

Original Article

Streptococcus mutans, sugar consumption, and oral hygiene: Which one has more effect on decayed, missing, and filled teeth (DMFT) score in Iranian adults?

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ABSTRACT

Background: *Streptococcus mutans* as an acid-generator of biofilm, sugar as a caries-conductive environment, and oral hygiene have been implicated as major etiological agents in dental caries. This study was designed to assess the association and impact of *S. mutans*, sugar consumption, and tooth brushing on decayed, missing, and filled teeth (DMFT) score in Iranian 20–30-year-old individuals and compare the effect of the three mentioned factors to find the most effective one.

Materials and Methods: In this cross-sectional study, 459 adults completed a Sugar Frequency Questionnaire and were examined for dental caries using DMFT index, sugar consumption level, and tooth brushing frequency per day. Saliva and plaque samples were collected, and the target population without *Streptococcus sobrinus* in their microbial oral community was selected using polymerase chain reaction technique. Data were analyzed by one-way analysis of variance and multiple linear regression tests ($\alpha = 0.05$).

Results: Nearly 77.1% of the study population were harboring *S. mutans*. Mean DMFT of the population was 6.62. Mean comparison analysis showed that there is a strong relationship between *S. mutans* existence in mouth flora and DMFT scores ($P < 0.0001$). Multiple linear regression test showed higher percentage of *S. mutans* contribution (28.2%) in DMFT score changes than sugar consumption (3.6%) and tooth brushing (0.7%).

Conclusion: This study provides a recent report from *S. mutans* frequency and DMFT score in Iranian adult population. It is also the first study that shows significantly higher impact of *S. mutans* in microbial population of mouth microflora on caries development than sugar consumption and oral hygiene. Accordingly, *S. mutans* screening program should be more highlighted in preventive strategies.

Key Words: Decayed, missing and filled teeth, Iran, *Streptococcus mutans*, tooth brushing

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INTRODUCTION

Oral diseases are categorized in the top 100 causes of disability-adjusted life years.^[1] Among them,

dental caries affects 60%–90% of children as well as majority of adults and shows serious economic,

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social, and functional impacts on individuals.^[2,3] According to the World Health Organization (WHO), oral diseases are the fourth-most expensive disease to be treated in industrialized countries.^[2]

Based on ecological plaque hypotheses, there is a dynamic relationship between the composition of dental plaque (biofilm and microbial community) and the environment. Hence, caries is the consequence of bacterial unfavorable shift from beneficial organisms (that preferentially grow at neutral pH) to acid producer (tolerant to the acidic conditions) driven by changes in the dental environment to caries-conducive environment (sugar accessibility and/or low saliva flow). Hence, dental caries can be controlled by bacterial activity inhibition and interfering with the factors that drive the microbiota shifts (i.e., reducing the amount and frequency of sugar intake).^[4]

Mutans streptococci (MS) including *Streptococcus mutans* and *Streptococcus sobrinus* as members of acid-generating and acid-tolerating bacterial community using glucosyltransferases (Gtfs) metabolize sucrose as a substrate to synthesize insoluble and soluble glucans, which change the extracellular composition of the biofilm structure and increase bacterial adhesion and biofilm accumulation on dental surface.^[5,6] Studies have been conducted on special populations to investigate the determinative role of MS in dental caries and the association of *S. mutans* and *S. sobrinus* with caries formation.^[7-9]

There is also an agreement that sugars are implicated in dental caries.^[10-12] Sugar by pH decrease can change the composition of the oral microflora toward caries-promoting one and increase the colonization and outgrowth of MS.^[4] It is shown that people with higher level of sugar consumption have significantly higher incidence of dental caries than those with lower level of sugar consumption in their diet.^[13-16]

In addition, tooth brushing can modulate the number of MS^[13] and the impact of sugar on dental caries.^[14] It is shown that poor oral hygiene practices can be a risk factor for DMFT scores and preventive measurements such as fissure sealant and fluoride therapy can prevent dental caries occurrence.^[15,16]

To prevent dental caries or estimate future caries activity, caries etiology revisiting is necessary. Numerous studies have shown the relationship between mouth microflora/sugar/tooth brushing and caries in children,^[7,8,12,14-20] and such estimations in adults have been largely ignored. This cross-sectional

study which was conducted on 20–30-year-old adults, for the first time in Iran, evaluates the association of *S. mutans*, sugar consumption, and oral hygiene with DMFT scores, and is aimed to compare the influence of the aforementioned factors on DMFT score changes. In other words, the presence or absence of *S. mutans* (as a member of biofilm community) and its influence on DMFT scores were compared with sugar consumption, an environmental-driven factor for microbial shift, and with tooth brushing.

