RANDOMIZED CONTROL TRIAL

# Effect of Fluoride Varnish in Caries Prevention on Permanent First Molars: A 36-Month Cluster Randomized Controlled Trial

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**Abstract:** ***Purpose:*** *The purpose of this study was to evaluate the effect of fluoride varnish in preventing dental caries of permanent first molars (PFMs).* ***Methods:*** *The study was designed to be a stratified-cluster randomized controlled trial with classes used as the unit of randomization. Classes stratified by district were followed for 36 months. All eligible children of the selected classes were included in the trial. The children in the test group were applied fluoride varnish biannually. The outcomes were measured at the individual level.* ***Results:*** *In total, 107 classes (51 in the test group, 56 in the control group) were recruited for the trial. Among the 5,397 total subjects, 5,005 and 4,596 children completed the 24-month and 36-month course, respectively. There were no group differences at baseline (P>0.05). The mean decayed and filled surfaces scores of the test group were significantly lower than those of the control group at the 36-month follow-up (P<0.05). The caries processing speed of PFMs increased from 24 months to 36 months; however, group differences were not significant (β equals 0.01; P>0.05).* ***Conclusions:*** *Biannual application of fluoride varnish can effectively prevent dental caries of six- to seven-year-old children. Nevertheless, the use of fluoride varnish with additional treatments (such as pit and fissure sealants) should be considered for optimized benefit after 24 months.* *(Pediatr Dent 2021;43(2):82-7)*
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Dental caries is among the most prevalent chronic diseases worldwide.[1] The prevalence of dental caries varies among countries.[2] In China, data from the fourth National Oral Health Survey showed that the mean decayed, missing, and filled permanent teeth (**DMFT**) scores for 12-year-olds was 0.86 and the prevalence of dental caries was 38.5 percent.[3] Though the DMFT level at 12 years old was very low in China,[3,4] the increasing rate of dental caries was 59.3 percent from 2005 to 2015.[3,5] "The Healthy China Plan for 2030," issued by the General Office of the State Council of China, stated that the objective of this plan was to reduce the prevalence of dental caries in 12-year-olds to under 30 percent by 2025 and to 25 percent by 2030,[6,7] which presents a significant challenge to dental professionals and the public.

Previous studies found that over 90 percent of dental caries occurred on permanent first molars (**PFMs**) among school-aged children, mainly affecting pit and fissures.[8-10] The time of tooth eruption for PFMs of children is approximately six years of age, with a mean 15-month duration of eruption.[11] The eruption process is more caries susceptible due to dental plaque accumulation.[12,13] Therefore, PFMs should be protected from the very beginning of tooth emergence.

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Previous studies found that fluoride varnish could effectively prevent dental caries from allowing PFMs to erupt.[14-18] However, the duration of these clinical trials was limited to less than 24 months. Although these findings are encouraging, they provide no insight into the effect of fluoride varnish for periods longer than 24 months. Some clinical trials reported that the patterns of change in the mean response throughout the longitudinal study is not simply linear.[19-21] Intensive changes in the mean response often occur in a short period, while the long-term effect may be relatively minimal.[19,20]

The purpose of the present study was to evaluate the caries-preventive effect of the semi-annual application of fluoride varnish on permanent first molars in a 36-month study course, with a focus on the effect after 24 months. The null hypothesis was that there was no significant difference in the caries-preventive effect on PFMs between the test group and the control group over 36 months.

## Methods

**Trial design.** This study was designed as a stratified-cluster randomized controlled trial with classes as the unit of randomization. Classes stratified by district were followed for 36 months. The study adhered to CONSORT guidelines[22]. The design and conduct of this trial were approved by the Ethics Committee of the Chinese Stomatological Association (Beijing, China, approval number 2014-003) and registered on the Chinese Clinical Trial Registry (ChiCTR-IIR-17013897, retrospectively registered). The local education bureaus and school administrations also approved the trial.

