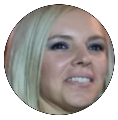
A prospective longitudinal cohort study of the effectiveness of 25% xylitol toothpaste on mutans streptococci in high caries-risk young children



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Abstract

Aim To compare the prevalence of mutans streptococci (SM) prevalence and categorical distribution in the saliva of high caries-risk children after their 24 months exposure to 25% xylitol toothpaste applied twice a day. To also examine the early caries susceptibility at baseline.

Study design An observational design with two times of collection. A total of 270 mother-infant dyads (mean age of infant 6.7 months) were initially recruited. At baseline, a dental exam looked for any early clinical signs of caries, and saliva collections were performed to assess SM prevalence. Two years later, other saliva collections were performed on a residual sample of 102 young children at the endpoint.

Method Two hundred four saliva samples were analysed with Dentocult, a commercial kit estimating in four categories, the SM distribution, expressed as the number of colonies forming units per milliliter of saliva (CFU/ml). The dental exam used the ICDAS system to include initial carious lesions (d1-2). The dentist administrated a maternal socio-economic questionnaire as the study collector.

Results The dramatic sample attrition between the two collection timepoints was likely caused by the fact that the first saliva collection made the children cry.

ICDAS system applications initially found that 14.4% of the infants aged 6.7 months experienced a total of 216 carious lesions, with 161 being initial and 55 being cavitated.

A statistically significant (0.001) difference in SM prevalence and distribution was found between the two collection times. The most impacting categories showed optimal benefits: bacteria-free increased (from 27% to 44%) and SM>10^5 decreased (from 17% to 5%).

Conclusion A domestic strategy using a natural toothpaste containing 25% of xylitol as a vehicle, and tooth brushing as a habit, showed a promising antibacterial cariogenic effect. This approach could be a relevant alternative to in-home care to prevent ECC and early SM contamination.

KEYWORDS caries, mutans streptococci, xylitol

Introduction

Early childhood caries (ECC) is an aggressive form of dental caries, which, if left untreated, can result in rapid and extensive cavitation in teeth (rampant caries) that is painful and costly to treat [Paul et al., 2009]. Dental problems were shown to be the main reason for the hospitalisation of children in Australia in 2015 [Chrisopoulos, 2016].

Despite evidencebacked recommendations for adopting more biological measures to counter the disease, a significant proportion of dentists continue following traditional caries management guidelines in their daily clinical practice [Philip, 2018]. Also, even after removing or restoring carious teeth, children remain at high risk for future recurrences, despite intervention such as topical fluoride/antimicrobial applications or recommendations to alter caries promoting feeding behaviours [Li Y, 2015].

The World Health Organization (WHO) has proposed that at least 80% of children aged six years should be caries-free by 2000 [Chen et al., 2015]. However, in terms of primary dentition, many children are found to be very far from this aim [Begzati et al., 2010]. Therefore, it was suggested that more effective preventive strategies are critically and urgently needed [Xiao et al., 2019].

ECC as a disease is a prime example of the consequences arising from the complex, dynamic interactions between microorganisms, host, and diet, leading to highly pathogenic (cariogenic) biofilms [Hajishengallis et al., 2019]. The bacterial group mutans streptococci (SM) is associated with the development of dental caries that has been used successfully as a marker for risk assessment, as well as monitoring the outcome of caries prevention [Tanzer et al., 2001].

Properties enabling S. mutants (SM) to colonise the oral cavity include the ability to survive in an acidic environment and specific interaction with other microorganisms colonising this ecosystem [Krzyściak et al., 2014]. This has inspired the formulation of medical strategies aimed to control MS colonisation in caries prevention rather than mechanical approaches [Köhler, 2012]. When we look at the antibacterial approaches for a medical ECC treatment, we observe limited options, primarily professional ones [Zhan et al., 2006]. We need an earlyintervention as suggested by [Xiao et al., 2019] since the peak of ECC onset occurs at three years of age, and there is a significant increase in incidence between the age of two and three years [Javed et al., 2017].

A new toothpaste formulation containing 25% of xylitol was proposed as a potential antibacterial agent against ECC causal bacteria. The current study hypothesised that children exposed long-term to this toothpaste might benefit from a protective flora effect regarding cariogenic bacteria prevalence.

Method

Study population

We used a cohort design with a 24-month follow-up. The ethics committee approved the study of Pristina University, Kosovo (Ref Nr 1851). The children were recruited almost from birth as part of RCT and recalled every three months.