According to the results of the studies, the incidence of caries is higher in the co-existence of *S. mutans* and *S. sobrinus* in mouth microflora.^[7,9,17,21] It has also been shown that *S. sobrinus*, unlike *S. mutans*, is not detected in any healthy controls, and may be a more effective factor of caries than *S. mutans*.^[21] As the purpose of the present study is to estimate the presence of *S. mutans* and its relationship with higher caries experience and to compare its effect with sugar consumption and tooth brushing factors, molecular detection of *S. mutans* and *S. sobrinus* from saliva and plaque samples was performed, and samples negative for *S. sobrinus* were selected as the study population. The identification of *S. mutans* and *S. sobrinus* was based on *gtfB* and *gtfI* gene detection for *S. mutans* and *S. sobrinus*, respectively. These genes express important cariogenic factors and have nucleotide sequences specific to *S. mutans* and *S. sobrinus*.^[22,23]

MATERIALS AND METHODS

Study population, dental caries, sugar consumption, and tooth brushing habit assessments

A total of 459 individuals aged 20–30 years (in Tehran province) were included in this cross-sectional study. People who were excluded were as follows: (a) those who used fluoride or taken antibiotics within the previous 3 months or with systemic diseases; (b) had <24 permanent teeth; and (c) used dental appliances (orthodontic appliances and dentures). It should be mentioned that, in Iran, water supply and food, salt, or milk fluoridation is not conducted, so in the studied population, the only exposure to fluoride is the fluoride in the toothpaste. Dental examinations are performed using a plane dental mirror and an explorer under artificial light. The DMFT value was calculated following the WHO's caries diagnostic criteria.^[24] The research assistants were encouraged to review the caries scoring criteria protocol. Calibration or interrater reliability analysis

using the Kappa statistic was performed (with 13 individuals) by comparison with a dentist who did not participate in the fieldwork to determine consistency among raters.^[25]

A Sugar Frequency Questionnaire (SFQ) consisting of 22 items on commonly consumed snacks in Iran was designed [Appendix A]. The internal consistency of the questionnaire was assessed by Cronbach's alpha and the items in which their removal would increase the alpha amount were reconsidered. Each participant was asked how often in a month, week, or day each item was consumed. A unit of sweet food consumption per day (unit/day) and a mean of unit per day were calculated for each participant. A score for the number of consumed sweet foods was also recorded (from 1 to 22) for each participant. Population grouping to low-, intermediate-, and high-level sugar consumption was done according to the numbers calculated for each person using the sugar consumption score (sugar consumption score: mean unit/day/score from 1 to 22) [Appendix A]. Population grouping for sugar consumption based on the yielded sugar consumption scores was as follows:

- Low: 0.44/4–0.76/10
- Intermediate: 0.76/10–1.12/18
- High: 1.12/18–1.5/24.

Each study participant was inquired about his/her tooth brushing frequency, and it was categorized as never or rarely, once a day (1/d), twice a day (2/d), and three times a day (3/d).

Streptococcus mutans and *Streptococcus sobrinus* detection

The plaque samples were collected by rubbing the vestibular face of the smooth and proximal surfaces of the central and lateral incisors, canines, premolars, and the first and second molars with a sterile swab. Sample size was calculated based on Okada *et al.*'s study.^[26] The mean concentration of DNA recovered from the saliva and plaque samples with approximately 660 µg/ml was sufficient for performing a polymerase chain reaction (PCR)-based survey. Each plaque sample was immediately placed in a sterile tube containing saliva sample of the same participant. Tubes were then vortexed for 30 s for content homogenization and processed within 2–3 h after collection.