**Participants.** The classes were recruited from three low-fluoridated county-level cities (Dahua, Linxia, and Linxiang) in China. Dahua is located in the southern part of China, Linxia in the northwest, and Linxiang in the middle of the country. Individuals of Han ethnicity live in Linxiang. Many minority groups reside in Dahua and Linxia and have quite different

dietary constructions and living habits. The fluoride level in the local water supply was under 0.2 mg/L. These county-level cities belong to rural areas and have poor dental care due to the lack of dental manpower and sophisticated dental equipment. Public health measures, including pit and fissure sealants, were not commonly applied in these cities.

The participants were recruited by three coordinators. First, all districts of the county-level city were randomly sequenced by computer, and those with even numbers were selected. Second, all grade one classes of large-scale public primary schools (at least 450 students) of the selected districts were invited. All classes that agreed to participate in the trial were randomized into a test or control group.

The parents' meeting was held one month before the start of the study in order to inform parents about the trial. The child's participation was voluntary throughout the study, and the child could withdraw from the study at any time. If the study participant could not follow the study protocol, he or she would be excluded from the study by the investigators. Any adverse events occurring during the study course were carefully recorded and followed.

Eligible participants were six- to seven-year-olds with no acute or chronic systematic disorders, no gingivitis or ulcers, no allergy history, and no participation in other trials during these 24 months; additionally, to participate all children and their parents had to sign an informed consent. Those with hypoplastic defects, fluorosis, or pit and fissure sealed PFMs were excluded.

**Sample size.** The pretrial sample size was calculated without accounting for clustering and based on the caries prevalence reported in the dental literature. The minimum sample size needed was calculated as 614 children per group, with an alpha level of 0.05 and a power of 90 percent; caries incidence was anticipated to be nine percent in the test group and 15 percent in the control group.[8,18] To account for the anticipated dropout rate during the study (estimated at 30 percent), approximately 800 children were aimed to be enrolled in each group. To further evaluate the effect of fluoride varnish on the prevention of dental caries in three county-level cities, respectively, approximately 4,800 children were aimed to be enrolled in total.

**Interventions.** Oral health education was given to all children and their parents each year in the classrooms, including instruction on toothbrushing and dietary counseling. All children were encouraged to brush their teeth twice a day with fluoride toothpaste throughout the study. The oral hygiene instructions were repeatedly given every six months by providing the same toothbrush and fluoride toothpaste. Children who were allocated to the test group were scheduled for the application of fluoride varnish. Children in the control group would receive no other treatment.

The fluoride varnish was applied by two dentists and assistants at schools in each county-level city. Duraphat (Colgate-Palmolive [UK] Limited, Waltrop, Germany) was used in this study. Duraphat is a fluoride varnish containing five percent sodium fluoride (**NaF**; 2.26 percent fluoride) in an alcohol suspension of natural resins.[23] It was commercially available in China in 2012. The children in the test group were scheduled for topical application of fluoride varnish at baseline and then every six months. A total of six applications were given during the 36-month study course. Every child was given 0.25 ml of fluoride varnish according to a standard card, corresponding to 5.65 mg of fluoride per application. After isolating the teeth with cotton rolls and drying with swabs, the varnish was then applied on all accessible tooth surfaces of PFMs using a disposable microbrush and dried by air. The rest of the varnish was applied to other teeth. The child was told not to eat for at least two hours after the application and did not brush their teeth that day.

