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Effects of short-term xylitol chewing gum on pro-inflammatory cytokines and *Streptococcus mutans*: A randomised, placebo-controlled trial

Özer Akgül[1] | Aslı Topaloğlu Ak[2] | Sevgi Zorlu[2] | Didem Öner Özdaş[2] | Melisa Uslu[2] | Dilara Çayirgan[2]

[1]Department of Medical Microbiology, Faculty of Medicine, Istanbul Aydin University, Istanbul, Turkey

[2]Department of Pedodontics, School of Dentistry, Istanbul Aydin University, Istanbul, Turkey

Correspondence
Özer Akgül, Department of Medical Microbiology, Faculty of Medicine, Istanbul Aydin University, Istanbul, Turkey.
Email: akgulozer@hotmail.com

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Abstract

Introduction: Dental caries is an infectious disease with predominantly of cariogenic bacteria such as *Streptococcus mutans* (*S mutans*). Xylitol is considered as one of the effective agents that can limit this dental infection. In this randomised, placebo-controlled trial, we aimed to evaluate the potential reflection of short-term xylitol consumption on pro-inflammatory cytokines (TNF-α, IL-6 and IL-8) and *S mutans* counts by ELISA and qPCR (Quantitative real-time PCR), respectively.

Methods: In this study, 154 participants were assigned to two groups, control and xylitol. Dental examination, saliva and swab samples were done at baseline and at 3-week for clinical and microbiological assessment.

Results: In xylitol group at the end of 3-week, gingival and plaque index scores were significantly decreased with respect to baseline values ($P < .001$ and $P < .05$, respectively). The salivary concentration of TNF-α, IL-6 and IL-8 were statistically declined at 3-week, more so than those at baseline in xylitol group ($P < .001$). *S mutans* expression was reduced about fivefold at 3-week use of xylitol and it was a statistically significant difference compared to baseline ($P < .001$).

Conclusion: Intriguingly, even short-term consumption of xylitol might play a favourable role in maintaining the oral health status, possibly as a result of decreasing the release of pro-inflammatory cytokines and the counts of *S mutans*. Nonetheless, this investigation warrants further endorsement.

1 | INTRODUCTION

The global increase in sugar consumption resulted with systemic health problems, such as heart disease, some cancers and tooth decay, which have led to the use of sweeteners.[1] At the beginning of 1960s, a sugar alcohol called xylitol was first approved by FDA (Food and Drug Administration) to use in foods.[2] It has been reported to have positive effects on oral health, since it cannot be fermented by cariogenic bacteria. Subsequently, microbial dental plaque formation and *Streptococcus mutans* (*S mutans*) have been reported to reduce.[3] Its use has been effective in oral health programs in schools and thus it has been recommended in the prevention of dental caries.[4]

Cochrane analysis of randomised studies demonstrated that xylitol-containing fluoride toothpastes are more effective than fluoride toothpastes in dental caries prevention among children.[5] In addition, xylitol-containing products have been found to be effective in children and adults. Recent research focuses on xylitol chewing gums due to its ease of use, particularly among children and disabled individuals.[6,7]

A possible role of xylitol in caries prevention has been shown to persist after the active intervention period. 44%-59% reduction in caries prevalence was reported among children who consumed xylitol chewing gum for two years compared to those who did not chew after 3 years of the study. However, in the dental literature conflicting

results are present about the efficacy of xylitol on oral health due to different study designs, dose and frequency of xylitol use.[5]

On the other hand, link between carious lesion initiation and the response of immune cells have been revealed. Activated monocyte-macrophage cells produce cytokines which are significant mediators of inflammation. These cells express a vast number of pro-inflammatory cytokines, such as tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-8 (IL-8). It is generally agreed that pro-inflammatory cytokine concentrations are increased within both serum and saliva of people with periodontal inflammation.[8] To date, there has been no specific randomised, placebo-controlled article published regarding the effect of xylitol on pro-inflammatory cytokines.

