of this secondary analysis was to investigate the caries-preventive effects of MI Varnish™ among the following coronal tooth surfaces: smooth (buccal and lingual), occlusal, and proximal surfaces.

2. Materials and Methods

A complete and detailed description of this clinical trial according to the CONSORT checklist has been presented in our previous publication [6]. This secondary analysis contains additional data gained in the same clinical trial.

2.1. Interventions

Prior to examination, all proximal surfaces were cleaned using non-fluoridated dental floss. Each examined surface was placed under one of the following classifications to enable the calculation of DMFS and DFS: sound, non-cavitated (n/c) lesions (ICDAS II 1 and 2), cavitated (c) lesions (ICDAS II 3–6), filled surfaces (F), non-cavitated lesions around restorations (CARn/c), and cavitated lesions around restorations (CARc) (https://www.iccms-web.com) (1 September 2015) [9]. No additional magnification was used in examining the tooth surfaces.

All coronal surfaces were categorized into proximal, occlusal, and smooth (buccal/lingual) surfaces. For statistical purposes, the buccal/lingual surfaces were considered one surface, all unerupted surfaces at baseline were not included in further statistical analysis, and the ICDAS-II data were transformed into decayed (ICDAS II 1–6 + CARn/c + CARc) and, as such, decayed + filled (ICDAS II 1–6 + CARn/c + CARc + F). It is important to note that the majority of the data concerning caries status were collected on mixed dentition in 6-year-olds and on permanent teeth- in 12-year-olds. It is necessary to emphasize that unerupted surfaces of teeth at baseline were not included in further statistical analysis.

The official dental patient chart was used to place collected data regarding caries and oral hygiene habits. This further statistical analysis did not include the results of bitewing radiographs.

After examination, the groups undergoing treatment (Groups 1 and 3) received an application of MI Varnish™ (GC Corp., Tokyo, Japan) containing 5% NaF and CPP-ACP, while the control groups (Groups 2 and 4) did not receive varnish treatment. The application and three-month re-application were completely described in our previous publication [6]. The application of the MI Varnish™ was carried out in accordance with the manufacturer's instructions. The manufacturer's post-varnish instructions were given to all children and their parents.

2.2. Assessment of Oral Hygiene

A questionnaire with a variety of open-ended and close-ended questions was used to obtain information on oral hygiene habits and was administered to all participants at baseline and at the 36-month visits.

Children and/or their parents were questioned about toothbrushing habits; the name of the toothpaste they used; the Fluoride content of the toothpaste they used; the use of F-containing tablets; and the use of dental floss, type of dental floss (with or without Fluoride), and frequency of use.

The method through which the questionnaire was administered and filled out as well as the parental involvement in answering questions were identical to the questionnaire on dietary habits in our previous publication [6]. The participants were generally advised to use toothpaste with a fluoride concentration of 1450 ppm, which was provided at baseline and at the 36-month period as a final step after the examination had been completed [6].

The daily use of non-fluoridated dental floss was recommended, preferably in the evening. No particular brand of dental floss was recommended. The use of fluoride-containing tablets was prohibited for all children during the study. The questionnaire included information on whether they had used any tablets before they began partaking in the examination (at baseline) and if they have continued using them. The aim was to collect information only.

2.3. Statistical Methods

The same software package used for our previous publication [6] was used in this study's analysis, where *p*-values less than 0.05 were considered statistically significant [6]. Primary outcomes were analyzed by following tests. A t-test (Welch test) was used to calculate and compare the mean values of various affected surfaces at baseline and at 36 months in all groups. Oral hygiene habits were analyzed using the Chi-square test at baseline and at 36 months. Also, the risk ratio test was performed over a period of 36 months. For secondary analysis, correlation analysis using the Pearson correlation coefficient ($\alpha = 0.05$) was used to detect statistically significant differences between different tooth surfaces at baseline and at the end of the 3-year period. The registration number of the clinical trial was ISRCTN10584414 (https://www.isrctn.com/ISRCTN10584414 (7 March 2019)).

3. Results

The flow diagram, number of analyzed participants, dropout rate, and demographical changes were completely described and represented in our previous publication [6].

3.1. Primary Outcomes

3.1.1. Caries Increment

The results of ICDAS-II data obtained via the calculation of mean values with a 95% Confidence Interval (95% CI) are presented in Tables 1 and 2 for the 6- and 12-year-olds, respectively. Also, decreases or increases in caries increment are shown in Figure 1 for the 6-year-olds, and Figure 2 demonstrates the changes in caries increment among the 12-year-olds.

Table 1. Mean values (SD) of ICDAS II data in interproximal, occlusal, and buccal/lingual surfaces at baseline and at 36 months among 6-year-old children (Group 1 and Group 2).

	Group 1 (n = 48) (MI Varnish) 6 Year Olds			**Group 2 (n = 48) (Control) 6 Year Olds**		
	At Baseline (%)	**At 36 Months (%)**	***p* Values**	**At Baseline (%)**	**At 36 Months (%)**	***p* Values**
Interproximal ICDAS 1 and 2	15.38 (5.49) 95% CI [13.8, 16.9]	14.02 (5.67) 95% CI [12.4, 15.6]	Decrease 0.245	15.73 (5.18) 95% CI [14.3, 17.2]	16.25 (6.60) 95% CI [14.4, 18.1]	Increase 0.614
Interproximal ICDAS 3–6	3.71 (4.14) 95% CI [2.54, 4.88]	1.35(1.64) 95% CI [0.886, 1.81]	Decrease <0.001	3.29(4.45) 95% CI [2.03, 4.55]	1.46 (1.68) 95% CI [0.985, 1.94]	Decrease 0.003
Interproximal F	2.65 (2.65) 95% CI [1.9, 3.4]	2.35 (2.32) 95% CI [1.69, 3.01]	Decrease 0.495	1.65 (2.23) 95% CI [1.02, 2.28]	1.67(2.05) 95% CI [1.09, 2.25]	Increase 0.954
Interproximal CAR n/c	0.06 (0.25) 95% CI [−0.0107, 0.131]	0 (0)	Decrease 0.083	0 (0)	0.02 (0.14) 95% CI [−0.0196, 0.0596]	Increase 0.322
Interproximal CAR/c	0.44(0.77) 95% CI [0.222, 0.658]	0.02 (0.14) 95% CI [−0.0196, 0.0596]	Decrease <0.001	0.27 (0.82) 95% CI [0.038, 0.502]	0.06 (0.25) 95% CI [−0.0107, 0.131]	Decrease 0.096
Interproximal decayed (ICDAS 1–6+ CARn/c + CARc)	19.58 (6.89) 95% CI [17.6, 21.5]	15.40 (5.86) 95% CI [13.7, 17.1]	Decrease 0.002	19.29 (6.36) 95% CI [17.5, 21.1]	17.79 (6.82) 95% CI [15.9, 19.7]	Decrease 0.248
Interproximal decayed and filled (ICDAS 1–6)	22.23 (6.91) 95% CI [20.3, 24.2]	17.75 (6.46) 95% CI [15.9, 19.6]	Decrease 0.002	20.94 (6.30) 95% CI [19.2, 22.7]	19.46 (6.94) 95% CI [17.5, 21.4]	Decrease 0.291
Occlusal ICDAS 1 and 2	0.96 (1.34) 95% CI [0.581, 1.34]	1.31 (1.45) 95% CI [0.9, 1.72]	Increase 0.061	1.13 (1.28) 95% CI [0.768, 1.49]	1.02 (1.25) 95% CI [0.666, 1.37]	Decrease 0.646

