RESEARCH PROTOCOL

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a) Title:

Glass ionomer sealant versus fluoride varnish application to prevent occlusal caries among preschool children - A randomized controlled trial.

b) Introduction:

Problems encountered in clinical work

Dental caries has been considered as a significant public health problem worldwide (Selwitz et al., 2007; Peterson 2005). Despite that its prevalence might have declined in many developed countries (Selwitz et al., 2007), epidemiological data have shown a considerable rise in global caries rate, posing a significant point of issue in public health (Bagramian et al., 2009; Peterson 2005).

In Hong Kong, the problem of dental caries among preschool children remains unsolved and the severity has exacerbated. The number of decayed, missing and filled teeth among pre-school children had increased from 2.3 in 2001 (Hong Kong Department of Health, 2002) to 2.5 in 2011 (Hong Kong Department of Health, 2012). The latest government oral health survey found that more than 50% of the preschool children had caries experience, with over 90% of the decayed teeth being untreated and that one in every 20 children had even developed dental abscesses from extensive carious teeth (Hong Kong Department of Health, 2012).

Preventing caries development in primary molars is especially important, as they would remain in the oral cavity for more than 10

years before exfoliation. Premature loss of primary molars can seriously affect the development of permanent dentition, including space loss and malocclusion. Moreover, second primary molars were reported to be the most susceptible to caries development at the 4 and 5-year-olds in studies conducted in different countries around the globe (Elfrink et al., 2006; Autio-Gold & Tomar, 2005; Holt, 1995, Holland & Crowley, 1982), with a predilection for their occlusal surfaces (Douglass et al., 1995; Bimstein et al., 1981). Pits and fissures on the occlusal surface of permanent molars are considered to favor biofilm formation and retention, which the rate of occlusal caries development and progression are comparatively higher than that of smooth surface caries (Batchelor et al., 2004; Brown, 1995). In primary second molars, the generally deeper pits and fissures, as well as the presence of buccal pits and palatal fissures at mandibular and maxillary second primary molars respectively, may also contribute to its caries prevalence (Bimstein 1981).

Incipient carious lesions occur when minerals from the enamel subsurface are being lost initially, which may progress to cavitated carious lesions requiring restorations if left untreated. Calcium and phosphate ions can be redeposited to demineralized enamel via the process of remineralization as crystal voids, enhancing the strength by creating a net mineral gain (Cochrane et al., 2010). Remineralization with appropriate agents has proven effective to treat incipient carious lesions (Mohd Said et al., 2017; Cochrane et al., 2010). The use of fluoride promotes the remineralization in incipient carious lesion. Fluoride facilitates adsorption of calcium ions to demineralized enamel surface, as well as substituting hydroxyl ions (OH-) to form fluorapatite, that have a strong acid resistance against demineralization (Featherstone, 1999).

Topical fluoride varnish (TFV) with 5% sodium fluoride in its content has proven effective in preventing caries among children and

adolescents (Marinho et al., 2009; Marinho et al., 2002). Its reduction in DMFT is well supported by Cochrane reviews and clinical trials (Ahovuo-Saloranta et al., 2016; Marinho et al., 2013). When the varnish containing high concentration of fluoride (22,600ppm) is applied to the tooth surface, it forms a fast-setting base that releases fluoride subsequently (Marinho et al., 2002). It also increases the retention of fluoride on tooth surface, allowing a continuous flow of fluoride ions to enamel, dentine, plaque and saliva (Mohd Said et al., 2017). However, its effect in preventing pit and fissure caries in primary dentition has not been assessed (Ahovuo-Saloranta et al., 2016).

