The effect of MI Varnish™ on Caries Increment among 6 and 12 Years Old Children in Riga, Latvia. A 3 Year Study.

Objectives:

This randomized controlled trial will investigate the effect of MI Varnish™ (5% sodium fluoride GC Corp., Tokyo, Japan) on caries increment in 6- and 12-year-old children in Riga, Latvia within 36 months.

Subject recruitment

This will be a randomized controlled study. The study will be performed at the RSU Institute of Stomatology, in Riga on a population of 6- and 12-year old children, inhabitants of Riga, who will visit the RSU Institute of Stomatology for dental treatment.

All children will be recruited into the study during their regular dental check-ups in a random manner as follows. During the visit, children will be listed in serial numbers in the order of their arrival time at the reception desk of the RSU Institute of Stomatology. Recruitment will be facilitated by exempting the volunteers from the two-year wait time required for regular complete (including radiographic examination) dental check-up financially supported by government.

Written informed consent and assent will be obtained from parent and child respectively. On meeting the examiner, odd numbers of both age groups will be recruited into the Varnish group (6 years old into group 1; 12 years old into group 3), while even numbers will be recruited into the control groups (6 years old into group 2; 12 years old into group 4). Also the information about this study will be delivered to the patients and their parents at the time of recruitment. The children's demographic information will be collected, including their full name with the first two letters of their surname, available mobile telephone number, and an e- mail address, and these will be filled in the specially prepared study folder for each subject. Complete names and surnames will be provided only in the signed written informed consent before the examination procedure started.

Every child within the chosen ages, that will visit the RSU Institute of Stomatology, will be eligible to be enrolled irrespective of their caries status, except if children and/or their parents refuse to participate in the study anymore, the families move away from Riga, or children or parents do not answer the 3 telephone calls confirming their appointments. Children and their parents will be informed about the precise time of attending the dentist by using the mobile telephone number provided at a baseline. All calls will be carried out in one week before to arrange the appropriate

time and the date of visiting the dental office, and then the information will be reminded one day prior the visit. Also excluded will be children wearing orthodontic braces or diagnosed of general ill health within the study period.

Examiner calibration

Prior to the clinical examination, the examiner will be calibrated on visual examination by a caries detection expert using the first 15 patients who will not be included in the study. Agreement to the set standard will be quantified by Kappa analysis. The Kappa scores for intra-examiner and inter-examiner (examiner-calibrator) will be 0.81 and 0.87 respectively (any score > 0.70 will be considered to be acceptable as an adequate agreement). Only one calibrated dentist-examiner (JG) will be involved in examining the children.

The dental examiner will be blinded to children study group (varnished or not varnished). The examiner will use the caries assessment criteria of the ICDAS II to distinguish between initial (non-cavitated) caries lesions and developmental defects of enamel. The scoring criteria according to ICDAS-II (https://www.iccms-web.com), will be: score 0: sound tooth surface; score 1: first visual change (opacity or discoloration) in enamel hardly visible on the wet surface but distinctly visible after air drying; score 2: distinct visual change (opacity or discoloration) in enamel, visible without air drying; score 3: localized enamel breakdown without visible dentin; score 4: underlying dark shadow from dentin without cavitation; score 5: distinct cavity with visible dentin; score 6: extensive distinct cavity with visible dentin. The same scoring criteria will be used for caries around restorations. Detected lesions will be recorded in a specially designed case report form (CRF). A tooth will deemed to be present in the oral cavity when part of its occlusal surfaces visible without the need for gingival displacement.

Oral Examination procedure

The children will be examined on a dental chair. A visual-tactile examination of all teeth will be conducted using the dental operatory light and with prior drying, by qualified dentist using a standard dental mirror and rounded dental probe. The examination environment, procedure and sequence employed during normal dental check-up will be maintained throughout the study. Face masks, disposable latex gloves and pre-packed sterile examination sets will be used for each child.

All examinations as well as MI Varnish™ (5% sodium fluoride GC Corp., Tokyo, Japan) procedures will be performed by one and the same clinical Examiner in all groups at the baseline, and 36 months examination. The clinical Examiner as well as the Recorder will be blinded at each examination visit with regards to each child's study group (MI Varnish™ varnish or Control

groups). Visual caries assessment will be performed on every surfaces of each tooth on all subjects. Prior to examination, subjects will have their teeth brushed with non-fluoridated professional toothpaste. Following brushing, visual examination will be carried out on clean, plaque-free teeth with careful drying of the tooth surface to identify early lesions. Each examined surface will be placed under one of the following classifications: Sound, non-cavitated (n/c) lesion (ICDAS 1 & 2), non-cavitated lesion around restorations (CARn/c), cavitated (c) lesion (ICDAS II 3-6) or cavitated lesion around restorations (CARc).