Table 1. *Cont.*

	Group 1 (*n* = 48) (MI Varnish) 6 Year Olds			**Group 2 (*n* = 48) (Control) 6 Year Olds**		
	At Baseline (%)	**At 36 Months (%)**	***p* Values**	**At Baseline (%)**	**At 36 Months (%)**	***p* Values**
Occlusal ICDAS 3–6	0.98 (1.38) 95% CI [0.59, 1.37]	0.46 (0.77) 95% CI [0.242, 0.678]	Decrease 0.010	0.81 (1.51) 95% CI [0.383, 1.24]	0.35 (0.57) 95% CI [0.189, 0.511]	Decrease 0.049
F	3.33 (2.41) 95% CI [2.65, 4.01]	3.25 (2.14) 95% CI [2.65, 3.85]	Decrease 0.820	2.45 (2.20) 95% CI [1.83, 3.07]	3.02 (2.56) 95% CI [2.3, 3.74]	Increase 0.121
CAR n/c	0.02 (0.14) 95% CI [−0.0196, 0.0596]	0 (0)	Decrease 0.322	0.08 (0.28) 95% CI [0.0008, 0.159]	0.02 (0.14) 95% CI [−0.0196, 0.0596]	Decrease 0.083
CAR/c	0.42 (0.65) 95% CI [0.236, 0.604]	0.06 (0.25) 95% CI [−0.0107, 0.131]	Decrease 0.001	0.42 (0.96) 95% CI [0.148, 0.692]	0.10 (0.37) 95% CI [−0.005, 0.205]	Decrease 0.046
Occlusal decayed (ICDAS 1–6+ CARn/c + CARc)	2.38 (1.78) 95% CI [1.88, 2.88]	1.83 (1.74) 95% CI [1.34, 2.32]	Decrease 0.019	2.44 (2.03) 95% CI [1.87, 3.01]	1.50 (1.61) 95% CI [1.04, 1.96]	Decrease 0.012
Occlusal decayed and filled (ICDAS 1–6+)	5.71 (2.43) 95% CI [5.02, 6.4]	5.08 (2.37) 95% CI [4.41, 5.75]	Decrease 0.103	4.88 (2.66) 95% CI [4.13, 5.63]	4.52 (2.99) 95% CI [3.67, 5.37]	Decrease 0.407
Buccal/Lingual ICDAS 1 and 2	11.27 (6.25) 95% CI [9.5, 13]	12.75 (5.35) 95% CI [11.2, 14.3]	Increase 0.126	9.19 (5.64) 95% CI [7.59, 10.8]	9.77 (4.97) 95% CI [8.36, 11.2]	Increase 0.487
Buccal/Lingual ICDAS 3–6	1.96 (3.07) 95% CI [1.09, 2.83]	1.33 (1.80) 95% CI [0.821, 1.84]	Decrease 0.037	1.71 (2.72) 95% CI [0.941, 2.48]	0.42 (0.92) 95% CI [0.16, 0.68]	Decrease <0.001
F	1.17 (1.79) 95% CI [0.664, 1.68]	0.77 (1.29) 95% CI [0.405, 1.14]	Decrease 0.097	0.35 (1.21) 95% CI [0.008, 0.692]	0.4 (0.89) 95% CI [0.148, 0.652]	Increase 0.808

Table 1. *Cont.*

	Group 1 (n = 48) (MI Varnish) 6 Year Olds			**Group 2 (n = 48) (Control) 6 Year Olds**		
	At Baseline (%)	**At 36 Months (%)**	**p Values**	**At Baseline (%)**	**At 36 Months (%)**	**p Values**
CAR n/c	0.02 (0.14) 95% CI [−0.0196, 0.0596]	0.04 (0.20) 95% CI [−0.0166, 0.0966]	Increase 0.569	0.13 (0.39) 95% CI [0.02, 0.24]	0.10 (0.37) 95% CI [−0.005, 0.205]	Decrease 0.743
CAR/c	0.19 (0.53) 95% CI [0.04, 0.34]	0.02 (0.14) 95% CI [−0.0196, 0.0596]	Decrease 0.031	0.08 (0.28) 95% CI [0.0008, 0.159]	0.02 (0.14) 95% CI [−0.0196, 0.0596]	Decrease 0.182
Buccal/Lingual decayed (ICDAS 1–6+ CARn/c + CARc)	13.44 (7.71) 95% CI [11.3, 15.6]	14.15 (5.97) 95% CI [12.5, 15.8]	Increase 0.458	11.10 (7.14) 95% CI [9.08, 13.1]	10.31 (5.42) 95% CI [8.78, 11.8]	Decrease 0.410
Buccal/Lingual decayed and filled (ICDAS 1–6)	14.60 (8.50) 95% CI [12.2, 17]	14.92 (6.18) 95% CI [13.2, 16.7]	Increase 0.772	11.46 (7.43) 95% CI [9.36, 13.6]	10.71 (5.57) 95% CI [9.13, 12.3]	Decrease 0.464
Interproximal total	39.33 (3.77) 95% CI [38.3, 40.4]	41.71 (4.81) 95% CI [40.4, 43.1]	Increase 0.009	37.79 (5.26) 95% CI [36.3, 39.3]	43.17 (4.79) 95% CI [41.8, 44.5]	Increase <0.001
Occlusal total	10.27 (1.71) 95% CI [9.79, 10.8]	10.21 (1.66) 95% CI [9.74, 10.7]	Decrease 0.858	9.52 (1.83) 95% CI [9, 10]	10.71 (1.64) 95% CI [10.2, 11.2]	Increase 0.002
Buccal/Lingual total	39.33 (3.77) 95% CI [38.3, 40.4]	41.71 (4.81) 95% CI [40.4, 43.1]	Increase 0.009	37.79(5.26) 95% CI [36.3, 39.3]	43.17 (4.79) 95% CI [41.8, 44.5]	Increase <0.001