**Randomization and blinding.** The class randomization was carried out by school administrators according to coin-flipping results. Children from the same class were assigned to the same group. Each child was given an identification (**ID**) number at the first visit and identified by this ID number throughout the study. The examiners, assistants for data recording, and data analyst were blind to the allocation. Allocation lists were provided to the varnish providers and their assistants. However, they did not take part in the dental caries examination, data recording, or analysis. The participants were

Table 1. ERUPTION STAGES OF PERMANENT FIRST MOLARS AT BASELINE (N=5,397)

| Eruption stage | | Test group (%) | Control group (%) | *P*-value* |
|---|---|---|---|---|
| 0 | Fully erupted occlusal surface and fully exposed crown, established antagonist contact | 43.0 | 41.8 | 0.26 |
| 1 | Fully erupted occlusal surface, partially exposed crown | 13.2 | 13.3 | |
| 2 | Partially erupted occlusal surface | 9.5 | 10.4 | |
| 3 | Only cusp erupted | 2.0 | 2.0 | |
| 4 | No eruption | 32.3 | 32.5 | |

* Chi-square test.

Table 2. BASELINE CHARACTERISTICS OF THE PARTICIPANTS

| Variables | Test group (N=2,657) | Control group (N=2,740) | *P*-value |
|---|---|---|---|
| *Age (years)* | | | |
| Mean±(SD) | 6.81±0.42 | 6.85±0.42 | 0.87* |
| *Sex (%)* | | | |
| Males | 55.3 | 53.0 | 0.09† |
| Females | 44.7 | 47.0 | |
| *Toothbrushing habit (%)* | | | |
| ≥2x/day | 28.7 | 28.9 | 0.85† |
| <2x/day | 71.3 | 71.1 | |
| *Sweet consumption (%)* | | | |
| ≥1x/day | 42.8 | 44.4 | 0.25† |
| <1x/day | 57.2 | 55.6 | |
| *Caries experience of primary dentition* | | | |
| Prevalence (%) | 87.3 | 85.7 | 0.096† |
| *Caries experience of permanent first molars* | | | |
| Decayed filled surfaces (Mean± [SD]) | 0.03±0.35 | 0.04±0.32 | 0.49* |

* Two-sample *t*-test. † Chi-square test.

likely to be aware of the allocation due to the physical nature of Duraphat. Randomization was revealed after statistical analysis to ensure concealment of allocation.

**Data collection.** All parents were required to complete a questionnaire during the meeting at the first visit. The questionnaire included: (1) child's demographic characteristics (e.g., sex and birth date); and (2) child's oral health-related behavior (e.g., sweets intake, frequency of toothbrush with or without fluoride toothpaste).

Clinical examinations were carried out by two examiners with the help of two assistants for data recording. The examination was conducted using a head lamp, plane surface dental mirrors, and CPI probes at the schools in each county-level city. The examiners were all dentists with at least two years of clinical experience. After toothbrushing, with children in a supine position, the examiners dried the surface of the teeth with cotton rolls and swabs. No radiographic examinations were performed. World Health Organization criteria[24] were used for caries examination. The data were collected at three time points: (1) baseline; (2) at the end of 24 months; and (3) at the end of 36 months. The eruption stages of PFMs were classified as stage zero, one, two, three, and four, respectively (Table 1). After caries examinations, a report was sent to the child's caretakers to inform them if the child needed treatment. To ensure the validity and reliability of the data collection, examiners were trained and calibrated before the baseline oral examination and recalibrated before the follow-up examination. Five percent of the subjects were selected randomly for repeated tests by examiners to measure their consistency. Compared with the reference examiner's results, the six examiners demonstrated good reliability for caries diagnosis, with kappa-coefficient values greater than 0.8 at baseline, 24-month, and 36-month follow-up examinations. The intraexaminer consistencies reached over 90.0 percent agreements for all six examiners.