Cells from 500-µl homogenized plaque and saliva mixture were pelleted down and washed twice with TE buffer (10 mM trisaminomethane hydrochloride [Tris-HCl], 1 mM ethylenediaminetetraacetic acid

[EDTA], pH: 8), suspended in 200 µl boiling buffer (Tris-HCl 10 mM, pH: 8, EDTA 40 mM, pH: 8, sodium dodecyl sulfate [SDS] 0.2%, pH: 7.2), boiled for 10 min, and immediately transferred to ice to release DNA from cells or incubated in lysis buffer (Tris-HCl 10 mM, pH: 8; EDTA 40 mM, pH: 8; SDS 0.2%, pH: 7.2; lysozyme 2 mg/ml) for 2 h at 37°C with occasional inversion. The cell suspension was then mixed with 200-µl proteinase k solution (Tris-HCl 10 mM, pH: 8; proteinase K 2 mg/ml) and incubated at 50°C for 2 h with occasional inversion. Extra incubation at 68°C for 30 min was performed for proteinase K inactivation. Each suspension was mixed with 300-µl equilibrated phenol solution (Sigma-Aldrich, USA) by vigorous shaking for 5 min and centrifuged (12,000 × g, 15 min). The upper DNA-containing layer was mixed (by shaking) with 300 µl of equal volume of phenol: chloroform (1:1 v/v) and also treated with 30 µl 3 M sodium acetate buffer (pH 5.2). The DNA was precipitated with 2.5–3 volumes of ice-cold 95% ethanol and allowed to precipitate overnight. After wash by 70% ethanol and removal of the ethanol, the DNA dissolved in 30-µl autoclaved TE buffer (Tris-HCl 10 mM, EDTA 1 mM, pH: 8). The DNA was quantified spectrophotometrically by calculating the A 260/A 280 ratios and the A 260 values to determine protein impurities and DNA concentrations.

Standard PCR amplification of *gtfB* and *gtfI* gene for *S. mutans* and *S. sobrinus* detection, respectively, was performed using 20-µl PCR buffer containing 2 µl PCR buffer (10 mM Tris, 50 mM KCl, pH: 8), 0.2 mM of each of the deoxy nucleoside triphosphates, 2.5 mM MgCl₂, 10 pM of primers^[22,23] [Table 1], 0.5 U of Taq DNA polymerase, and 10 ng of target DNA. A total of 35 cycles of amplification were performed with template DNA denaturation at 94°C for 2 min, primer annealing at 60°C for 30 s, and primer extension at 72°C for 2 min. The PCR products were analyzed by electrophoresis on 1.5% agarose gel (Sigma, Germany) containing ethidium bromide (0.5 µg/ml) (CinnaGen, Iran).

Statistical analysis

Data were processed for analysis using SPSS software (version 20.0/PC; Chicago, IL, USA), and statistical significance was set at $P < 0.05$. As mentioned in the materials and methods section, calibration or interrater reliability analysis for DMFT score recording was done using the Kappa statistic, and the

internal consistency of the questionnaire was assessed by Cronbach's alpha.

The frequency of *S. mutans* existence in individuals with and without caries lesion was analyzed by Chi-square test. Data from the samples were recorded as DMFT score (dependent variable), and the three independent variables were as follows: *S. mutans* existence in saliva and plaque (two categories of positive or negative), sugar consumption (three categories of low, intermediate, and high), and tooth brushing habits (four categories of never or rarely, 1/d, 2/d, and 3/d). The significant differences of DMFT scores for the two categories of *S. mutans* variable were assessed using independent sample *t*-test. For sugar consumption and tooth brushing habits, one-way analysis of variance was performed to verify statistical differences, and Tukey's honestly significant difference test was used for multiple mean comparisons ($\alpha = 0.05$). As the DMFT score distribution was normal, linear multiple regression was used to study the influence of independent variables (*S. mutans*, sugar consumption, and tooth brushing habits) on DMFT scores.

Ethical issues

This study was conducted with the understanding and written consent of each participant and in full accordance with the World Medical Association Declaration of Helsinki. It was independently

reviewed and approved by Islamic Azad Medical University's ethics committee (ethical code: IR.IAU.TMU.REC.1396.299). Volunteers' data were anonymized prior to the analysis.

RESULTS

PCR reaction by *gtfB* and *gtfI* gene-specific primers produced DNA fragments of 517 bp and 712 bp from *S. mutans* and *S. sobrinus*, respectively [Figure 1].

Out of the 459 samples, 381 samples (83%) negative for *S. sobrinus* were selected as the study population. Out of the 381 samples, 294 samples (77.1%) were positive for *S. mutans* and the remaining were negative. Of the 294 individuals with *S. mutans*, 276 (86.7%) had caries lesion. *S. mutans* was not present in 42 individuals (13.2%) with caries lesion and was detected in 18 individuals (28.5%) without caries lesion [Table 2]. Statistical analysis by Chi-square test showed a strong relationship between *S. mutans* existence and caries experience ($P < 0.05$) [Table 2]. DMFT mean from the studied population was 6.62.