The 5% sodium fluoride varnish containing 22,600 ppm fluoride is the only fluoride agent that is considered safe-to-use for children aged below 6 years (Maguire, 2014; Weyant et al., 2013; American Dental Association Council on Scientific Affairs, 2006). As the quantity of fluoride contained in a single application dose of 5% sodium fluoride varnish is relatively small (5.65mg in 0.25mL), it is well below the probable toxic dose (5mg/kg) (Lee, 2015). Comparing with fluoride gels or other fluoride agents, the potential risks associated with inadvertent ingestions in young children is also reduced (Weyant et al., 2013; American Dental Association Council on Scientific Affairs, 2006). The current recommended dosage of 5% sodium fluoride varnish for children between 2-5 years old is 0.25mL, applying at intervals of every 3-6 months, depending on the caries risk assessed (Lee, 2015; American Dental Association Council on Scientific Affairs, 2006).

Pit and fissure sealants have been a recommended procedure in preventing pit-and-fissure caries development in permanent molars. Both resin-based and glass-ionomer sealant serve as a physical barrier between enamel and the oral environment, reducing plaque retention by sealing occlusal pit and fissures (Nunn *et al*., 2000). Multiple systematic reviews and clinical trials have concluded that

fissure sealants are significantly more effective than topical fluoride varnishes in preventing occlusal caries in permanent molars (Levy, 2012; Liu et al., 2012). However, a recent Cochrane review suggested that there was still insufficient evidence to determine superiority of resin or glass ionomer fissure sealants over topical fluoride varnishes for occlusal caries prevention, due to the lack of reliable results and low quality of evidence (Ahovuo-Saloranta et al., 2016).

Also, most studies were conducted on permanent first molars among school children (Ahovuo-Saloranta et al., 2016), whom cooperation and moisture control can be easily achieved. However, application of fissure sealants, especially resin-based sealant, in very young children can be a technique-sensitive procedure. Compared with a mean treatment time of less than 3 minutes for varnish application, application of resin-based sealants required over 15 minutes (Splieth et al., 2001). Moisture control is also of paramount importance for the retention and success of resin-based sealants (Gooch et al., 2009), which may be difficult among young preschool children.

Glass-ionomer sealants can chemically bond to enamel and are more tolerable to inadequate moisture control (Antonson et al., 2012). As its application requires less clinical steps, glass-ionomer sealant is comparatively more acceptable to younger patients and can be used in outreach settings with a large number of participants. The fluoride releasing ability is also beneficial in preventing caries especially at adjacent tooth surfaces (Cagetti et al., 2014). However, its retention rate is significantly lower than that of resin-based sealants (Antonson et al., 2012), its success rate in preventing fissure caries in very young patients is still unknown.

c) Aims and Hypotheses to be Tested:

Aims

To compare the efficacy of glass ionomer fissure sealant versus topical application of 5% sodium fluoride varnish in prevention of occlusal caries among preschool children.

Hypotheses to be tested

Greater reduction in occlusal caries in preschool children by glass ionomer fissure sealant compared to topical application of 5% sodium fluoride varnish.

d) Plan of Investigation:

(i) Subjects

The proposed study will be conducted in kindergartens in Hong Kong. The communal water supply in Hong Kong is fluoridated at a concentration of 0.5 ppm. Most child formula toothpastes available in Hong Kong contain fluoride at a concentration of 500–600 ppm. Generally healthy children aged between 3-4 years with no remarkable medical history will be invited to participate in this study. Children with at least one of the following criteria, deemed to be at moderate to high caries risk (American Academy of Paediatric Dentistry, 2013), will be included in this study:

Moderate risk	The child is a recent immigrant.
	The child has plaque on teeth.
High risk	The parent/ caregiver has low socioeconomic status.
	The child has >3 between meal sugar-containing snacks or beverages per day.
	The child is put to bed with a bottle containing natural or added sugar.
	The child has >1 decayed/missing/filled

	surfaces.
	The child has white spot lesions or enamel defects.
	The child has visible cavities or fillings.

They are thus qualified to receive fissure sealants and topical fluoride application regularly every 3 months (Lee, 2015; AAPD, 2013).