Altogether, there were 102 mother-infant dyads from the previous study available for recruitment into the present study. Inclusive criteria for this cohort were: child age ≤ 12 months old, daily exposure of the child (twice a day) to a 25% xylitol toothpaste for 24 months, saliva collection, and parental acceptance for this study design during their attendance to the main center family medicine of Pristina through the immunisation department. Children aged one to 12 months were recruited and included the 24-month study follow-up.

Study variables

The study's primary dependent variable was the differential SM prevalence pre/post assessed in the 0 category (no SM detectable). The secondary outcome was the pre/post highly SM infected category (≥105) difference. In young children, these two bacterial categories are seen [Thibodeau et al., 1995; Alaluusua et al., 1987] as the most relevant to avoid future carious lesions. Independent variables might be pre SM child's prevalence, familial socio-economic status, and maternal level of SM infection.

Patient evaluation caries index

The caries experiences of participant children were assessed using the ICDAS system [Pitts, 2002]. Our cohort sample was evaluated at baseline with these criteria given their young age.

- 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 enamel visual change (opacity/ discoloration), seen without air-drying;
- Score 3: localised enamel breakdown without visible dentin.

Patient toothpaste exposure management

At the initial meeting, all cohort mothers received instructions on tooth brushing (daily, twice a day, using a pea-sized amount). All the mothers were contacted by phone to visit the dental

clinic at Pristina University to receive a 25% xylitol toothpaste tube and were instructed to implement the toothpaste for three months of use.

The study assistants were responsible for the supply. The 24-month follow-up led to a total of seven maternal visits for supplies.

Collection of microbial samples

We collected saliva from participant infants using the rough surface of dentocult SM saliva strip [Pienihäkkinen., 1995] at two study time points: at baseline, when the infant had an average age of 6.7 months, and two years later. After pressing for 30 seconds against the tongue's saliva, the strip was removed through gently closed lips.

The strips were placed in the selective culture broth with the smooth surface clipped and attached to the cap. The vials were incubated in an upright position at 37 °C for 48 hours with the cap opened one quarter to allow the growth of microorganisms.

Patient microbial evaluation of the samples: Participants were exposed to two microbial evaluations corresponding to the timings of collection. The growth on the strips was assessed according to the manufacturer's categorizing chart [Jauhari et al., 2015].

1. no SM detectable;
2. ≤102 SM CFU/ml of saliva
3. = 103-4 SM CFU/ml of saliva
4. ≥105 SM CFU/ml of saliva.

Each participant had two pre/post microbial samples.

Statistical analysis

All the data collected were calculated and statistically analysed. Values were expressed in the form of the mean and standard deviation of the mean. The comparisons were made by applying Chi-square test.

Results

Study Population: Table 1

The study included 102 children and their caregivers. At the start, the child's mean age was 6.7 (±3.7) months and, at the endpoint, it became 30.8 (±3.9) months. Study participation involved 43 girls and 59 boys.

At recruitment, the majority (59%) of children had no teeth Half of the caregivers (50.1%) were ready to brush their child's teeth as soon as the first tooth appeared in the mouth as recommended [Paglia., 2017]. About 29% of the children had siblings with a previous dental extraction. The proportion of children who were two or three times ill in their life so far was 20.6%.

Caregivers, on average, were aged 30.4 years, with a university education level for 48% of the sample. The majority of families fall in the lowest monthly income category (less than 500 EUR).

Finally, Table 1 shows the SM infection level trend of mothers included in the cohort with 60 participants, with 66.6% being in the highest categories (≥103 of SM prevalence). All variables were homogeneously distributed.