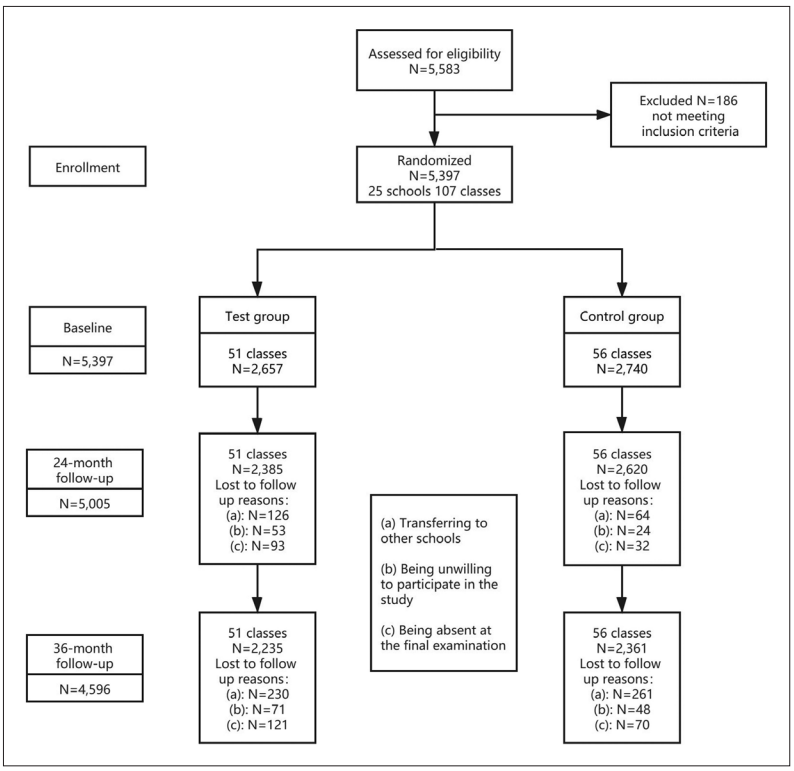


Figure. Flowchart of the participants of the clinical trial.

**Outcomes.** Although randomization was at the unit level, outcomes were measured at the individual level. The primary outcome measure was the mean decayed and filled surfaces (**DFS**) of PFMs at each time point (baseline, 24 months, and 36 months). The secondary outcome measure was the change in the mean DFS of PFMs for one unit of time (ΔDFS per year).

**Statistical analysis.** The data were analyzed using SAS 9.4 software (SAS Institute Inc., Cary, N.C., USA). Descriptive analysis was conducted to summarize the baseline characteristics of the participants. The caries experience of PFMs was calculated as the DFS scores. Missing tooth surfaces owing to caries were not included in the analysis since tooth loss rarely occurred in these mixed dentition ages. Concerning continuous variables (age and mean DFS scores of PFMs), a two-sample *t*-test was used to assess the statistical significance of the differences between the test group and the control group. In terms of binary variables (e.g., sex, toothbrushing habits, sweets consumption) and the eruption stages of PFMs, a chi-squared test was used. The *t*-test was employed to compare the mean DFS of PFMs between the two groups at the 24-month follow-up and 36-month follow-up, respectively. Piecewise linear mixed-effects (**PLME**) modeling was used to evaluate the patterns of change in the mean DFS of PFMs over time in each group for the period between baseline and 24 months (phase one) and the period between 24 months and 36 months (phase two). Modeling allowed for comparisons within groups (by evaluating the direction and significance of the change in slope) and between groups (by comparing the difference between group slopes). The slope represented the change in DFS scores for one unit of time (ΔDFS per year). All available data at each time point (baseline, 24 months, and 36 months) were included in the models. The statistical significance level for all tests was set at 0.05.

## Results

The trial was conducted from October 20, 2014, to December 20, 2017. A total of 107 classes were randomly assigned to a test group or control group. Between October 20 and December 10, 2014, 5,583 children were assessed for eligibility (Figure). Of these children, 2,657 children were enrolled in the test group and 2,740 children were enrolled in the control group. At baseline, there was no statistically significant difference between the two groups in terms of age, sex, frequency of toothbrushing, frequency of sugar consumption, caries experience of primary dentition, eruption stages, and caries experience of the PFMs ($P>0.05$; Tables 1 and 2).