The interrater reliability for the raters was found to be kappa = 0.73 with $P < 0.001$. Cronbach's alpha analysis showed that the questionnaire has acceptable reliability, $\alpha = 0.8$.

Table 1: Polymerase chain reaction primers for *Streptococcus mutans* and *Streptococcus sobrinus* detection

Bacterium	Primer	Sequence (5'-3')	Location	GenBank accession number	Amplicon size (bp)	Reference
<i>S. mutans</i>	GTFB-F	ACT ACA CTT TCG GGT GGC TTG G	793-814	M17361	517	[22]
	GTFB-R	CAG TAT AAG CGC CAG TTT CAT	1288-1309			
<i>S. sobrinus</i>	GTFI-F	GAT AAC TAC CTG ACA GCT GAC T	871-892	D90213	712	[23]
	GTFI-R	AAG CTG CCT TAA GGT AAT CAC T	1561-1582			

GTFB: Glucosyltransferase B; GTFI: Glucosyltransferase I; *S. mutans*: *Streptococcus mutans*; *S. sobrinus*: *Streptococcus sobrinus*

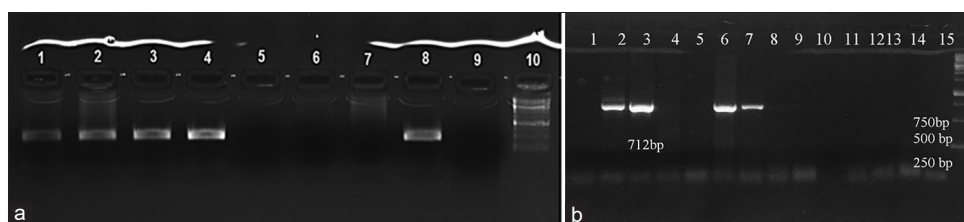


Figure 1: Detection of *Streptococcus mutans* in saliva and dental plaque samples by polymerase chain reaction amplification. (a) Glucosyltransferase B gene (517 bp) amplification from saliva and plaque samples. Lanes 1–4: Polymerase chain reaction products from *Streptococcus mutans*-positive samples. Lane 5–7: Polymerase chain reaction product from *Streptococcus mutans*-negative samples. Lane 8: Control chromosomal DNA from *Streptococcus mutans* (PTCC 1683) (positive control). Lane 9: No chromosomal DNA (negative control). Lane 10: 1 kb DNA ladder (fermentase). (b) Polymerase chain reaction amplification of glucosyltransferase I gene (712 bp) from saliva and plaque samples. Lane 1: No chromosomal DNA (negative control). Lane 2 Control chromosomal DNA from *Streptococcus sobrinus* (PTCC 1601) (positive control). Lanes 3, 6, and 7: Polymerase chain reaction product from *Streptococcus sobrinus*-positive samples. Lanes 4, 5, and 8–14: Polymerase chain reaction product from *Streptococcus sobrinus*-negative samples. Lane 15: 1 kb DNA ladder (fermentase).

The mean comparison of DMFT scores for the two categories of *S. mutans* variable showed statistically significant difference in DMFT scores between the two categories ($P < 0.05$) [Table 3]. The differences between the categories of sugar consumption and tooth brushing habits were also statistically significant ($P < 0.05$) [Table 3].

Linear multiple regression analysis results showed that, from the three evaluated factors, bacterial adhesion and sugar consumption were found to significantly affect DMFT score ($P < 0.05$) [Table 4]. According to standardized regression coefficients, bacterial adhesion had the highest effect on DMFT score ($\beta = 0.474$) followed by sugar consumption ($\beta = 1.076$). Relative adjusted R^2 also indicated that 28.2% of DMFT changes is explained by *S. mutans*

Table 2: *Streptococcus mutans* existence frequency in individuals with and without caries lesion

Category	Caries active, n (%)	Caries free, n (%)	Total, n (%)	P	Mean DMFT
<i>S. mutans</i> +	276 (86.7)	18 (28.5)	294 (77.1)		
<i>S. mutans</i> -	42 (13.2)	45 (71.4)	87 (22.8)		
Total	318 (83.4)	63 (16.5)	381 (100)	<0.001	6.22

$P < 0.001$: Statistically significant difference for *S. mutans*' existence frequency in individuals with and without caries lesion. DMFT: Decayed, Missing, and Filled teeth; *S. mutans*: *Streptococcus mutans*