The following will be excluded from this study:

1. Children with the presence of caries of ICDAS code 3, 4, 5, or 6 on other primary teeth.
2. Children who had received professional fluoride application in the past 6 months.
3. Children with serious systemic disease or taking long term medication.
4. Children who are uncooperative or refuse the treatment.
5. Second primary molars that are (i) with caries in dentin indicated by ICDAS code 4, 5 or 6; (ii) partially erupted; (iii) with fillings and/or sealants; and (iv) hypoplastic or hypomineralized.

(ii) Methods

Sample size calculation

The primary outcome of this clinical trial is carious cavity development into dentin (ICDAS code 4,5 and 6) on occlusal surfaces of primary second molars. Sample size is calculated to be 256 children with a type I error of 0.05 and a power of 90%. This is based on the results of a previous study on primary molars (Honkala et al., 2015), where the real difference between fluoride varnish and sealant effect on occlusal caries reduction was 30%. Calculating with a 5% statistical significance level and a 90% power, a minimum of 102 children in each group will be required. To allow for loss of power due to drop-out, the initial sample size should be around 25%

larger, i.e. 128 children are needed in each group at the beginning of the study. Thus, the total initial sample size will be 256 (128 x 2 groups).

Ethical approval

Ethical approval will be obtained from the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster and the trial will be registered in the HKU Clinical trials registry.

Recruitment and screening

Children studying in kindergarten 1 and Nursery 2 will be invited to participate in this proposed study. An invitation letter will be sent to their parents, explaining the objectives and procedures of the study. Only those children whose parents have signed the informed consent will be included in this study. Children will be screened for the following aspects:

1. Plaque assessment using visible plaque index (VPI) (Ainamo & Bay, 1975)
2. Overall tooth status with diagnostic criteria recommended by WHO in 2013, in the form of:

Classification	***Description***
Decay (d)	presence of dentin carious lesion(s) with or without a restoration.
Missing (m)	the tooth is extracted due to caries.
Filling (f)	presence of a temporary or permanent filing due to caries with no decay.

3. Dental caries status based on ICDAS code score (International Caries Detection and Assessment System Coordinating Committee, 2009; Ismail et al., 2007), which is summarized below:

Code	***Classification***	***Description***
0	Smooth & intact tooth surfaces	No evidence of dental caries or questionable changes in translucency of enamel after prolonged drying (around 5 seconds).
1	Visual change in enamel	Color changes due to caries which is not consistent with the clinical appearance of sound enamel, which is seen only after prolonged drying.
2	Distinct visual change	Carious opacity (white spot lesion) and/or brown carious discoloration which is wider than the natural fissure/fossa that is not consistent with the clinical appearance of sound enamel, when seen wet and after drying.
3	Localized enamel breakdown due to caries with no visible dentin or underlying shadow	The tooth viewed wet might have a clear carious opacity and/or brown carious discoloration which is wider than the natural fissure/fossa that is not consistent with the clinical appearance of sound enamel.
4	Underlying dark shadow from dentin with or without localized enamel breakdown	The carious lesion appeared like a shadow of discolored dentin appeared through an apparently intact enamel, which might show signs of localized breakdown.
5	Distinct cavity with visible dentin	Cavitation in opaque or discolored enamel exposing the dentin beneath, involving less than half of the tooth

		surface.
6	Extensive distinct cavity with visible dentin	Cavitation in opaque or discolored enamel exposing the dentin beneath, involving at least half of the tooth surface. Obvious loss of tooth structure and dentin is clearly visible on the walls and at the base in a cavity that involved at least half of the tooth surface.

Questionnaire survey

Parents are required to complete a validated questionnaire before the baseline examination, which consists of the following sections:

(1) The child's personal information - gender, age, place of birth, medical history of systemic disease and long term medication

(2) The child's oral health-related behaviors – snacking habit, frequency of toothbrushing, parent's supervision in toothbrushing, use of toothpaste and night bottle habit

(3) The child's dental history – past dental treatment received

(4) The child's socio-economic background - parents' age, education level, household income and number of children

(iii) <u>Study design</u>

Clinical exam

Clinical examination will be carried with the child lying supine on table provided by the kindergarten.