In total, 5,005 children completed the 24-month course and 4,596 children completed the 36-month course. At the 24-month follow-up examination, the dropout rate for the test group was 10.2 percent (272 out of 2,657) versus 4.4 percent (120 out of 2,740) for the control group. At the 36-month follow-up examination, the dropout rate was 15.9 percent (422 out of 2,657) and 13.8 percent (379 out of 2,740) for the test group and control group, respectively.

At the 24-month follow-up examination, 98.5 percent of the PFMs had fully erupted. The mean DFS scores of the PFMs in the test group were 0.41 (SD 1.22) and 0.64 (SD 1.64) for the control group. There was a statistcally significant difference

Table 3. MEAN DECAYED AND FILLED SCORES (DFS) OF PERMANENT FIRST MOLARS AT THE 24-MONTH FOLLOW-UP AND 36-MONTH FOLLOW-UP

| | Test group | Control group | *t*-value | *P*-values* |
|---|---|---|---|---|
| *24-month follow-up* | | | | |
| N | 2,385 | 2,620 | | |
| DFS (mean±[SD]) | 0.41±1.22 | 0.64±1.64 | -6.53 | <0.001 |
| *36-month follow-up* | | | | |
| N | 2,235 | 2,361 | | |
| DFS (mean±[SD]) | 0.67±1.64 | 1.03±2.07 | -5.68 | <0.001 |

* *t*-test.

Table 4. PIECEWISE LINEAR MIXED-EFFECTS (PLME) MODELS WITH RANDOM INTERCEPT

| | Randomization | Parameter estimate | Standard error | *P*-values* |
|---|---|---|---|---|
| Intercept | Control | 0.04 | 0.01 | <0.001 |
| | Test | 0.03 | 0.01 | <0.001 |
| | Difference | -0.01 | 0.01 | 0.49 |
| Slope 1 | Control | 0.30 | 0.01 | <0.001 |
| | Test | 0.19 | 0.01 | <0.001 |
| | Difference | -0.11 | 0.02 | <0.001 |
| Slope 2 | Control | 0.38 | 0.02 | <0.001 |
| | Test | 0.25 | 0.02 | <0.001 |
| | Difference | -0.13 | 0.03 | <0.001 |
| Slope 3 | Control | -0.08 | 0.03 | 0.004 |
| | Test | -0.06 | 0.03 | 0.02 |
| | Difference | 0.01 | 0.04 | 0.71 |

* *P*-values are from PLME models. *P*-values for intercept are for baseline estimates relative to the mean decayed and filled scores for permanent first molars based on data collected at baseline examination. *P*-values for slope one are for slope estimates in comparison to zero, indicating whether there was a significant change in phase one for the control group, test group, or a comparison between the control group and test group slopes (difference), respectively. *P*-values for slope to are for slope estimates in comparison to zero, indicating whether there was a significant change in phase two for the control group, test group, or a comparison between the control group and test group slopes (difference), respectively. *P*-values for slope three are for slope change estimates in comparison of the slope before and after 24 months, indicating whether there was a significant change between the period from phase one to phase two for the control group, test group, or a comparison between the control group and test group slope changes (difference), respectively.

between the two groups ($P<0.001$). At the 36-month follow-up examination, all PFMs had fully erupted. The mean DFS scores of the PFMs were 0.67 (1.64) and 1.03 (2.07) for the test group and control group, respectively. There was a statistically significant difference between the two groups ($P<0.001$; Table 3).

As shown in Table 4, PLME models also confirmed that there was no significant difference in mean DFS scores of PFMs at baseline between the test group and the control group (β equals -0.006, $P>0.05$). In phase one, the control group demonstrated a significantly greater processing speed of mean DFS than that of the test group (β equals 0.19, $P<0.001$). In phase two, both the test group and the control group showed a steeper increase of the mean DFS of PFMs (test group: β equals 0.25, $P<0.001$; control group: β equals 0.38, $P<0.001$), and the difference between the two groups was significant (β equals -0.13, $P<0.001$). However, the difference of the change of the increasing rate of the mean DFS showed no statistically significant difference between the two groups from phase one to phase two (β equals 0.01, $P>0.05$).