Numerous studies have supported the idea that *S mutans* plays a critical role in caries process.[9,10] One such study reveals that *S mutans* is detected more in counts in high caries activity groups in comparison to caries free individuals. Hence, bacteria counts may be applied as a microbiological parameter for screening the caries risk status. Likewise, the presence of salivary *S mutans* trigger the production of pro-inflammatory cytokines.[11]

The conventional (culture-based) researches have focalised on the impact of xylitol usage on *S mutans* presence in oral cavity. The results obtained from these studies can only provide limited information about the possible effects of xylitol on *S mutans*.[12,13] Among the reasons for the limited data obtained by culture-based methods are *S mutans* can only be identified phenotypically, the sensitivity/specificity rates of these methods are low and false negative results can be obtained. Despite the fact that the conventional polymerase chain reaction (PCR) provides valid results, it unable to screen exact quantification. Quantitative real-time PCR (qPCR) can ensure a sensitive technique for not only detection, but also for quantification of *S mutans* populations.[14] *S mutans* quantification in saliva is superior to over previous qualitative molecular methods. In qPCR, microbiological information is gained not only about the presence of *S mutans* but also about the number of *S mutans* in saliva sample that might be interconnected with immunological and/or dental caries status.

There is no clinical study investigating the impacts of short-term xylitol consumption on pro-inflammatory cytokine concentrations and *S mutans* counts by qPCR. As a resust of lack of data in this area, the present randomised controlled clinical study aimed to interpret the effects of short-term xylitol consumption on pro-inflammatory cytokines namely, TNF-α, IL-6 and IL-8 and *S mutans* counts by qPCR method.

What's known

Dental caries occurs with predominantly *Streptococcus mutans* (*S mutans*). Xylitol is considered as one of the effective agents that can limit this dental infection.

What new

The short-term use of xylitol may play a favourable role in protect oral health, possibly as a result of decreasing the release of pro-inflammatory cytokines and the counts of *S mutans*. The xylitol-containing chewing gum might be an effective agent in maintaining oral health, decreasing cytokines release and *S mutans* counts.

2 | METHODS

In this prospective, randomised controlled, single-blind study, the effect of using xylitol was compared to the control group.

2.1 | Study design and sample size calculation

This study was approved by the Istanbul Aydin University Clinical Research Ethics Committee with 2019/3 protocol number in terms of the study methods and protocols prior to implementation. All participants gave informed consent before taking part in the study. We have adhered to the Consolidated Standards of Reporting Trials (CONSORT) guideline for parallel-group randomised trials.[15] For the randomised study design and using the outcome measures, the statistical power analysis indicated that a total sample of n = 67 in each group would have the assumption of 95% CI and power of 80%. A nominal $\alpha = 0.05$ and two-tailed tests were used in the power calculations.

2.2 | Study participants and intervention

A total of 230 dental students accepted to participate and were screened for eligibility, of whom 154 (66.9%) were eligible for enrolment. Participants were eligible if they reported good health systemically, had no allergy to xylitol, and between 18 and 65 years old. Exclusion criteria included using topical fluoride application for the last 4-week, using antibiotics in last 3-month before the evaluation, having untreated caries lesion or gingival/periodontal disease. A total of 154 subjects participated in the study and in the second week of the study, seven subjects (three in control; four in xylitol groups) were excluded from the study because of the use of antibiotics. They were then randomised by a biostatistician using a computer-generated randomisation program to either control or xylitol groups; participants were allocated 1:1 into the study arms. The flow diagram, showed in Figure 1, explains the design of the study. In terms of standardise the possible caries progress, dental students with similar brushing habits and sugar intake were included this research. The subjects included in the study were trained to brush with 1450 ppm fluoride toothpaste twice a day in the morning and evening. The subjects were asked to continue their normal dietary intake. The subjects were asked to chew the gums that were distributed to them in small locked packages on a weekly basis. In control and xylitol groups, distributed gums had a same shape, amount and packaging. The subjects were instructed to chew for at least ten minutes, two gums three times a day (after each meal). The xylitol group