The Visible Plaque Index (VPI) (Ainamo & Bay, 1975) will be used to measure the oral hygiene status of the child. Buccal and palatal/ lingual surfaces of 6 index teeth (teeth 55, 51, 63, 71, 75, 83) will be examined. The presence or absence of visible plaque on the tooth surfaces mentioned above will be recorded as "1" or "0" respectively. The VPI score of the child will be obtained by dividing the number of

tooth surfaces recorded as “1” with the total number of tooth surfaces recorded as “0” and “1”, i.e.

$$\text{VPI} = \frac{\text{number of tooth surfaces recorded as “1”}}{\text{total number of tooth surfaces recorded as “0” and “1”}}$$

b. Tooth status

All tooth surfaces will be first wiped clean and dry by clean gauze and cotton swab for visual inspection of caries. A basic dental charting will be first performed, which the overall primary dentition will be further assessed and recorded based on the presence of decayed (d) missing (m), and filled (f) teeth, as recommended by the WHO in 2013.

Then the ball end of the CPI probes will be used to slide through the pits and fissures of primary molars to detect any enamel cavities or discontinuities. The status of the occlusal surfaces of primary molars will be recorded based on the ICDAS coding system (International Caries Detection and Assessment System Corrdinating Committee, 2009; Ismail et al., 2007).

Children who do not meet all the inclusion criteria will be given an examination report. Parents will be informed regarding the carious teeth and will be advised to seek treatment from private dental practitioners or specialist paediatric dentists promptly. Children with second primary molar(s) that fulfill all the criteria will be included in the study.

The occlusal fissures of each of the upper and lower molars included in the study will be assessed, and the fissure morphology (deep/shallow) and ICDAS score will be recorded.

Randomization and treatment allocation

By using the random numbers generated by stratified block randomization method in a personal computer, children will be randomly allocated to one of the two study groups:

(1) NaF varnish

— Application of a 5% NaF varnish (Duraphat, Colgate-Palmolive Ltd, Waltrop, Germany) on the occlusal surfaces of primary second molars and all other teeth, every 3 months during the study period;

(2) GI sealant

-- Glass ionomer sealant (GC Fuji VII® (pink)) on all primary second molars included in the studies, with no further repair/replacement of the sealant

Interventions

Immediately after the oral examination, a chair-side dental assistant will prepare the materials respective to the groups allocated on the random allocation list.

For the sealant group, glass ionomer sealants will be applied using the finger pressure technique described in the WHO manual for atraumatic restorative treatment (ART) (Frencken et al.; 1997). The primary second molars included in the study will first be cleaned and dried by wiping with gauze and cotton pellets. Following the manufacturer's instructions, further cleaning of the fissures will be performed by applying GC cavity conditioner with micro-applicator for 10-15 seconds, then clean with wet cotton pellets for several times. The surface will be dried with cotton pellets. The dental assistant will mix the glass ionomer sealants (GC Fuji VII) in a standardized proportion with an amalgamator. Glass ionomer sealants will be applied to the occlusal surface with blunt end of an applier and slightly overfilled. A gloved finger with petroleum jelly will be used to push and rub the materials into the pits and fissures, as well as removing the excess sealants from the tooth surfaces. The sealants will be covered and protected by a layer of petroleum jelly (Vaseline®).

In the NaF varnish groups, 0.25mL (one drop) of the varnish will be placed in a plastic dappen dish. A disposable microbrush will be used to apply the varnish onto the second primary molars included in the study and all other teeth. The child will be instructed not to eat or drink after the application of varnish for at least half an hour.