Table 2. Mean values (SD) of ICDAS II data in interproximal, occlusal, and buccal/lingual surfaces at baseline and 36 months among 12-year-old children (Group 3 and Group 4).

	Group 3 (*n* = 47) (MI Varnish) 12 Year Olds			**Group 4 (*n* = 37) (Control) 12 Year Olds**		
	At Baseline (%)	**At 36 Months (%)**	***p* Values**	**At Baseline (%)**	**At 36 Months (%)**	***p* Values**
Interproximal ICDAS 1 and 2	23.81 (8.50) 95% CI [21.4, 26.2]	32.40 (8.34) 95% CI [30, 34.8]	Increase <0.001	19.05 (9.62) 95% CI [16, 22.2]	29.51 (6.32) 95% CI [27.5, 31.6]	Increase <0.001
Interproximal ICDAS 3–6	1.40 (2.75) 95% CI [0.614, 2.19]	1.02 (1.92) 95% CI [0.471, 1.57]	Decrease 0.371	0.68 (1.18) 95% CI [0.3, 1.06]	0.98 (1.85) 95% CI [0.384, 1.58]	Increase 0.488
F	2.06 (3.44) 95% CI [1.08, 3.04]	3.92 (7.37) 95% CI [1.81, 6.03]	Increase 0.006	0.92 (1.38) 95% CI [0.475, 1.36]	1.35 (1.86) 95% CI [0.751, 1.95]	Increase 0.069
CAR n/c	0.02 (0.15) 95% CI [−0.0229, 0.0629]	0 (0)	Decrease 0.323	0 (0)	0 (0)	NaN
CAR/c	0.12 (0.38) 95% CI [0.011, 0.229]	0.04 (0.20) 95% CI [−0.0172, 0.0972]	Decrease 0.323	0.03 (0.16) 95% CI [−0.0216, 0.0816]	0.05 (0.23) 95% CI [−0.0241, 0.124]	Increase 0.571
Interproximal decayed (ICDAS 1–6+ CARn/c + CARc)	25.34 (8.66) 95% CI [22.9, 27.8]	33.47 (8.76) 95% CI [31, 36]	Increase <0.001	19.76 (9.78) 95% CI [16.6, 22.9]	30.49 (7.08) 95% CI [28.2, 32.8]	Increase <0.001
Interproximal decayed and filled (ICDAS 1–6)	27.40(10.43) 95%CI [24.4, 30.4]	37.38 (10.22) 95% CI [34.5, 40.3]	Increase <0.001	20.68 (10.12) 95% CI [17.4, 23.9]	31.84 (7.38) 95% CI [29.5, 34.2]	Increase <0.001
Occlusal ICDAS 1 and 2	2.34 (1.72) 95%CI [1.85, 2.83]	3.09 (2.16) 95% CI [2.47, 3.71]	Increase 0.008	1.78 (1.65) 95% CI [1.25, 2.31]	2.62 (1.96) 95% CI [1.99, 3.25]	Increase 0.016
Occlusal ICDAS 3–6	1 (1.34) 95%CI [0.617, 1.38]	0.72 (0.99) 95% CI [0.437, 1]	Decrease 0.102	0.76 (1.38) 95% CI [0.315, 1.21]	0.65 (1.23) 95% CI [0.254, 1.05]	Decrease 0.512

Table 2. *Cont.*

	Group 3 (*n* = 47) (MI Varnish) 12 Year Olds			**Group 4 (*n* = 37) (Control) 12 Year Olds**		
	At Baseline (%)	**At 36 Months (%)**	***p* Values**	**At Baseline (%)**	**At 36 Months (%)**	***p* Values**
F	2.66 (3.15) 95%CI [1.76, 3.56]	3.85(4.03) 95% CI [2.7, 5]	Increase <0.001	2.14(1.99) 95% CI [1.5, 2.78]	3.14(2.52) 95% CI [2.33, 3.95]	Increase 0.001
CAR n/c	0 (0)	0.02 (0.15) 95% CI [−0.0229, 0.0629]	Increase 0.323	0.08 (0.28) 95% CI [−0.0102, 0.17]	0 (0)	Decrease 0.083
CAR/c	0.17 (0.52) 95% CI [0.021, 0.319]	0.09(0.35) 95% CI [−0.01, 0.19]	Decrease 0.351	0.05(0.23) 95% CI [−0.0241, 0.124]	0 (0)	Decrease 0.160
Occlusal decayed (ICDAS 1–6+ CARn/c + CARc)	3.51 (2.09) 95% CI [2.91, 4.11]	3.91 (2.59) 95% CI [3.17, 4.65]	Increase 0.211	2.68 (2.45) 95% CI [1.89, 3.47]	3.27 (2.35) 95% CI [2.51, 4.03]	Increase 0.131
Occlusal decayed and filled (ICDAS 1–6)	6.17 (3.81) 95% CI [5.08, 7.26]	7.77 (4.28) 95% CI [6.55, 8.99]	Increase <0.001	4.81 (3.15) 95% CI [3.8, 5.82]	6.41 (3.51) 95% CI [5.28, 7.54]	Increase <0.001
Buccal/Lingual ICDAS 1 and 2	12.36 (7.19) 95% CI [10.3, 14.4]	19.53 (8.29) 95% CI [17.2, 21.9]	Increase <0.001	9.89 (6.15) 95% CI [7.91, 11.9]	14.68(6.33) 95% CI [12.6, 16.7]	Increase <0.001
Buccal/Lingual ICDAS 3–6	1.21 (1.92) 95% CI [0.661, 1.76]	1.02 (1.23) 95% CI [0.668, 1.37]	Decrease 0.386	0.49 (0.84) 95% CI [0.219, 0.761]	0.62 (1.66) 95% CI [0.085, 1.16]	Increase 0.549
F	1.51 (2.01) 95% CI [0.935, 2.08]	2.62 (3.72) 95% CI [1.56, 3.68]	Increase 0.003	0.62 (1.01) 95% CI [0.295, 0.945]	1 (1.35) 95% CI [0.565, 1.44]	Increase 0.029
CAR n/c	0.55(1.21) 95% CI [0.204, 0.896]	0.79(1.44) 95% CI [0.378, 1.2]	Increase 0.109	0.24(0.55) 95% CI [0.063, 0.417]	0.32(0.71) 95% CI [0.091, 0.549]	Increase 0.262