None of the children experienced any adverse effects related to the application of fluoride varnish during the 36-month study course. Only one child complained about the taste of the fluoride varnish. In that case, the taste did not cause any nausea or vomiting.

## Discussion

The study subjects had diverse diets and living habits from three county-level cities. Moreover, a sufficient sample size of children was recruited in all cities. Therefore, the study evaluated the effect of fluoride varnish in large groups of children and produced powerful statistical results. Instead of randomizing individual participants, a higher-level unit (classes) facilitated the organization and promotion of the trial at primary schools. Also, randomization by cluster managed to avoid contamination between the two groups. The intracluster correlation was also considered for sample size estimation, which could significantly improve the validity and reliability of the overall results.

However, there were some limitations to the study. First, the estimation of the sample size was not based on a conventional calculation for cluster randomized controlled trials, as a value for the intracluster correlation coefficient was not available. However, a lower than expected dropout rate (15.9 versus 30 percent, according to a pretrial sample size calculation) may compensate for this fact. Second, the randomization was performed on the class level but analyses were conducted on the individual level and the clustering was not adjusted in the statistical analysis. Third, the potential lack of continuity of participation could cause data fluctuation. Fourth, since there was no placebo in the study design, the participants in the test group may have known their allocation, which might cause a potential for placebo effect or affect the dropout rate.

The results of the study showed that five percent NaF varnish prevented dental caries of PFMs among children aged six to seven years over 36 months. However, there was no significant difference in caries prevention between the two groups beyond the 24-month intervention. Therefore, the caries-preventive effect of five percent NaF varnish on PFMs was nonlinear over a long-term monitoring period. A notable effect with a significant difference was achieved only at the beginning of 24 months. It could then be concluded that the observed caries-preventive effect of five percent NaF varnish on PFMs at the 36-month follow-up was mainly ascribed to the first 24 months of the 36-month period. The null hypothesis was rejected.

Both Milsom and Hardman failed to demonstrate that biannual application of five percent NaF varnish provided at school could reduce dental caries in school-aged children. The authors attributed their failed outcome to high dropout rates and a lower-than-expected caries increment[25,26] However, the present study had a large sample size and low dropout rate. The eruption stages of PFMs may be correlated to the patterns of change. Approximately 60 percent of PFMs were in the

erupting stages in phase one, while all PFMs had fully erupted in phase two. The partially erupted PFMs were more susceptible to caries than PFMs with full occlusion.[12,27,28] It can be deduced that the fully erupted PFMs may suffer a relatively lower risk for dental caries than the erupting ones. Previous studies found that fluoride varnish demonstrated a lower caries-preventive efficacy in a low-risk population than in a high-risk population.[29-31] Therefore, once PFMs reach full occlusion they may be less sensitive to five percent NaF for caries prevention. However, Liu et al. reported that five percent NaF varnish could effectively prevent dental caries in fully erupted PFMs.[32] The difference could be the cause of the participant variation in different studies. In Liu et al.'s study, children who had at least one sound PFM with deep fissure or fissures with signs of early caries were included,[32] whereas in the present study children with all patterns of pit and fissure morphology were included. Moreover, the sample size in their study was much smaller than the present study, which may also explain the different results between the two studies.

Accurately estimating the effect of an intervention over time is a common challenge for researchers.[19] Comparing the results from different studies could lead to inaccurate conclusions due to investigational variations. PLME models capture the variations in slopes of various phases,[19] which reveals the precise nature of the effect of five percent NaF varnish in preventing dental caries of PFMs over time. Once the accurate effect of fluoride varnish is known, interventions can be more appropriately conducted. According to the findings of the current study, an additional 12-month application of five percent NaF varnish on PFMs after initial 24-month intervention seemed less meaningful since only a modest benefit was found at the expense of clinical material and time involved. To maintain a prolonged caries-preventive effect, the use of fluoride varnish with additional treatments, such as pit and fissure sealants, should be considered to optimize benefits after a 24-month intervention.