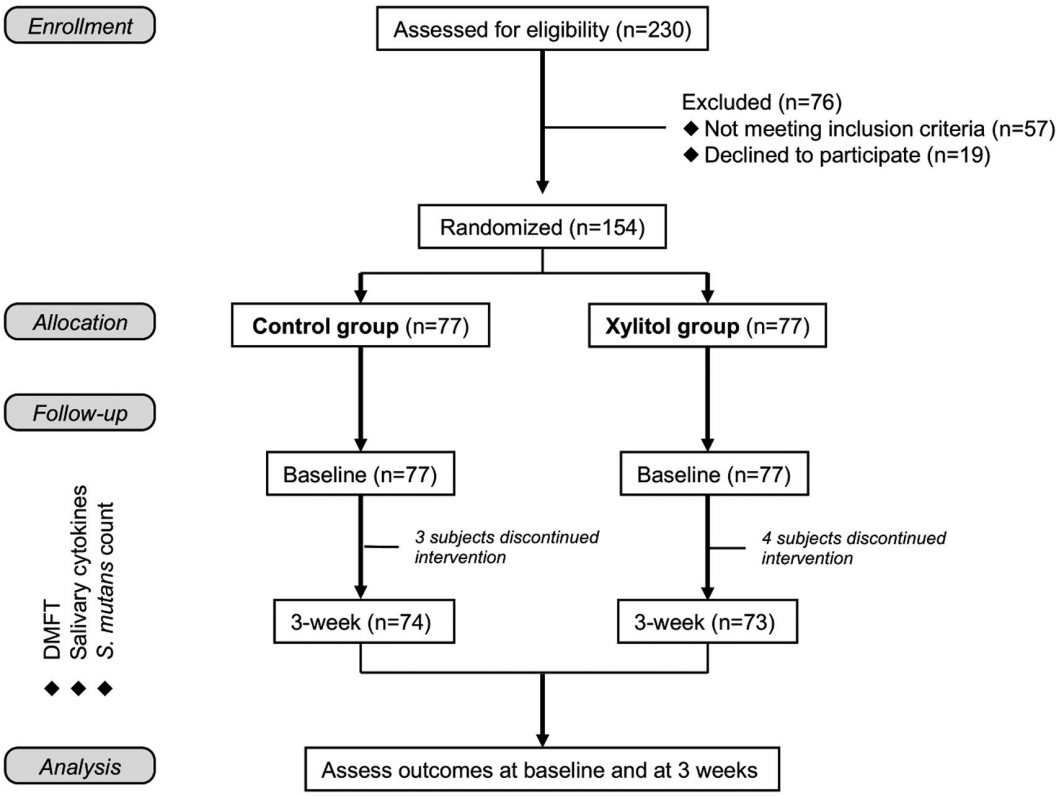


FIGURE 1 Flow diagram of the study

was asked to chew xylitol-containing chewing gums (Vivident Xylit, Perfetti van Melle Food Co.). Since each chewing gum contained 0.9 g xylitol, the participants received 5.4 g xylitol daily. At least one reminder message was sent every day to chew the gums. During the study, all participants were asked to strictly avoid using other commercially available gums.

2.3 | Oral clinical evaluation

Intra oral examination was performed by one dentist using mirror and probe with cotton pellets and suctions under reflector light. DMFT (decayed, missing, filled teeth) scores were recorded as stated in WHO standard (1997), whereas Gingival Index and Plaque Index were used to adjust the microbial plaque and gingival tissue.[16,17] Intra oral examinations were done prior to having breakfast and tooth brushing. Dental plaque and gingival indices of the patients were recorded for each tooth and the scores were added up and compared in groups at baseline and 3-week time points.

2.4 | Analysis of cytokines

The following cytokine analysis in saliva were performed: IL-6, IL-8 and TNF-α with unstimulated saliva collection method and stored at −80°C prior to analyse. The concentrations of above-mentioned cytokines were simultaneously measured by using the commercial ELISA (Enzyme-linked immunosorbent assay) kits (Boster). The cytokines were measured following the manufacturers' instructions, for which the sensitivity limit was <1 pg/mL. Cytokines were calculated as pg/mL based on the mean fluorescent intensities.

2.5 | DNA extraction

For PCR-quantification, the microbial swab samples were collected 80 mm^2 on oral cavity. DNA was extracted using GeneMark GMPure Swab DNA isolation kit (BM Labosis) according to the manufacturers' instructions. Spectrophotometer were used to measure DNA concentration and purity at 260/280 nm in extraction samples (Nanodrop 2000C Thermo Scientific). The extracted DNA was stored at −20°C prior to testing.