Table 3: Mean comparisons of Decayed, Missing, and Filled teeth

Variable	Category	n (%)	DMFT* (mean±SD)	P
<i>S. mutans</i>	+	294 (77.1)	8.1±4.7 ^a	<0.001
	-	87 (22.8)	1.66±2.35 ^b	
Sugar consumption	Low	171 (44.8)	4.8±4.47 ^b	<0.001
	Intermediate	102 (26.7)	7.02±4.22 ^{ab}	
	High	108 (28.3)	9.14±5.61 ^a	
Tooth brushing habits	None	24 (6.2)	9.75±3.58 ^a	0.016
	1/day	231 (60.6)	6.87±4.17 ^{a,b}	
	2/day	96 (25.1)	5.84±3.99 ^{a,b}	
	3/day	30 (7.8)	4.8±3.71 ^b	

*Different letters indicate significant differences between categories based on Tukey's test ($P < 0.05$). $P < 0.05$: Statistically significant difference between categories. DMFT: Decayed, Missing, and Filled teeth; *S. mutans*: *Streptococcus mutans*; SD: Standard deviation

Table 4: Linear multiple regression for Decayed, Missing, and Filled teeth^a

Independent variable	B	β	F	Adjusted R^2	
				Relative	Cumulative
<i>S. mutans</i>	5.692	0.474	50.56	0.282	0.282
Sugar consumption	1.076	0.179	30.332	0.036	0.318
Tooth brushing habits	-0.823	-0.117	21.222	0.007	0.325

^aDependent variable: DMFT. DMFT: Decayed, Missing, and Filled teeth; *S. mutans*: *Streptococcus mutans*

variable. Sugar consumption variable was the next in rank with 3.6% contribution in DMFT changes. The effect of tooth brushing habits on DMFT was not statistically significant ($P > 0.05$) [Table 4]. Cumulative adjusted R^2 of 0.325 showed that 32.5% of DMFT changes could be explained by three factors, namely presence of *S. mutans*, sugar consumption, and tooth brushing habits [Table 4].

DISCUSSION

Dental caries is a multifactorial incident, and its prevalence in a population is influenced by a number of factors including microflora, sugar consumption, genetic, socioeconomic status, and oral hygiene habits.^[18,27] Microbiota of the dental plaque, being exposed by sugars, makes plaque environment to be acidogenic and initiates dental caries.^[4] Moreover, diets rich in sucrose can change the population of the oral microflora toward caries-promoting one.^[4] In this study, in addition to the new report of *S. mutans* epidemiology and DMFT scores and the evaluation of association of *S. mutans* with sugar consumption and tooth brushing habits with DMFT scores in Iranian adults, the effect of the three mentioned factors in caries occurrence was compared using DMFT scores analysis, to find the most effective one.

The mean DMFT score found in the present study was 6.62. The prevalence of *S. mutans*, found in this study, was 77.1%, and the frequency of the presence of *S. mutans* in caries-active group was statistically significantly higher than the caries-free group ($P < 0.001$) [Table 2]. This study also showed that there is a significant difference in mean DMFT scores between *S. mutans*-positive and *S. mutans*-negative categories ($P < 0.001$) [Table 3]. No epidemiological studies have been conducted in Iran to investigate the frequency of *S. mutans* in adults. In a study by Ghasempour *et al.*^[19] on 4–6-year-old children, 65% of the children were harboring *S. mutans* in their saliva, and the frequency of *S. mutans* in caries-active children was significantly higher than that of caries-free one ($P = 0.046$). Similar observations regarding the association of *S. mutans* with higher DMFT scores have been made in other parts of the world in different age groups.^[8,10,18,28]

While in this study *S. mutans* showed a strong relationship with caries incidence and DMFT, it is not present in all individuals with caries (13.2% of the population with dental caries were not harboring

S. mutans). This finding supports the “nonspecific plaque hypothesis,” in which caries is the consequence of the net metabolic activity of the biofilm,^[29] which can be attributed to other contributing factors in caries occurrence or other members of complex microbial community including *Streptococcus salivarius*, *Streptococcus vestibularis*, *Streptococcus parasanguinis*, *Lactobacillus*, *Veillonella*, *Actinomyces*, *Propionibacterium*, *Atopobium*, and *Bifidobacterium dentium*.^[21,30-32]

S. mutans was detected in 28.5% of individuals without caries lesion, and this may be because of the differences between *S. mutans* strains’ capabilities in adherence to hydroxyapatite or bacteriocin production.^[33] In addition, the importance of prominent microbial community members that contribute in promoting health by ammonia production and pH homeostasis^[34] should be considered as an explanation.