The present study provides some initial evidence for the 36-month effect of five percent NaF varnish on caries prevention of PFMs among children aged six to seven years. More studies that demonstrate the 36-month effect, as well as a generalization of the results, is needed. In addition, whether the 24 months was the inflection point in the nonlinear trajectory should also be ascertained in future studies.

## Conclusions

Based on the present study's results, the following conclusions can be made:

1. Semiannual application of five percent sodium fluoride varnish can effectively prevent dental caries in permanent first molars among six- to seven-year-olds.
2. To maintain a prolonged caries-preventive effect, the use of fluoride varnish with additional treatments, such as pit and fissure sealants, should be considered for optimized benefit after 24 months of semiannual application of fluoride varnish on PFMs.

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## References

1. Petersen PE, Bourgeois D, Ogawa H, Estupinan-Day S, Ndiaye C. The global burden of oral diseases and risks to oral health. Bull World Health Organ 2005;83(9):661-9.
2. World Health Organization oral health database. Oral Health Country/Area Profile Project. Available at: "https://capp.mau.se/country-areas/". Accessed January 12, 2020.
3. Wang X. Report of the Fourth National Oral Health Survey. Beijing, China. People's Medical Publishing House; 2018:39.
4. World Health Organization, Oral Health Programme. Basic global indicator of oral health 2000. DMFT Levels at 12 years. Geneva, Switzerland. World Health Organization; 1996:1.
5. Qi X. Report of The Third National Oral Health Survey. Beijing, China. People's Medical Publishing House; 2008: 16.
6. The General Office of the State Council of China. Policy issued by the General Office of the State Council of China. Available at: "http://www.gov.cn/zhengce/content/2017-02/14/content_5167886.htm". Accessed January 12, 2020.
7. The General Office of the State Council of China. Policy issued by the General Office of the State Council of China. Available at: "http://www.gov.cn/zhengce/2016-10/25/content_5124174.htm". Accessed January 12, 2020.
8. Jing Q, Liu Y, Wang M, Wang Y, Zhao X, Rong W. Prevalence and related factors of dental caries in 7-9-year-old children in the development district in Dalian. Journal of Practical Stomatology 2012;28(06):760-765.
9. Tu R, Zhong Y, Li X, Hu D, He T. Three years follow-up observation and analysis of caries status of primary and permanent teeth among 652 6-year-old children in Sichuan Province. West China Journal of Stomatology 2015;33(1):46-9.
10. Huang W, Tai B, Du M, Jiang H, Guo Y, Yuan B, Xu X: Survey and analysis of oral health status of 1898 6-7-year-old children in Yichang, Hubei Province. Journal of Oral Science Research 2002;(05):335.
11. Ekstrand KR, Christiansen J, Christiansen ME. Time and duration of eruption of first and second permanent molars: A longitudinal investigation. Community Dent Oral Epidemiol 2003;31(5):344-50.
12. Carvalho JC, Ekstrand KR, Thylstrup A. Dental plaque and caries on occlusal surfaces of first permanent molars in relation to stage of eruption. J Dent Res 1989;68(5): 773-9.