2.6 | Quantitative Real-time PCR of *S mutans*

The quantitative real-time PCR (qPCR) of *S mutans* was performed in the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules) using *S mutans* specific primers listed in Table 1 as previously described.[18] Reaction mixture contained 10.4 μL of nuclease-free water, 4 μL of 5X GM SYBR qPCR Master Mix (GMbiolab, Taiwan), 0.3 μL of 10 μmol/L forward and reverse primer and 5 μL DNA Template. The thermal cycling was performed at 45 cycles (Enzyme activation at 95°C for 3 minutes, denaturing at 95°C for

20 seconds, annealing at 63°C for 30 seconds and extension at 72°C for 45 seconds). Melting curves were generated from 60 to 95°C and read every 0.5°C for 5 seconds. For the relative quantification in qPCR, $2^{-\Delta CT}$ mathematical model were used to calculate *S mutans gtfB* gene expression.[19]

2.7 | Statistical analysis

Clinical profile, cytokine and gene expression were evaluated using SPSS version 23.0 (IBM Corp.). The differences between control and 3-week groups were determined by applying a one-way analysis of variance. The clinical profile and cytokines are presented as the mean ± standard deviation (SD). In the gene expression experiments statistical analysis was performed using Wilcoxon analysis of variance at a significance level. The figures display the mean and standard error. A $P < .05$ was considered to indicate a statistically significant difference. Statistical significance is denoted by an asterisk.

3 | RESULTS

The average age of the 154 participants (79 female, 75 male) was 23.43 ± 2.3. Mean gingival and plaque index score changes in study groups at different time points (baseline and 3-week) are given in Table 2. At baseline, plaque and gingival index cores were similar in the groups, with no statistically significant differences. No statistically significant differences were calculated in control group at baseline and 3-week time points. A statistically significant decrease was noted for both gingival ($P < .001$) and plaque index scores ($P < .05$) in xylitol group at the end of 3-week time point.

Similar to the results obtained in the clinical profile, a statistically significant decrease in cytokines was observed at 3-week period of xylitol group. In control group, salivary TNF-α, IL-6 and IL-8 concentrations were similar at baseline and 3-week time points, with no statistically significant differences. The TNF-α concentration was statistically significantly decreased in xylitol group, ranging from 204.66 ± 173.49 pg/mL at baseline to 81.86 ± 75.77 pg/mL at 3-week evaluation ($P < .001$). Similar to TNF-α, the concentrations of IL-6 and IL-8 were decreased at 3-week compared to baseline. The level of salivary IL-6 was 159.71 ± 116.39 pg/mL at baseline and 45.62 ± 21.27 pg/mL at 3-week ($P < .001$). The most dramatic reduction in evaluated cytokines was observed at salivary IL-8 concentration (381.9 ± 254.83 at baseline and 113.41 ± 92.8 at 3-week, $P < .001$). The results of this study clearly showed that the concentration of TNF-α, IL-6 and IL-8 in xylitol group at 3-week decreased statistically significantly compared to baseline (Figure 2). These cytokine results indicate that xylitol might have a suppressor effect of inflammatory cytokine production.

The Sm F5 and Sm R4 primers which anneal to conserved sequences of *gtfB* gene of *S mutans* were selected and compared its expression in each tested group at different time points (baseline and 3-week). Standard curves were utilised to screen the exact number of *S mutans* tested while melting peaks (Figure 3). The calculated $2^{-\Delta CT}$ values in control group at baseline and 3-week were similar, while a significant decrease was recorded in xylitol group (21.98 ± 12.5 at baseline and 4.11 ± 3.73 at 3-week). As presented in Figure 4, the qPCR result evaluated that *S mutans* expression was decreased about 5-fold in xylitol group at the end of 3-week evaluation, and it was a significant difference compared with baseline value ($P < .001$).

TABLE 1 Nucleotide sequences, positions and melting temperatures of the primers used in this study

Primer	Sequence (5′-3′)	Position	T_m[a] (^{0}C)	Fragment size (bp)
Sm F5	AGCCATGCGCAATCAACAGGTT	2007-2028[b]	69	415
Sm R4	CGCAACGCGAACATCTTGATCAG	2421-2399[b]	70	

[a]Melting temperature calculated by Primer Express (Applied Biosystems) at 1 WM of each primer.
[b]The position of the nucleotide sequence from the ATG codon of the gtfB gene (accession number: D88651).