Table 2. *Cont.*

	Group 3 (*n* = 47) (MI Varnish) 12 Year Olds			**Group 4 (*n* = 37) (Control) 12 Year Olds**		
	At Baseline (%)	**At 36 Months (%)**	***p* Values**	**At Baseline (%)**	**At 36 Months (%)**	***p* Values**
CAR/c	0.06 (0.25) 95% CI [−0.0115, 0.132]	0.09 (0.41) 95% CI [−0.027, 0.207]	Increase 0.710	0.08 (0.49) 95% CI [−0.078, 0.238]	0 (0)	Decrease 0.324
Buccal/Lingual decayed (ICDAS 1–6+ CARn/c + CARc)	14.19 (8.27) 95% CI [11.8, 16.6]	21.43 (8.74) 95% CI [18.9, 23.9]	Increase <0.001	10.70 (6.47) 95% CI [8.62, 12.8]	15.62 (6.94) 95% CI [13.4, 17.9]	Increase <0.001
Buccal/Lingual decayed and filled (ICDAS 1–6)	15.70 (9.04) 95% CI [13.1, 18.3]	24.04 (10.72) 95% CI [21, 27.1]	Increase <0.001	11.32 (6.49) 95% CI [9.23, 13.4]	16.62 (7.34) 95% CI [14.3, 19]	Increase <0.001
Interproximal total	51.32 (5.23) 95% CI [49.8, 52.8]	54.72 (3.13) 95% CI [53.8, 55.6]	Increase <0.001	50.0 (5.23) 95% CI [48.3, 51.7]	55.35 (1.57) 95% CI [54.8, 55.9]	Increase <0.001
Occlusal total	14.0 (2.18) 95% CI [13.4, 14.6]	17.6 (14.74) 95% CI [13.4, 21.8]	Increase 0.099	13.51 (2.18) 95% CI [12.8, 14.2]	15.76 (0.72) 95% CI [15.5, 16]	Increase <0.001
Buccal/Lingual total	51.32 (5.23) 95% CI [49.8, 52.8]	54.72(3.13) 95% CI [53.8, 55.6]	Increase <0.001	50.0 (5.23) 95% CI [48.3, 51.7]	55.35 (1.57) 95% CI [54.8, 55.9]	Increase <0.001

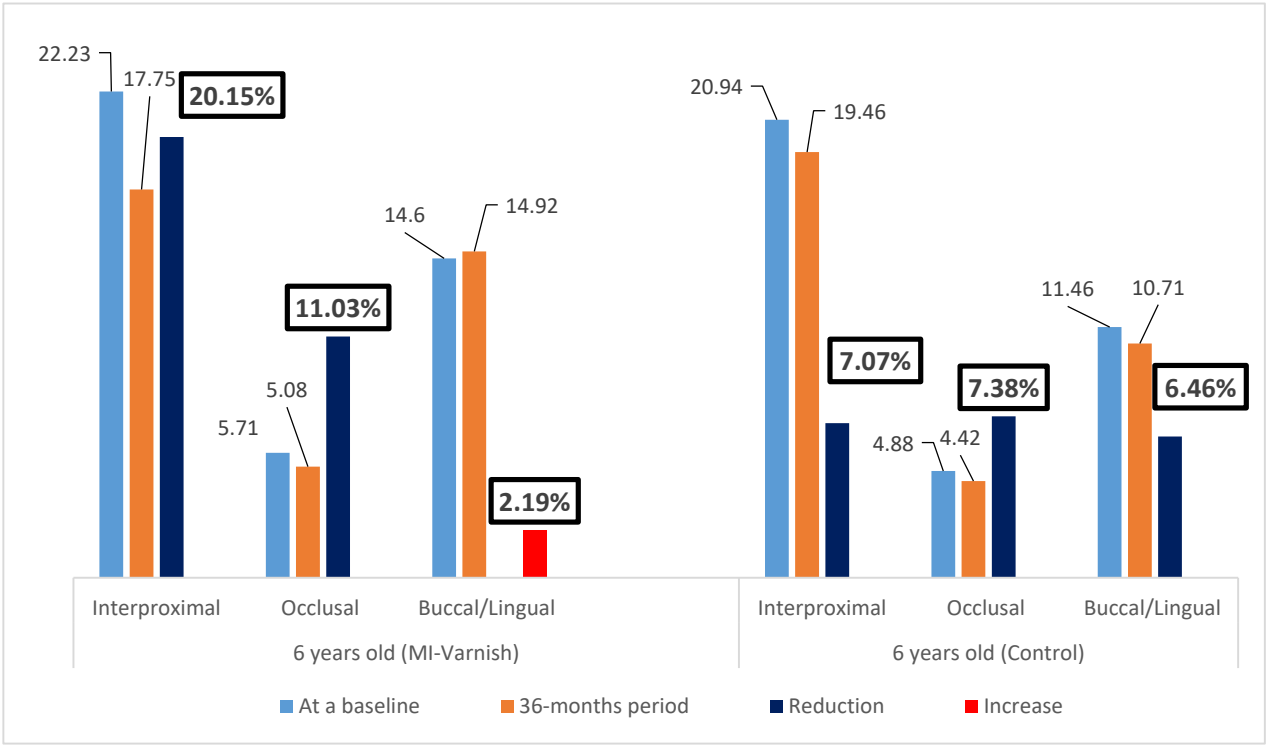

Figure 1. Changes in decayed and filled surfaces in 6-year-old children over a period of 36 months.