Child Caries Experiences: Table 2

At the average age of 6.7 months, and based on the ICDAS

Variables	Category	Cohort	P-value
Number of participants		N = 102	
Mean age in months at recruitment		6.7 (3.7)	*0.001
Endpoint mean age in months		30.8 (3.9)	*0.001
Gender	Male	58.0%	**0.47
	Female	42.3%	
Does the child have any teeth?	Yes	41.3%	**0.77
	No	58.7%	
When should you start cleaning your child's teeth?	As soon as the first tooth appears	50.1%	**0.79
	When the child is 1 year old	27.4%	
	When the child is 3 years old	15.7%	
	I don't know	6.9%	
Sibling had an extraction in the past?	Yes	29.0%	**0.44
	No	71.0%	
How many times in his/her life has your child been ill?	Never	58.8%	**0.06
	Once	18.6%	
	Two or three times	20.6%	
	More	2.0%	
Maternal age in years		30.6 (±3.8)	*0.001
Maternal level of education	Graduate from primary school	15.7%	**0.82
	Graduate from high school	32.3%	
	College	3.9%	
	University	48.0%	
Monthly familial income	Less than 500 €	52.9%	**0.31
	600-1000 €	35.3%	
	1000-2000 €	9.8%	
	2000-3000 €	1.2%	
Some mothers and level of SM?[1]	0	6.0%	**0.50
	1	31.4%	
	2	33.0%	
	3	33.6%	

[1] Sixty mothers of the 102 dyads were randomly selected to evaluate their SM level

TABLE 1 Socio-economic variables of cohort participants

system evaluations, caries prevalence affected 12.4% of children at baseline.

The mean caries experiences was 0.70 (2.42) on average. The majority (74.2%) of the carious lesions were initial, although 21 lesions (25.8%) were classified as cavitated.

Bacterial Flora

Baseline Child Levels of SM Infection: Table 3

Based on the manufacturer's categorising chart, four infection levels were documented. At baseline, 72.6% of children were SM infected. The primary dependent study variable, level 1 or 0 SM detectable, was initially estimated of 28 children (27.4%). The secondary dependent study variable, level 4 or a prevalence ≥105 SM was at baseline, of 17 children.

Other categories, level 2 and 3 (102-4 SM) gathered 56% of the initial cohort. This SM infection categorical distribution was significantly different.

Variables	Clinical Indicators
Number of participants	N = 102
Mean age of participants in months	6.7 (3.8)
Mean dmfs	0.70 (2.48)
% of dmfs ≠ 0	12.4%
All lesions	n=82
Initial (01-02) lesions	n=61
Initial lesions mean	0.60 (1.68)
Cavitated lesions	n=21
Mean cavitated lesions	0.20 (1.08)

TABLE 2 Cohort initial caries experiences description

Variables	SM prevalence by category Frequency/ proportion	P-value
Number of participants	N = 102	
0	28 (27.4%)	0.02
10^2 UFC/ml	37 (36.3%)	
10^{2-4} UFC/m	20 (19.7%)	
$10^{\geq 5}$ UFC/m	17 (17.1%)	

TABLE 3 Cohort baseline SM infection at 6.7 months old, N=102

Endpoint Child Levels of SM Infection: Table 4

At the endpoint, 53.5% of children had detectable SM.

The primary outcome variable, level 1 or 0 SM detectable, showed a final proportion of 44.5%, corresponding to 45 participants. The secondary outcome study variable, level 4 or a prevalence ≥105 SM changed for an endpoint proportion of 5% associated with five participating children. Other categories, level 2 and 3 (102-4 SM) gathered 50.5% of the final cohort. The categorical distributions were highly statistically significant.

Comparison of Pre/Post-Study Outcome

Distributions Generally speaking, after the 24 months of 25% xylitol toothpaste exposure, the protective level of children SM-free increased from 27.4% to 44.5 % for a net 19.1% improvement. The secondary outcome concerned the highest SM prevalence category, where the study follow-up led to a net 12.2 % reduction. The other categories decreased from 56.% to 50.5%. The 25 % xylitol toothpaste exposure significantly changed the global pre/post categorical distributions.

Discussion

SM was reported and suggested to play a significant role as an etiologic factor in the cariogenic process [Tanzer et al., 2001] by its colonisation of biofilm on tooth surfaces [Zou et al., 2008] and being one of the critical risk factors of the development of ECC [Berkowitz., 1996]. Previous studies have shown that xylitol is effective in inhibiting SM [Soderling.,

Variables	SM endpoint Frequency/ proportion	*P-value*
Number of participants	N = 102	
0	45 (44.5%)	0.0001
10^2 UFC/ml	23 (22.8%)	
10^{2-4} UFC/m	28 (27.7%)	
$10^{\geq 5}$ UFC/m	5 (4.9%)	