TABLE 2 Gingival and plaque index scores in control and xylitol groups at baseline and 3-week time points

		Control		Xylitol		
		Subjects (n)	Mean ± SD	Subjects (n)	Mean ± SD	*P*-value
Gingival Index	Baseline	77	7.48 ± 3.93	77	6.83 ± 4.2	.17
	3-week	74	8.81 ± 2.14	73	2.06 ± 0.5	**<.01**
	P-value		0.31		**<0.01**	
Plaque Index	Baseline	77	18.82 ± 9.2	77	20.71 ± 11.4	.24
	3-week	74	17.11 ± 8.17	73	11.21 ± 5.43	**<.05**
	P-value		.87		**<.05**	

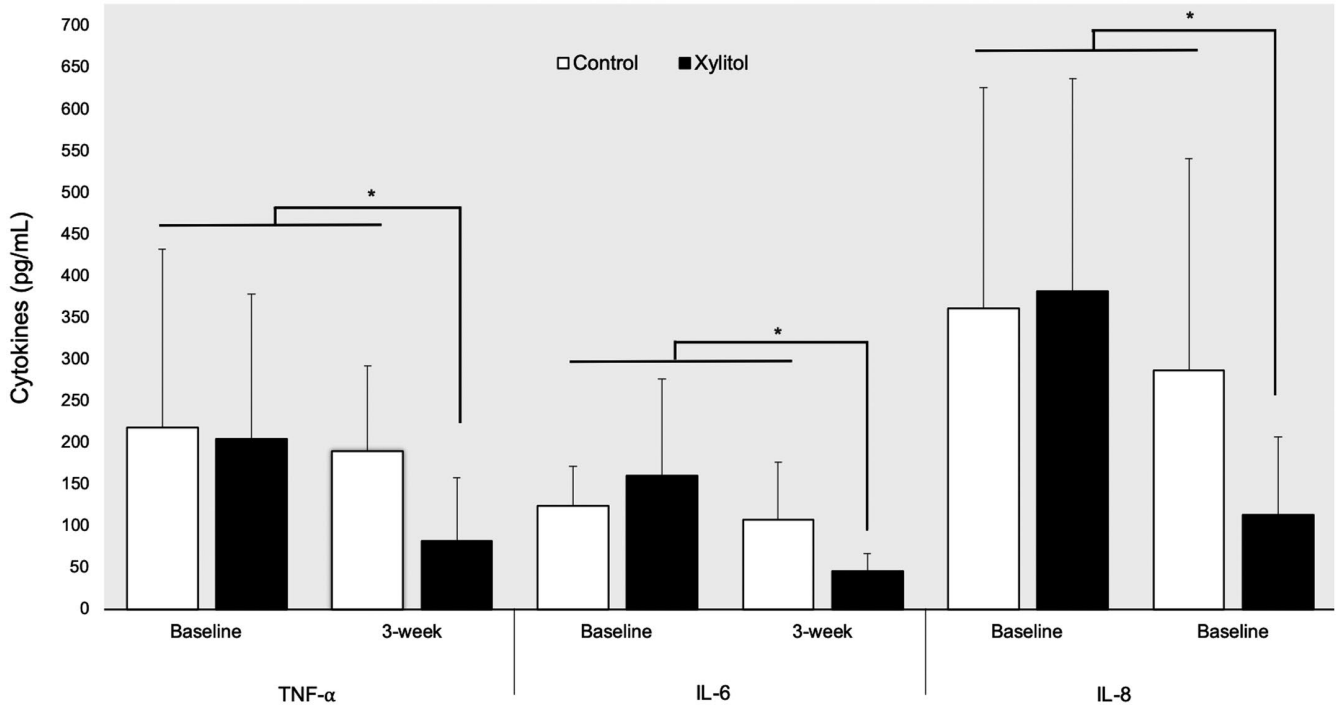
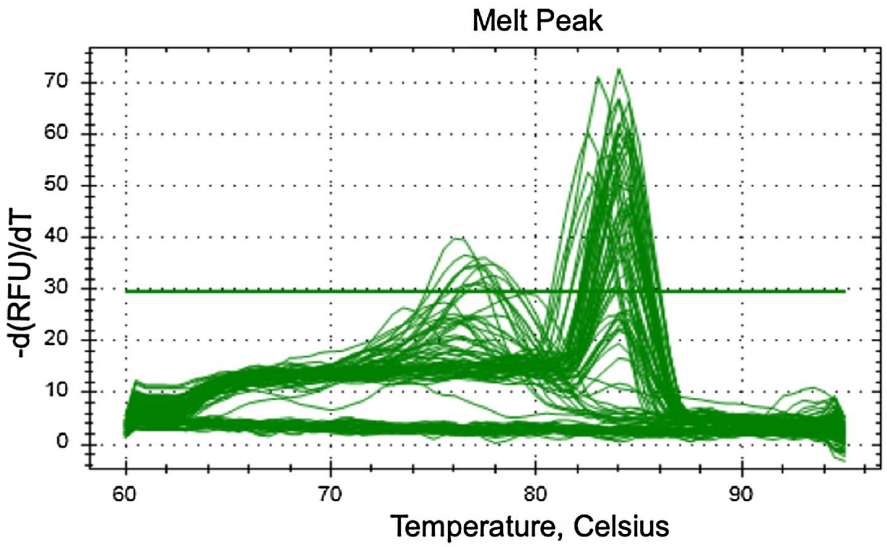