13. Brailsford SR, Sheehy EC, Gilbert SC, et al. The microflora of the erupting first permanent molar. Caries Res 2005;39(1):78-84.
14. Abreu-Placeres N, Garrido LE, Castillo Jáquez I, Féliz-Matos LE. Does Applying fluoride varnish every three months better prevent caries lesions in erupting first permanent Molars? A randomised clinical trial. Oral Health Prev Dent 2019;17(6):541-6.
15. Zhang LD, Zhou Y, Zhu YQ. Evaluation of effects of fluoride varnish on prevention of first permanent molar caries. Shanghai Kou Qiang Yi Xue 2017;26(6):641-5.
16. Suwansingha O, Rirattanapong P. Effect of fluoride varnish on caries prevention of partially erupted of permanent molar in high caries risk. Southeast Asian J Trop Med Public Health 2012;43(3):808-13.
17. Gugwad SC, Shah P, Lodaya R, et al. Caries prevention effect of intensive application of sodium fluoride varnish in molars in children between age 6 and 7 years. J Contemp Dent Pract 2011;12(6):408-13.
18. Marinho VC, Worthington HV, Walsh T, Clarkson JE. Fluoride varnishes for preventing dental caries in children and adolescents. Cochrane Database Syst Rev 2013;(7): CD002279.
19. Kohli N, Sullivan AL, Sadeh S, Zopluoglu C. Longitudinal mathematics development of students with learning disabilities and students without disabilities: A comparison of linear, quadratic, and piecewise linear mixed effects models. J Sch Psychol 2015;53(2):105-20.
20. Conklin HM, Ashford JM, Clark KN, et al. Long-term efficacy of computerized cognitive training among survivors of childhood cancer: A single-blind randomized controlled trial. J Pediatr Psychol 2017;42(2):220-31.
21. Moog NK, Buss C, Entringer S, et al. Maternal Exposure to childhood trauma is associated during pregnancy with placental-fetal stress physiology. Biol Psychiatry 2016;79 (10):831-9.
22. Schulz KF, Altman DG, Moher D, CONSORT Group. CONSORT 2010 statement: Updated guidelines for reporting parallel group randomised trials. BMJ 2010;340: c332.
23. Clark DC, Stamm JW, Quee TC, Robert G. Results of the Sherbrooke-Lac Mégantic fluoride varnish study after 20 months. Community Dent Oral Epidemiol 1985;13 (2):61-4.
24. World Health Organization. Basic methods. Oral Health Survey. 5th ed. Geneva, Switzerland. World Health Organization; 2013:42.
25. Milsom KM, Blinkhorn AS, Walsh T, et al. A cluster-randomized controlled trial: Fluoride varnish in school children. J Dent Res 2011;90(11):1306-11.
26. Hardman MC, Davies GM, Duxbury JT, Davies RM. A cluster randomised controlled trial to evaluate the effectiveness of fluoride varnish as a public health measure to reduce caries in children. Caries Res 2007;41(5):371-6.
27. Zenkner JE, Alves LS, de Oliveira RS, Bica RH, Wagner MB, Maltz M. Influence of eruption stage and biofilm accumulation on occlusal caries in permanent molars: A generalized estimating equations logistic approach. Caries Res 2013;47(3):177-82.
28. Yaegaki K, Masuda T, Suetaka T, Akamatu T. Correlations between first molar eruption, caries incidence and caries experience in primary school children. Shigaku 1989;77 (2):672-81.
29. Schwendicke F, Splieth CH, Thomson WM, Reda S, Stolpe M, Foster Page L. Cost-effectiveness of caries-preventive fluoride varnish applications in clinic settings among patients of low, moderate and high risk. Community Dent Oral Epidemiol 2018;46(1):8-16.
30. Devji T. Application of fluoride varnish during childhood and adolescence seems to be more cost-effective than doing nothing for patients with medium and high caries risk. J Am Dent Assoc 2018;149(1):e14.
31. Tagliaferro EP, Pardi V, Ambrosano GM, Meneghim Mde C, da Silva SR, Pereira AC. Occlusal caries prevention in high and low risk schoolchildren. A clinical trial. Am J Dent 2011;24(2):109-14.
32. Liu BY, Lo EC, Chu CH, Lin HC. Randomized trial on fluorides and sealants for fissure caries prevention. J Dent Res 2012;91(8):753-8.