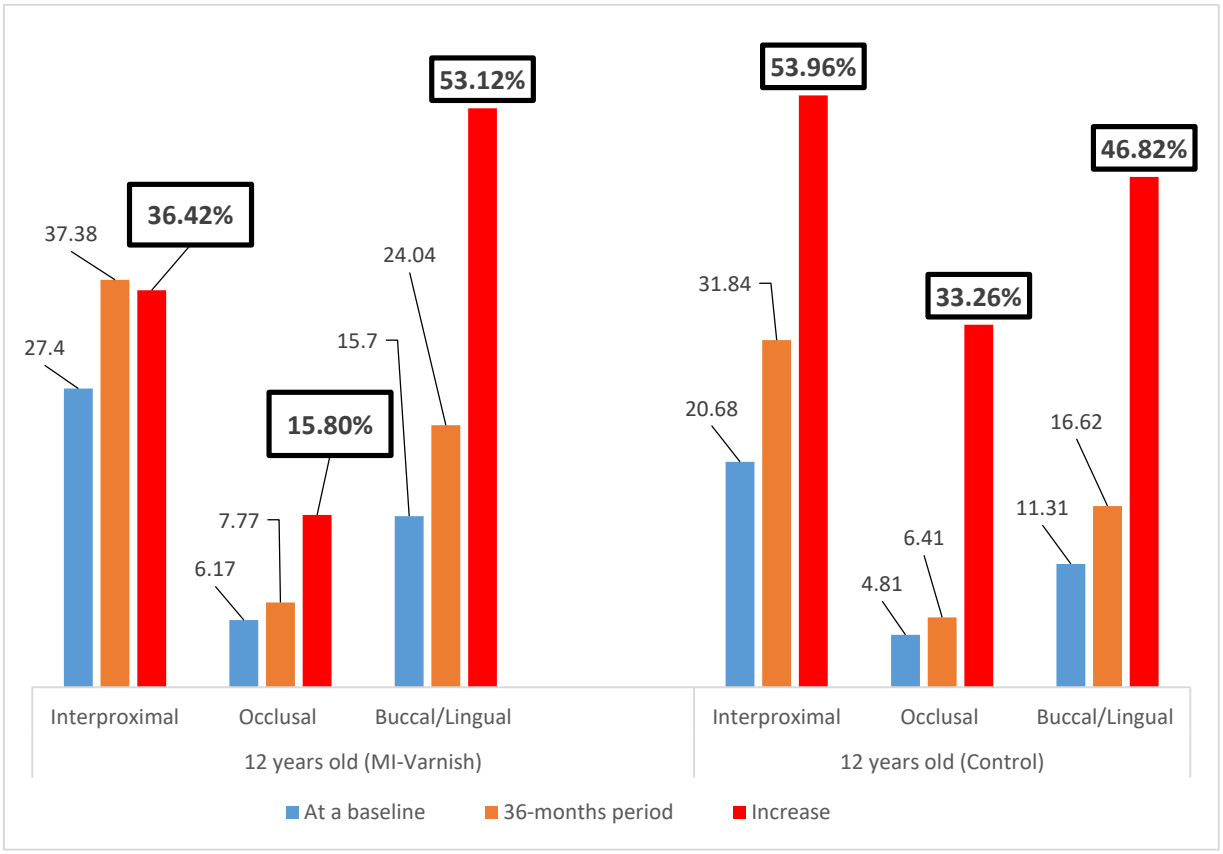

Figure 2. Changes in decayed and filled surfaces in 12-year-old children over a period of 36 months.

The six-year-olds in the control group (Group 2) demonstrated a higher percentage of caries reduction in occlusal surfaces than those of the same age group in Group 1 (a group that received varnish treatment) (Table 1). Similarly, the number of 'Filled' occlusal surfaces in the control group (Group 2) increased within the same period (Table 1). Thus, the higher percentage of caries reduction in occlusal surfaces could be attributed to the increased number of restored occlusal surfaces in Group 2 (Table 1) within the 36-month study period. Similarly, a higher increase in percentage in 'Decayed' buccal/lingual surfaces was observed in Group 1 compared to Group 2 (Table 1), but again, the increase in the mean values of 'Decayed' B/L surfaces reached only 0.7 (less than one surface) in Group 1 (Table 1). It is worth mentioning that the changes observed in the mean values of the 'Decayed' B/L surfaces were not statistically significant in both groups of 6-year-olds (Table 1). Our analysis of caries increment in 'Decayed and Filled' surfaces showed a decrease in caries increment in almost all types of surfaces in both groups of 6-year-olds, with the exception of B/L surfaces in Group 1 (Figure 1). The changes observed in the mean values of 'Decayed and Filled' B/L surfaces differed (at baseline to 36 months) only in 0.32 surfaces (Table 1, Figure 1).

We observed changes in caries increment in almost all types of 'Decayed' surfaces; a positive caries-reducing effect was observed in the 12-year-olds treated with MI Varnish™ (Group 3) compared to the 12-year-olds who were not subjected to MI Varnish™ treatment (Group 4) (Table 2). Surprisingly, the percentage increase in caries in 'Decayed' B/L surfaces in Group 3 was higher compared to that of Group 4 (Figure 2).

3.1.2. Oral Hygiene Habits

The oral hygiene habits of the participants are shown in Tables 3 and 4. In 6-year-olds, only 6.25% (n = 3) in each group stated that they use F-containing tablets at baseline, and none (0%) stated that they use F-containing tablets at 36 months. At baseline and at 36 months, none of the children at the age of 12 stated that they use F-containing tablets. Dental floss was not used by all children at baseline or at the end of the 36-month period.