FIGURE 2 Salivary pro-inflammatory cytokines in control and xylitol groups at baseline and 3-week evaluation. Data are presented in terms of mean ± standard deviation. *$P < .001$

FIGURE 3 Melt curve profiles and melting temperatures for *S mutans*



4 | DISCUSSION

Xylitol has been approved originally as sweetener in chewing gum. In dental literature, proper use of xylitol-containing gum could be effective in preventing caries,[20] presumably by accelerating saliva flow and pH[21] and boosting remineralisation of enamel lesions.[20] Based on the findings in dental literature review, the change in selected pro-inflammatory cytokines (TNF-α, IL-6 and IL-8) and in molecular counts of *S mutans* was investigated in the present study. The volunteers included in the study were selected among dental students as they can be traced easily. They were also with similar tooth brushing habits and diet in an attempt for standardisation among all 154 included subjects in the study.

Impacts of xylitol chewing gum on dental health have been investigated for both long and short-term use[7,22,23] and the possible effects of xylitol on microbial dental plaque, *S mutans* counts and saliva pH were evaluated. In these clinical studies, it was clearly demonstrated that xylitol chewing gum was not effective when daily consumption was below 3.4 g.[24] Therefore, in the present study 5.4 g xylitol containing chewing gum was used three times after each meal for 10 minutes a day for 3-week period.

Xylitol is broadly preferred anti-cariogenic ingredient as *S mutans* unable to fermented it.[25,26] Shyama *et al*[7] evaluated the efficacy of long-term xylitol use on microbial dental plaque and gingival index scores in physically disabled students. One hundred

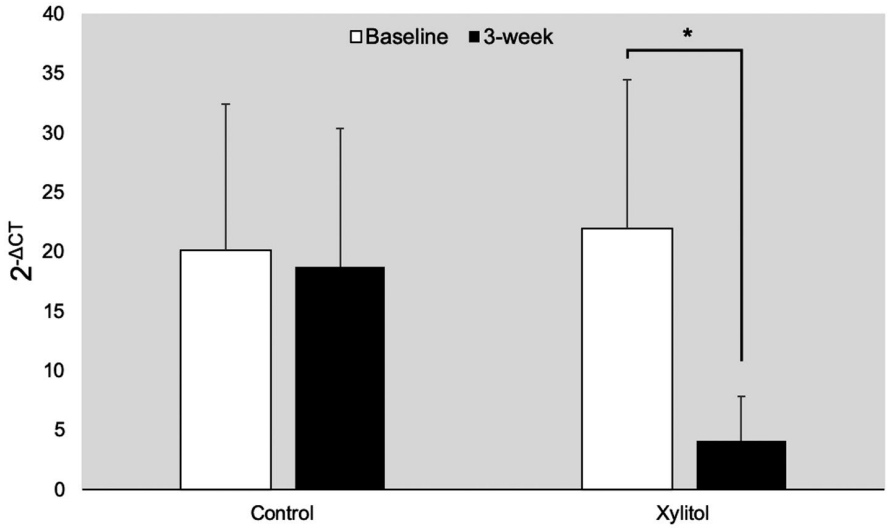


FIGURE 4 *S mutans gtfB* gene expression. Bars represent the mean expression levels; error bars represent the standard error limits. *$P < .001$