Following visual examination, digital bitewing (BW) X-rays will be performed on every child, and will be examined at the Department of Radiology of the RSU Institute of Stomatology where all subjects will be recruited for their primary examination and interested in earlier regular checkups. All collected data concerning caries status, including oral hygiene status will be placed in the official dental patient chart.

Table 1. International Caries Detection and Assessment System-II (ICDAS II) scoring criteria.

Score	Clinical criteria description
0	Sound tooth: no evidence of caries after prolonged air drying (5 s), surfaces with developmental defects (enamel hypoplasia, fluorosis), tooth wear (attrition, abrasion and erosion), and extrinsic or intrinsic stains will be recorded as sound.
1	First visual change in enamel: opacity or discoloration is visible after prolonged air drying, which is not or hardly seen on a wet surface.
2	Distinct visual change in enamel: opacity or discoloration distinctly visible when wet, lesion must still be visible when dry.
3	Localized enamel breakdown due to caries with no visible dentin or underlying shadow.
4	Underlying dark shadow from dentin with or without localized enamel breakdown.
5	Distinct cavity with visible dentin: visual evidence of demineralization and dentin exposed.
6	Extensive distinct cavity with visible dentin with more than half of the tooth surface involved or possibly reaching the pulp.

Study Treatment

Following the baseline caries examination, the treatment groups (group 1 & 3) will receive the application of MI Varnish™ while the control groups (Group 2 & 4) will not have varnish applied. Application of MI Varnish™ will be performed in accordance with the manufacturer's instruction. The post-varnish instruction as provided by the manufacturer will be given to all children and their parents. Neither children nor their parents in the Varnish (Groups 1 and 3) will be informed about the name of varnish (MI Varnish™) used, and the manufacturer's name. Subsequently, subjects in the treatment groups (group 1 & 3) will be recalled every 3 months for re-application of varnish within the 36 months study period. MI Varnish™ (5% sodium fluoride GC Corp., Tokyo, Japan) will be re-applied on all tooth surfaces. At the baseline and 3 monthly MI Varnish re-application visits, teeth were brushed with non-fluoridated professional toothpaste (Zircate Prophy Paste; Dentsply Caulk, Germany) and then MI Varnish™ (GC Corp., Tokyo, Japan) was re=applied on all tooth surfaces.

Oral hygiene

Green-Vermillion oral hygiene index (G-V index) as described by the World Health Organization [10] will be used to determine the oral hygiene level in all participants at a baseline and in a 36-months period. All subjects will receive general oral hygiene instruction at a baseline and in a period of 36-months.

Questionnaire on dietary habits

Questionnaire will be used to obtain information on dietary habits. Depending on age, children and/or their parents will be questioned about snacking habit, intake of chocolates, carbonated soft or sport drinks during the day, number of tea spoons of sugar (t.s.) per cup of tea, and the number of cups of tea consumed daily. Questionnaires will be administered while children with their parents will be waiting to take the radiograph, so although all questions about oral hygiene and dietary habits will be answered by children in both age groups, their parents may help with the response. All questions about dietary habits will be answered by children in 12 year olds, their parents may help with the response. Parents will provide all responses for the 6-year olds. Questionnaires will be administered to all participants at the baseline and in a 36 months period. No information about so called 'Teeth Healthy Diet' will be provided at the baseline and throughout the study period. But the advice to improve not teeth-healthy dietary habits will be provided *only at the last visit*, after questionnaire is filled and the oral examination completed. All children/parents will

receive the same so called “general” preventive advice at a baseline and in a period of 36 months.

Sample size calculation

A parallel study will be conducted in 6 and 12 year olds with the ratio 1:1 between experimental and control groups.

A total number of children per group will be estimated to detect a difference between groups, with a two-tailed α of 0.05 and a (1-β) of 0.80, for a comparison of 2 independent means if there is an absolute difference of 5 units outcome measure and standard deviation of 9 units. The drop out will be calculated as 25%.

Sample size will be calculated as follows:

Type I error = 5% implies constant 1,96

Type 2 error = 20% implies constant 0,84

$N=2*[((1,96+0,84)*SD)/(difference)]^2 = 2*(2,8*9/5)^2=50,8032\sim51$

N(with drop-out)=1,25* 51 = 63,75 ~ 64 children per each group

Simple randomization will be used in order to obtain main outcomes in both age groups within a 3 year period : as a primary outcomes -changes in caries increment, oral hygiene indices, and dietary habits; as a secodary outcomes - to figure out any statistcally significant relationship, if present, between different parameters used in the study.