TABLE 4 Cohort endpoint SM infection at 30.7 months old, N=102

Variables	SM Pre Frequency/ proportion	SM Post Frequency/ proportion	*P-value*
0	28 (27.4%)	45 (44.5%)	0.002
10^2 UFC/ml	37 (36.3%)	23 (22.8%)	
10^{2-4} UFC/m	20 (19.2%)	28 (27.7%)	
$10^{\geq 5}$ UFC/m	17 (17.1%)	5 (4.9%)	

TABLE 5 Parallel comparison of pre/post SM infection after 25 % xylitol toothpaste exposure

2009]. Traditional treatment does not lead to a significant decrease in the level, and recurrence of the disease is biologically possible [Berkowitz et al., 1997]. Reducing the number of SM colonies as the main culprit responsible for the development of tooth caries leads to non-invasive and can be easily tolerated by young children [Erkmen., 2020]. So far, professional approaches [Zhan et al., 2006; Bargarizan et al., 2019], were suggested as a potential antibacterial agent, but with questionable feasibility on very young children. Therefore, other domestic preventive methods and conventional restorative treatments are needed to reduce cariogenic bacteria [Gregory et al., 1998]. In this study, we tested an at home acquired daily routine: tooth brushing containing, as a world premiere, 25 % xylitol toothpaste. Our results on SM bacteria reduction are consistent with previous studies by using xylitol chewing gum regimens but in older participant [Autio., 2002, Aluckal., 2018]. In addition, we assessed the antibacterial effect by key-threshold of SM contamination with success in concordance of modern management of the caries process [Selwitz et al., 2007]. Overall, the treatment changed the pre/ post children's level of SM infection significantly.

This is the first time a specific xylitol toothpaste has been tested as a potential antibacterial agent against ECC main bacteria. There is no reason to believe that the children were exposed to clinically meaningful doses of xylitol from other sources on the Kosovar market since there is no other xylitol products available. We cannot formally compare our results to that of other studies. However, a recent search of the Cochrane Library [Yihong et al., 2015] on antibacterial intervention effect on microbiota associated with ECC revealed [Pienihäkkinen, 1995] systematic reviews related to fluoride and ECC, four reviews on chlorhexidine plus fluoride and dental caries, three reviews on xylitol, and five reviews on other interventions or treatments of ECC.

They concluded that antimicrobial interventions and treatments show temporary reductions in SM colonisation levels, but insufficient evidence indicates that the current antimicrobial therapeutic approaches produced sustainable effects on cariogenic microbiota or ECC reduction and prevention [Yihong et al., 2015]. The authors mentioned that the research mission established over a decade ago has not yet been accomplished. The ages of six to 12 months thus represent a period when key variables may be controlled to reduce SM colonisation [Plonka et al., 2012]. We decided to initiate this long-term cohort to assess the antimicrobial effect of daily xylitol toothpaste on cariogenic bacteria independently. Recruiting, on average six-month-old infants, the actual study was planned to prevent and decrease SM contamination to reduce the ECC risk. Our results show that we succeeded in both goals by changing the odds of being more infected with age.

This study had two antibacterial outcomes. The first one was the capacity of our treatment to increase the first category (no detectable SM) from toothpaste exposition between the ages of six and 30 months. The other categories of infected children changed the primary outcome category from 27.4% to 44.5% for a significant net increase of 38% of children without any detectable SM. The second outcome was the treatment capacity to change the highly infected status (≥105 SM) for all future caries risk benefits. The anti-bacterial treatment reduced this status by 71% (from 17% to 5% children).

Our results confirmed the high caries risk status of participated children since literature showed that children under two years old, having already detectable SM in their saliva, are at high risk of future carious lesions [Berkowitz., 1996; Alaluusua et al 1983]. Based on Plonka et al. [2012] early SM contamination is usually detected in early childhood high caries risk populations. After 24 months of 25% xylitol toothpaste exposure, the endpoint SM evaluation showed a global bacterial prevalence of 55.5%, corresponding to a net count decrease of 17.3%. Although fatality based on literature predicts a fulgurate contamination increasing with age, it seems that this 25% xylitol toothpaste was able to reverse this tendency.

In a systematic literature review [Yihong et al., 2015] identified research-based evidence for an effect of antimicrobial therapeutic approaches on the cariogenic microbiota and ECC outcomes looked at the xylitol product's effect. Several xylitol delivery vehicles were used, including chewing gums, tablets, wipes, and combined treatment with NaF. The age of the children studied ranged from six months to five years. The meta-analysis of xylitol-based interventions indicated an overall significant reduction of SM colonisation in young children.