forty-five students chewed xylitol-containing gum three times per day for 18 months. A statistically significant decrease was reported in the plaque and gingival index scores. Thus, xylitol chewing gum was recommended in addition to oral hygiene in physically disabled individuals. In line with these findings, the present study pointed out a significant decrease in gingival and plaque index scores after 3 weeks. Alamoudi *et al*[27] conducted an 18-month long-term follow-up study in which 60 mother-child couples were included. Mothers were given xylitol chewing gum whereas children were provided with chewable xylitol tablets three times a day for 3 months. After 6, 12 and 18 months, new caries formation, microbial dental plaque and *S mutans* counts were evaluated. Consequently, a statistically significant reduction in the counts of *S mutans*, mean plaque scores and new carious lesion formation were reported. However, there are different conclusions about the efficacy of xylitol in dental literature. Masoud *et al*[28] evaluated the effect of 6 grams of xylitol daily for 3 months among 30 volunteers with fixed orthodontic appliances. There was no significant decrease in plaque score, *S mutans* counts in dental plaque and saliva. This might be attributed to fixed orthodontic appliances which might shadow the effect of xylitol use. Although the design of our study was planned on short-term xylitol consumption, positive changes reflected in the gingival index and plaque index values are promising.

Cytokines may be one of the useful parameters for diagnosis and monitoring the dental health, and saliva could be utilised as a non-invasive diagnostic sample to measure pro-inflammatory cytokines released during caries initiation and progression. Cytokines regulate many aspects of the immune response. In this response, molecular patterns of oral pathogens, like *S mutans*, bind to specific receptors on oral cavity cells, which then trigger the immunological response with a release of cytokines. The selected pro-inflammatory cytokines including TNF-α and IL-6 are one of two cytokines that are grouped as notable among these pro-inflammatory cytokines. Also, TNF-α and IL-6 are mainly considered to be unique mediators of acute inflammation.[29,30] IL-8 is synthesised by a vast number of different immune cells. The main role of IL-8 is to mediate the neutrophil migration and then activate them.[31] However, the effect of short-term xylitol consumption on pro-inflammatory cytokines has not yet been investigated. Our findings clearly highlighted a statistically highly significant decrease in salivary TNF-α, IL-6 and IL-8 using ELISA after the short-term consumption of xylitol ($P < .001$).

This was the first randomised controlled study which determined to allow comparison the amount of *S mutans* at baseline and 3-week use of short-term xylitol consumption on oral swab samples using qPCR. In dental literature, elevated TNF-α, IL-6 and IL-8 have been correlated with high numbers of *S mutans* and active caries lesions.[8] Many previous studies have suggested that the xylitol consumption could be have a reducing effect on *S mutans* counts.[32-34] To our knowledge there has been no other published data on the effect of xylitol on pro-inflammatory cytokines and *S mutans* counts. Swab samples were collected instead of saliva, in order to determine the number of *S mutans* using qPCR for the first time in the present study. In this manner, standardisation was ensured to determine the number of *S mutans* more accurately and to make relevant comparison among the groups. Our results showed that 3-week xylitol consumption resulted with decrease in the number of *S mutans* on standardised swab samples using qPCR when compared to control ($P < .001$). In other words, it was thought that the decrease in *S mutans* counts after the use of xylitol may be in line with the decrease in pro-inflammatory cytokines.

5 | CONCLUSION

Taken together with these data, xylitol may be a promising candidate as an anti-caries agent. Our results suggest that xylitol can inhibit the scores of gingival and plaque indexes possibly induced by decreasing the pro-inflammatory cytokines. We have shown here

for the first time that the effect of short-term xylitol use could be related with decreasing number of *S mutans* using qPCR. Since all cytokines role in a spatiotemporal manner, randomised controlled studies including vast number of cytokines may be warranted for better understanding of possible long-term effects of xylito. Overall, this study supports the hypothesis that short-term effect of xylitol not only reduces the number of *S mutans*, but may also decrease the TNF-α, IL-6 and IL-8.

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DISCLOSURES

Authors do not have conflicts of interests.

ORCID

Özer Akgül https://orcid.org/0000-0002-3802-3270

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