Statistical methods

For the statistical analysis, the ICDAS-II data will be used to calculate the DMFS/dmfs (D = decayed surfaces, M = *Missing surfaces* and F = *Filled* surfaces), Decayed (D) as n/c+c+CARn/c+CARc [(non-cavitated (n/c) lesion (ICDAS 1 & 2), non-cavitated lesion around restorations (CARn/c), cavitated (c) lesion (ICDAS II 3-6) and cavitated lesion around restorations (CARc)]. Data was analysed using SPSS software package IMB SPSS Statistics v.22, RStudio v. 1.0.153 and Excel 2013, p values less than 0.05 will be considered statistically significant. For the primary outcomes, mean values of parameters concerning dental caries development and oral hygiene will be obtained using t-test at a baseline and in a 36 months period in all groups. The division in dietary habits at a baseline and in a 36 months period will be calculated using Chi-square test in all groups. The statistically significant difference between mean parameters in a period of a 36 months will be obtained using t-test for paired parameters (Welch test) ($\alpha=0.05$) in all groups. For secondary outcomes, the correlation analysis with Pearson coefficient ($\alpha=0.05$) will be used to obtain statistically significant changes, if present, between used parameters.

Recommendations

F-containing tooth paste (all groups, at a baseline and in a period of 36-months)

Every child will be advised to use a tooth paste with 1450-1500ppm F concentration twice daily.

Dental floss (all groups, at a baseline and in a period of 36-months)

Dental non-fluoride floss will be recommended for daily use after tooth brushing at least once a day, preferably in the evening.

Dietary habits (all groups, at a baseline and in a period of 36-months)

Patients will be asked to continue their normal dietary habits (within the study period).

Examination procedure in dental office

Examination period for all groups

All subjects will be examined at a baseline and 36 months period.

Re-application of MI Varnish™ – Groups 1(6 y.o.) and 3 (12 y.o.) - every 3 months

Primary examination (all groups):

1. To deliver information about this study to the patient and his/her parents and to sign the agreement to participate in it;
2. To deliver questionnaires containing information about dietary and oral hygiene habits to children and/or their parents.
3. To perform BW -2 (in 6 y.o.) and 4 (12 y.o.) digital radiographs in the Department of Radiology; to fill in delivered questionnaires.
4. To collect filled questionnaires
5. To detect the level of the oral hygiene : Green-Vermillion oral hygiene index.
6. To provide professional tooth brushing using rotary instruments and pumice (Zircate Prophy Paste; Dentsply Caulk, Germany) that are used usually during the visit to the hygienist.
7. To perform caries detection and diagnosis using the ICDAS II (https://www.iccms-web.com)
8. **CONCERNIING ONLY Groups1 and 3**- to apply MI Varnish™ (5% sodium fluoride GC Corp., Tokyo, Japan) on all surfaces of all teeth
9. To analyze BW, if there are cavitated lesions (ICDAS II) that need to be restored – to refer to the dentist.
10. Patients will be allowed to make pictures of BW by their mobile phones ‘camera.
11. To placed collected data in the original patient chart.

12. To give general recommendations about toothbrushing twice daily with F-containing toothpaste (1450-1500 ppm), to use non-fluoridated dental floss once daily as well as to continue normal/usual dietary habits.

Last examination (3 year period; All groups):

1. To deliver questionnaires containing information about oral hygiene habits to the patients.
2. To perform BW -2 (in 6 y.o.) and 4 (12 y.o.) digital radiographs in the Department of Radiology; to fill in delivered questionnaires
3. To collect filled questionnaires
4. To detect the level of the oral hygiene: Green-Vermillion oral hygiene index.
5. To provide professional tooth brushing using rotary instruments and pumice (Zircate Prophy Paste; Dentsply Caulk, Germany) that are used usually during the visit to the hygienist.
6. To perform caries detection and diagnosis using the ICDAS II (https://www.iccms-web.com)
13. To analyze BW, if there are cavitated lesions (ICDAS II) that need to be restored – to refer to the dentist.
7. Patients will be allowed to make pictures of BW by their mobile telephones 'camera.
8. To placed collected data in the original patient chart.
9. To analyze dietary questionnaires.
10. To give general recommendations about toothbrushing twice daily with F-containing toothpaste (1450-1500 ppm), to use non-fluoridated dental floss once daily, also to educate about "Teeth Healthy Diet".