3.2. Secondary Outcomes

3.2.1. Ratio Risk Calculations

Table 5 represents the risk of further caries development in both age groups after a period of 36 months, demonstrating a reduction in further caries development in all types of surfaces, except for the interproximal surfaces in 6-year-olds, for which the RR was almost equal to 1.0 (Table 5). For the RR of the interproximal surfaces, it was necessary to calculate caries-free and filling-free interproximal surfaces for Group 1 (23.96) and for Group 2 (23.71) and then divide the gained data on the total number of interproximal surfaces (prevalence of free surfaces) (Table 1) separately to each group (Group 1—0.57, Group 2—0.55) and then again to divide gained data on each other (Group 1/ Group 2—0.570.55). It is obvious that the prevalence of free surfaces in Group 1 is slightly higher (0.02) than in Group 2 (Table 1), though this could be counted as a non-significant difference. In 12-year-olds, a reduced risk of further caries development was obtained for all types of surfaces (Table 5).

Table 3. Oral hygiene habits among the 6-year-olds (Group 1 and Group 2) at baseline and at 36 months.

	Group 1 (*n* = 48)	**(MI Varnish)**	**Group 2 (*n* = 48)**	**(Control)**
	At Baseline (%)	**At 36 Months (%)**	**At Baseline (%)**	**At 36 Months (%)**
Frequency of daily toothbrushing	70.83 (*n* = 34)— 2 times daily 22.92 (*n* = 11)—once daily 6.25 (*n* = 3)—>2 times daily	60.42 (*n* = 29)— 2 times daily 37.5 (*n* = 18—once daily 2.08 (*n* = 1)—once a week	64.58 (*n* = 31)— 2 times daily 25 (*n* = 12)—once daily 10.42 (*n* = 5)—>2 times daily	75 (*n* = 36)— 2 times daily 22.92 (*n* = 11)—once daily 2.08 (*n* = 1)—once a week
The use of F-containing toothpaste (TP)	47.92 (*n* = 23)—confirmed that they use F-containing TP 6.25 (*n* = 3)—stated they do not use F-containing TP 45.83(*n* = 22)—do not know about F in used TP	39.58 (*n* = 19)— confirmed that they use F-containing TP 0 (*n* = 0)— stated they do not use F-containing TP 60.42 (*n* = 29)—do not know about F in used TP	45.83 (*n* = 22)— confirmed that they use F-containing TP 10.42 (*n* = 5)— stated they do not use F-containing TP 43.75 (*n* = 21)—do not know about F in used TP	31.25 (*n* = 19)— confirmed that they use F-containing TP 2.08 (*n* = 1)— stated they do not use F-containing TP 66.67 (*n* = 32)—did not know about F in used TP
Parental supervision	54.17 (*n* = 26)—supervised 45.83 (*n* = 22)— not supervised	37.5 (*n* = 18)—supervised 62.5 (*n* = 30)— not supervised	77.08 (*n* = 37)—supervised 22.92 (*n* = 11)— not supervised	31.25 (*n* = 15)—supervised 68.75 (*n* = 33)— not supervised
Name of used toothpaste	27.08 (*n* = 13)—know 72.92 (*n* = 35)—do not know	58.33 (*n* = 28)—know 41.67 (*n* = 20)—do not know	39.58 (*n* = 19)—know 60.41 (*n* = 29)—do not know	64.58 (*n* = 31)—know 35.42 (*n* = 17)—do not know

Table 4. Oral hygiene habits among the 12-year-olds (Group 3 and Group 4) at baseline and at 36 months.

	Group 3 (*n* = 47)	**(MI Varnish)**	**Group 4 (*n* = 37)**	**(Control)**
	At Baseline (%)	**At 36 Months (%)**	**At Baseline (%)**	**At 36 Months (%)**
Frequency of daily toothbrushing	53.19 (*n* = 25)— 2 times daily 44.68 (*n* = 21)—once daily 2.13 (*n* = 1)—> 2 times daily	57.45 (*n* = 27)— 2 times daily 36.17 (*n* = 17)—once daily 6.38 (*n* = 3)—> 2 times daily	51.35 (*n* = 19)— 2 times daily 40.54 (*n* = 15)—once daily 2.7 (*n* = 1)—> 2 times daily 5.41 (*n* = 2)—once a week	43.24 (*n* = 16)— 2 times daily 48.65 (*n* = 18)—once daily 8.11 (*n* = 3)—> 2 times daily
The use of F-containing toothpaste (TP)	31.92 (*n* = 15)— confirmed that they use F-containing TP 8.51 (*n* = 4)— stated they do not use using F-containing TP 59.57 (*n* = 28)—do not know about F in used TP	14.89 (*n* = 7)— confirmed that they use F-containing TP 6.39 (*n* = 3)— stated they do not use F-containing TP 78.72 (*n* = 37)-do not know about F in used TP	16.21 (*n* = 6)— confirmed that they use F-containing TP 2.7 (*n* = 1)— stated they do not use F-containing TP 89.91 (*n* = 33)—do not know about F in used TP	10.81 (*n* = 4)— confirmed that they use F-containing TP 0 (*n* = 0)— stated they do not use F-containing TP 89.91 (*n* = 33)—did not know about F in used TP
Name of used toothpaste	8.51 (*n* = 4)—know 91.49 (*n* = 43)—do not know	34.04 (*n* = 16)—know 65.96 (*n* = 31)—do not know	18.92 (*n* = 7)—know 81.08 (*n* = 30)—do not know	43.24 (*n* = 16)—know 56.76 (*n* = 21)—do not know

Table 5. Risk ratio (RR) in all types of surfaces in 6-year-old and 12-year-old children after a period of 36 months.

	6-Year Olds	**12 Year Olds**
	At 36 Months	**At 36 Months**
Interproximal surfaces (ICDAS 1–6+ CARn/c + CARc + F)	RR = 1.04 (increase 4%)	RR = 0.75 (reduction 25%)
Occlusal surfaces (ICDAS 1–6+ CARn/c + CARc + F)	RR = 0.87 (reduction 13%)	RR = 0.96 (reduction 4%)
Buccal/Lingual surfaces (ICDAS 1–6+ CARn/c + CARc + F)	RR = 0.85 (reduction 15%)	RR = 0.80 (reduction 20%)

3.2.2. Correlation Analysis

Tables 6 and 7 represent the correlation analysis of various types of surfaces in all groups at baseline and after the 36 month-period. We analyzed whether various caries-affected surfaces may influence further caries development on other surfaces. Regarding the 6-year-old participants, our correlation analysis showed that there was a relationship between the 'Decayed' data of various tooth surfaces at baseline in the group undergoing MI Varnish™ treatment (Group 1) and the Group without treatment (Group 2), except for the relationship between the interproximal and B/L surfaces in Group 2 (Table 6). Surprisingly, no statistical significance was observed between the 'Decayed' data of various surfaces after a period of 36 months in the group without treatment (Group 2). After examining the statistically significant correlation between 'Decayed and Filled' surfaces, it is obvious that the 'Filled' surfaces data give a very tight relationship between surfaces in all groups (Tables 6 and 7). In other words, the higher number of filled surfaces may increase the risk of further caries development on all surfaces (Tables 6 and 7).