Follow-up evaluation

Children in the NaF varnish group will be recalled at the 3rd, 6th, 9th, 12th, 15th,18th, 21st and 24th months for reapplication of fluoride varnish. Similar to baseline, 0.25mL (one drop) of the 5% sodium fluoride varnish will be applied using a disposable microbrush. At the 6th, 12th, 18th and 24th months, a clinical examination will be performed for all children in both groups. During the follow-up examination, overall VPI and dmft will be recorded. ICDAS score will be recorded for each primary second molar included in the study.

Sites with sealant will be evaluated in the following aspects:

Aspect	Code	Classification	Description
Retention	0	Fully intact	Sealants are fully intact without apparent loss of materials.
	1	Partial loss	Sealants are retained with partial loss
	2	Total loss	No sealant materials are found on tooth surface.
Caries	0	No caries	No caries found at the margin of the sealants.
	1	Presence of caries	Evidence of caries found at the margin of the restoration.

A 10% random sample will be re-examined during every examination to monitor intra-examiner reproducibility.

An oral health talk will be given to the parents of the participants. During the talk, the parents will be instructed to choose fluoridated toothpastes and child toothbrushes throughout the period of study. In case of any adverse event during the study, the parents will be advised to contact the principal investigator.

Outcome measures

The primary outcome measure is carious cavity development into dentin (ICDAS code 4, 5 and 6) on the occlusal surfaces of primary second molars over time.

Only one registered dentist will be the examiner in the kindergarten throughout the whole 24-month study period. She will receive training and calibration by epidemiologists before examining the subjects. The following dental equipment will be used:

(1) Disposable dental mirror attached to a handle with light-emitting diode intra-oral illumination
(2) World Health Organization CPI periodontal probe(405/WHO probe)

A 10% random sample will be re-examined during every examination to monitor intra-examiner reproducibility. The ICDAS score will be recorded based on the index proposed by Ismail et al., 2007.

Parents will be notified about the development of carious lesions. Advice on oral hygiene maintenance and seeking private dentists for respective dental treatments will be given to the parents in the form of a detailed report.

The proposed study will be a randomized controlled trial with 2 parallel groups. The unit of randomization will be each individual participant, but not each tooth of the mouth.

(iv) Data processing and analysis

Data analysis will be performed using SPSS® Statistics version 23.0 (SPSS Inc, Chicago, IL, USA). All children with missing data resulting from loss to follow-up or unrecorded data will be excluded from the analysis. Data proof-reading will be performed after data entry to identify and correct any errors before analyzing the data. An intention-to-treat analysis will be undertaken. Intra-examiner agreement in the diagnosis of dental caries will be assessed by Cohen's Kappa statistics.

Chi-square test will be used to assess the difference between groups according to the distribution of children's demographic characteristics such as gender, place of birth, oral health-related behavior, use of fluoride toothpaste, snacking habit, parents' education and family income.

The outcome measures are the mean change in the overall dmft scores and ICDAS code in primary second molars over time. Within-group comparison of changes in mean overall dmft scores and ICDAS scores at 24 months and mean change in overall dmft scores and ICDAS scores will be assessed by applying repeated analysis of variance (ANOVA) (or Friedman test for non-parametric data).

Student's t-test will be applied to differentiate the comparability between the treatment groups according to the subject's baseline conditions, including the child's age, dmft score and VPI score.

Since more than one molar will be included from one child, GLIMMIX procedure will be used to adjust for the clustering effect. The first level and second level will be the primary second molar and the subject

respectively. This analysis accounts for the correlation (clustering) between observations of multiple molars from the same child.

A multi-level logistic regression analysis will be performed to analyze the effects of independent variables on the ICDAS scores at the 6, 12, 18 and 24 month examinations. The independent variables included the child's demographic characteristics, oral health-related behaviors and clinical characteristics (gender, place of birth, frequency of tooth brushing, use of fluoride toothpaste, snacking habits, mother's education, father's education, family income, VPI score, dmft score and the treatment group). The level of statistical significance for all tests will be set 5%.

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