Autio et al. [2002] observed a shift in SM scores from high to low within three weeks in children who chewed xylitol gum. By contrast, Oscarson et al. [2006] reported no difference in SM levels between test and control groups after a two-year follow-up observation. The study started with a very low dose of xylitol during the first six-month period due to not being sure if these small children would have any problem handling the tablets. Chewing gums are not generally considered as safe in this age group, which also was the reason to refrain from using chewing gums in the intervention [Oscarson et al., 2006]. Seki et al. [2011] using older children, found that xylitol gum led to reduced SM in dental plaque and noted that over 10% of the children experienced diarrhea in the experimental group. Daily xylitol wipe applications did not lower salivary SM and Lactobacillus levels over a 12-month observation [Zhan et al., 2012].

Notably, the meta-analysis results seem to suggest that xylitol delivered by tablets had the least anti-microbial effect, perhaps due to the lack of proper interaction with the oral microflora, and was, therefore, less effective in reducing SM adhesion [Soderling., 2009] versus other modes of delivery lack of true comparative control groups in the clinical studies. Although there is strong evidence supporting xylitol-containing chewing gum to reduce dental caries in adolescent and adult populations, [Deshpande, 2008] one should not automatically assume that the gum will be as effective for preschool-aged children.

The meta-analysis highlighted the paucity of high quality randomized controlled clinical trials that demonstrated the efficacy of commonly used antimicrobial agents and procedures. Many of the tested agents have been evaluated in adult populations and were highly recommended by dental professional organisations; thus, it was assumed that the same agents would provide preventive benefits for young children [Yihong et al., 2015].

Previously, the etiology of caries was not fully identified due to limited knowledge of the oral microbiome [Corby et al., 2005]. Also, with stagnation [Jordan et al., 2016] or increasing [Smith et al., 2013] of epidemiological indicators of ECC, it becomes interesting to validate a new antibacterial agent integrating the daily childhood routine easily.

Our results showed an already established high level of detectable SM at baseline 6.7 months of age, with 72.6% of infants infected. These results align with previous studies on early SM colonisation [Plonka., 2012, Wan et al., 2001, Wan et al., 2003] in high-risk populations and Kosovar scientific publications on the same topic [Begzati et al., 2010; Begzati et al., 2014]. This early SM colonisation is associated with early carious lesions and S-ECC [Costa et al., 2017]. Also, early cariogenic contamination is typically associated with a pathologic flora establishment [Plonka., 2012]: our results differed from this fatality with fewer children (from 72.6% to 55.4%) infected two years later. Late SM colonisation seems to be protective for future carious lesions [Straeremans et al., 1998]. The SM prevalence in this study with young Kosovar children seems to be in phase with the SM count found in high-risk first Nations children [Agnello et al., 2017] or with children experimenting severe ECC [Meric et al., 2020].

Strengths

The cohort design showed the capacity of 25% xylitol toothpaste to inhibit the growth of oral cariogenic bacteria. Reporting in infection categories gave a detailed picture of the pre/post changes. The two outcomes (no detectable bacteria and highly infected) validated are the bacteria levels with the most caries impacts. The study sample size by itself was a strength of the design. In their systematic review to identify research-based evidence for an effect of antimicrobial therapeutic approaches on the cariogenic microbiota and early childhood caries ECC, Yihong and Anne [2015] identified related studies [Agnello., 2017]. Our study sample (n=102) was higher than 63% of the Agnello [2017] study and higher than six on eight studies concerning xylitol [Agnello et al.,2017; Aluckal et al., 2018; Autio JT, 2002; Oscarson et al., 2006; Deshpande A., 2008; Seki et al., 2012; Soderling E., 2009; Zhan et al., 2012].

The study's retention of participants for a long-term followup, considering the invasive saliva collection on very young children, is also a strength. Our early intervention at six months may influence alternatives to reduce caries risk in the following months of these children's lives.

Limits

As this study was a world premiere, without a control group, our goal was first to explore the potential of an increasing xylitol concentration using toothpaste as a vehicle in SM control and prevention. In these high caries risk populations, results were significant. The next research opportunity could extend this scientific knowledge by having a formal control group. By providing the mother three months' worth of toothpaste, we may create a Hawthorn and social desirability effect, increasing the treatment impact: introduction of tooth brushing can help prevent the colonisation by SM [Seow et al., 2003].

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