Table 6. Correlation analysis between various types of 'Decayed' and 'Decayed and Filled' surfaces in Group 1 and Group 2 at baseline and at 36 months.

	Group 3 (*n* = 47)	**(MI Varnish)**	**Group 4 (*n* = 37)**	**(Control)**
	At Baseline	**At 36 Months**	**At Baseline**	**At 36 Months**
Interproximal Decayed to Occlusal Decayed	r = 0.329 $p < 0.05$	r = 0.381 $p < 0.001$	r = 0.459 $p < 0.001$	r = 0.270 $p > 0.05$
Interproximal Decayed to Buccal/Lingual Decayed	r = 0.480 $p < 0.001$	r = 0.274 $p > 0.05$	r = 0.573 $p < 0.001$	r = 0.602 $p < 0.001$
Occlusal Decayed to Buccal/Lingual Decayed	r = 0.368 $p < 0.05$	r = 0.056 $p > 0.05$	r = 0.469 $p < 0.001$	r = 0.466 $p < 0.001$
Interproximal Decayed and Filled to Occlusal Decayed and Filled	r = 0.516 $p < 0.001$	r = 0.535 $p < 0.001$	r = 0.528 $p < 0.001$	r = 0.537 $p < 0.001$
Interproximal Decayed and Filled to Buccal/Lingual Decayed and Filled	r = 0.619 $p < 0.001$	r = 0.615 $p < 0.001$	r = 0.570 $p < 0.001$	r = 0.640 $p < 0.001$
Occlusal Decayed and Filled to Buccal/Lingual Decayed and Filled	r = 0.665 $p < 0.001$	r = 0.618 $p < 0.001$	r = 0.612 $p < 0.001$	r = 0.685 $p < 0.001$

Table 7. Correlation analysis between various types of Decayed and Decayed and Filled surfaces in Group 3 and Group 4 at baseline and at 36 months.

	Group 1 (*n* = 48)	**(MI Varnish)**	**Group 2 (*n* = 48)**	**(Control)**
	At Baseline	**At 36 Months**	**At Baseline**	**At 36 Months**
Interproximal Decayed to Occlusal Decayed	r = 0.449 $p < 0.001$	r = 0.461 $p < 0.001$	r = 0.140 $p < 0.05$	r = 0.227 $p > 0.05$
Interproximal Decayed to Buccal/Lingual Decayed	r = 0.527 $p < 0.001$	r = 0.610 $p < 0.001$	r = 0.280 $p > 0.05$	r = 0.265 $p > 0.05$
Occlusal Decayed to Buccal/Lingual Decayed	r = 0.420 $p < 0.001$	r = 0.358 $p < 0.05$	r = 0.336 $p < 0.05$	r = 0.028 $p > 0.05$
Interproximal Decayed and Filled To Occlusal Decayed and Filled	r = 0.439 $p < 0.001$	r = 0.469 $p < 0.001$	r = 0.329 $p < 0.05$	r = 0.351 $p < 0.05$
Interproximal Decayed and Filled to Buccal/Lingual Decayed and Filled	r = 0.484 $p < 0.001$	r = 0.695 $p < 0.001$	r = 0.398 $p < 0.001$	r = 0.312 $p < 0.05$
Occlusal Decayed and Filled to Buccal/Lingual Decayed and Filled	r = 0.406 $p < 0.001$	r = 0.598 $p < 0.00$	r = 0.635 $p < 0.001$	r = 0.459 $p < 0.001$

3.3. Adverse Effects

In a period of 3 years, the quarterly application of MI Varnish™ (5% sodium fluoride, GC Corp., Tokyo, Japan) had no adverse effects on both age groups [6].

4. Discussion

Fluoride varnishes (FVs) are professionally applied fluoride treatments that can stick to the tooth surfaces for a long time and slowly release fluoride, preventing mineral loss from teeth, enhancing remineralization, and reversing or slowing down the progression of early caries lesions [10]. However, fluoride needs calcium and phosphate ions to accomplish these functions. For this reason, MI Varnish™, which contains fluoride, calcium, and phosphate ions, was developed, and studies have shown that it provides additional anti-cariogenic effects compared to other fluoride varnishes without CPP-ACP [8]. It has been established that a greater caries-preventive effect could be achieved by the application of F varnish in medium- and high-risk patients [11]. For this reason, the present study, which demonstrated that the quarterly application of MI Varnish™ over a period of 3 years reduced caries increment, was conducted on high-caries-risk children from Riga, Latvia [6]. Secondary analysis further demonstrated a differential caries reduction effect on different tooth surfaces among the 6- and 12-year-olds. This was attributed to the potential of this varnish to stabilize high concentrations of calcium and phosphate ions, along with fluoride ions, at the tooth surfaces by binding to the pellicle and plaque [8]. Thus, it was not surprising that the achieved caries reduction occurred only in interproximal and occlusal surfaces, where plaque removal is usually difficult, even with flossing and toothbrushing. We believed that the component ions of the varnish (calcium, phosphate, and fluoride ions) were retained in the residual plaque, where they effected caries prevention (Table 2, Figure 2). Besides retention in the plaque, the morphology of the occlusal pits and fissures and the compact nature of the interproximal space may have facilitated the physical retention of the varnish in those areas in both groups of 12-year-olds (Table 2, Figure 2).

On the other hand, it is known that the B/L surfaces are easily cleansable by tooth-brushing, and this explains the low plaque stagnation and shorter period of varnish retention. Furthermore, the caries increase in B/L surfaces in Group 1 (Figure 1) may not be considered as a failure of the MI Varnish™. Upon observing the risk ratio (RR) of all surfaces, it can be seen that a reduction in further caries development occurs in almost all types of surfaces, except interproximal surfaces in 6-year-olds, where RR is almost equal to

1.0 (Table 5), suggesting little or no difference in risk between the two 6-year-old groups. This fact could be explained by the presence of mixed dentition in 6-year-olds and the exfoliation of primary teeth in both groups (Figure 2), as well as a 4% smaller number of total surfaces in Group 1 [6]. In a previous study, no statistically significant difference in preventing interproximal caries development in primary teeth was obtained with the bi-annual application of 5% NaF varnish [12]. The greater increase in caries on the B/L surfaces of the 12-year-olds that received varnish treatment (Group 3) compared to the control group (Table 2, Figure 2) could be attributed to the large percentage of dropouts in the control group (Group 4) [6], without which the data of caries increment could have been much higher in this control group. It is also pertinent to mention that at baseline and after observing caries status, the highest caries prevalence was achieved in interproximal surfaces in both age groups undergoing MI Varnish™ treatment (56.52% in Group 1, 53.39% in Group 3); thus, the highest RR was recorded for the interproximal surfaces, but only in the older age group (Table 6). This suggests that the quarterly application of MI Varnish™ was more effective on caries-prone surfaces in the older age group.

In 12-year-olds, a statistically significant relationship between the 'Decayed' data of various surfaces in both groups was present at baseline (Table 7). However, at 36 months, the relationship of the 'Decayed' data between the interproximal and the occlusal surfaces only remained in the group subjected to the quarterly application of MI Varnish™ (Table 7). Also, the statistical significance relationship of the 'Decayed' data between Interproximal and B/L surfaces, as well as Occlusal and B/L surfaces, disappeared in the group undergoing treatment (Group 3) but remained in the group without treatment (Group 4) (Table 7). In other words, quarterly MI Varnish™ application seems to be able to interrupt the established (at the baseline) statistically significant relationship between the caries-affected surfaces of various tooth surfaces and reduce the risk of further caries development. However, the statistical significance disappeared in the 'Decayed' data between Interproximal and occlusal surfaces in Group 4 but remained in Group 3. This can be attributed to the high dropout in Group 4. The interrupted statistically significant relationships between interproximal and B/L surfaces, as well as occlusal and B/L surfaces, in Group 3 (Table 7) is likely attributable to the cumulative effect of CPP-ACP and fluoride in MI Varnish™, which has antibacterial effect against salivary *S. mutans* [13,14]. At the same time, following our statistical analysis, it became clear that the difference of the mean values of the 'Filled' (baseline—36 months), Interproximal (1.86), occlusal (1.19), and B/L (1.11) surfaces (Table 2), the statistically significant relationships between the surfaces remained (Table 7). This means that the restoration of one type of surface may influence further caries development on another type of surface. The interrelationship of the surfaces could be explained by microbiological aspects, including major microorganisms such as Streptococcus (particularly *S. mutans*) [15,16] and the recently discovered Selenomanas Sputigena [16]. Also, it could be suggested that the placement of any restoration may have changed the content of microflora, leading to further dental caries development.

Good oral hygiene plays an important role in reducing dental plaque and ensures that plaque-free teeth do not decay [17–19]. The use of fluorides will not compensate for poor oral hygiene, so it is highly recommended to brush teeth twice daily [17–19]. In 6-year-olds, the majority of the children in both groups were brushing their teeth twice daily, and this did not change over a period of 36 months (Table 3). It is worth mentioning that, at baseline, the 6-year-old control group (Group 2) already showed a lower caries rate than the group receiving MI Varnish™ treatment (Group 1) [6]; over the course of this study the number of children brushing their teeth twice daily increased in 6-year-olds in the control group (Group 2), and the percentage of parent's ability to provide the name of toothpaste also increased compared to Group 1 (Table 3), indicating an increase in parental involvement in the children's dental care (Table 3). The decrease in toothbrushing frequency could most likely be explained by the fact that parents (Group 1) were more reliant on professional dental prophylaxis procedures (provided four times yearly in a dental office over 3 years) prior to MI Varnish™ application rather than regular daily toothbrushing at home. Also,

after reviewing the mean values of the affected surfaces observed at baseline (Table 1), we believe the lower rate of caries-affected surfaces could be explained by the fact that the parents of the children in Group 2 were more interested in the oral health of their children than the parents of the children in Group 1.

In the 3-year period of the study, the knowledge about the content of fluoride in the toothpaste used by the participants, together with parental supervision, reduced in both groups, while the number of parents capable of providing the name of the toothpaste their child used increased in both groups (Table 3). This may be due to the type of questions asked in the questionnaire at baseline. To achieve proper results, an appropriate toothbrushing procedure with F-containing toothpaste must be performed under parental supervision in children until at least twelve years of age [20]. After examining the data on hygiene habits gained via the questionnaire, older age groups showed almost no improvement in oral hygiene habits (Table 4) compared to the younger age groups (Table 3) within the 3-year study period. This might be due to decreased parental involvement in daily oral health care. Generally, our results show that oral hygiene habits did not improve over a 3-year period, which is similar to the results observed in our previous studies conducted in Riga [3,4].

Undoubtedly, our study has its weaknesses and strengths, and these are completely and thoroughly described in our previous publication [6]. However, the highest caries-preventive effect in the study 3-year period was achieved in interproximal (decayed and filled) surfaces in both age groups receiving MI Varnish™ treatment, (Figures 1 and 2) despite the children's frequent consumption of sugary snacks and carbonated soft drinks, as well as the consumption of tea sweetened with sugar [6] and inadequate self-implemented home-based preventive measures (Tables 3 and 4). It should be noted that the highest reduction in the risk of further caries development was achieved in approximal surfaces among the 12-year-olds (Table 5).

In conclusion, the application of MI Varnish™ every 3 months had a caries-reductive effect on interproximal and occlusal surfaces among 6- and 12-year-olds.

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Data Availability Statement: The dataset generated and analyzed during the current study is available upon request from the corresponding author, Jekaterina Gudkina (j.gudkina@inbox.lv). The data are not publicly available due to ethical reasons.

Conflicts of Interest: The authors declare no conflict of interest.

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