Sugar consumption quantity as an environmental aspect of ecological plaque hypotheses was investigated in 20–30-year-old adults in this study, and the results showed a statistically significant difference in mean DMFT scores between the three sugar consumption categories (low, intermediate, and high) ($P < 0.000$). In the SFQ, people were asked about the amount of extrinsic sugar consumed in different times, and the selection of items was based on Iranian people’s nutritional habits. The observed positive relationship between sugar consumption and caries scores, apart from the use of fluoride, is in accordance with other studies.^[11,12,14]

Tooth brushing habits (with fluoride toothpaste) as a factor related to dental caries score were also investigated among the studied population, and there was a positive relationship ($P = 0.016$) between the frequency of brushing per day and decreased DMFT score. Some studies have also shown lower DMFT scores in 3–5-year-old children with earlier initiation of tooth brushing in their life and more frequency of brushing per day.^[15] Tooth brushing frequency and its association with lower dental caries experience is also confirmed in adults.^[10]

In this study, the effect of *S. mutans*, sugar consumption, and tooth brushing on DMFT changes was compared using linear multiple regression test. The results showed that *S. mutans* has the greatest impact on DMFT score changes ($\beta = 0.474$) followed by sugar consumption with remarkable difference in beta value ($\beta = 0.179$). It is also shown that tooth

brushing has no effect on DMFT changes ($\beta = -0.117$) [Table 4]. In other words, tooth brushing was not found as a more preventative factor of caries than *S. mutans* and sugar consumption. Acid production and pH drop by MS occurs 5–10 min following sugar consumption.^[35] A significant effect of the time that sugar is available to mouth microflora is seen in DMFT scores^[20] so, tooth brushing can be a hindrance to caries-producing factors. However, there was no possibility to record the brushing time for each individual in this study to verify tooth brushing factor on its merit. This limitation can be a justification for the ineffectiveness of brushing frequency in DMFT changes in this study.

In accordance with the results of the previous studies that consider dental caries as a multifactorial incident,^[18,27] the adjusted R^2 of 0.325 in the present study showed that 32.5% of DMFT changes in the present study population could be explained by three factors that were entered into regression model including *S. mutans* (28.2%), sugar consumption (3.6%), and tooth brushing (0.7%), and additional factors such as other acidogenic species, genetic factors, and socioeconomic status may consist the remaining (67.5%). It should be mentioned that, in one population, different explanations may be relevant for each individual, age group, and each part of teeth.

This study provided the first report of *S. mutans* frequency and DMFT score in Iranian 20–30-year-old adults. More importantly, it revealed that *S. mutans* existence in mouth microflora of the studied population can act as a stronger factor in dental caries development than sugar consumption and tooth brushing. In other words, this study shows that, among oral bacteria, *S. mutans* is more powerful than sugar because in this study, there were high DMFT scores in *S. mutans*-positive people than the negative one with equal sugar consumption. Hence, more preventive solutions should be presented for *S. mutans* and its caries development mechanisms. The method used in this study is useful because it allows comparison of sugar consumption and *S. mutans*’ effects on DMFT score. The results of the present study which can be strengthened by similar surveys, using the presented method, on different populations, have notable clinical significance for preventive and therapeutic purposes.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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APPENDIX

Appendix A: Sugar Frequency Questionnaire

Name/code: _____

How often do you consume these items?	Never (0)	Times/2 months	Times/month	Times/week	Times/day	Unit/day ^a
Hard candy						
Sweet biscuits						
Ice cream/popsicle						
Jam/margarine						
Chocolates						
Jelly/creme caramel/pudding						
Soft drinks						
Power juice						
Orange juice/fruit drink (natural or processed)						
Milk chocolate						
Sugar-added tea						
Sugar-added coffee						
Cream cookies						
Smart beans						
Cereal						
Pastilles						
Cake						
Flavored milk						
Peanut butter						
Raisins						
Halva Ardeh						
Persian traditional candy (Nougat, Sohan, and Baghlava)						
	Mean unit per day					

^aUnit per day is calculated by conversion of times each 2 months, months, and weeks to times per day. Sugar consumption score: Mean unit per day/score from 22 items. Unit corresponding to the number of consumed sweet foods (score from 22 items): 1-22. Population grouping based on sugar consumption score: Low: 0.44/4-0.76/10, Intermediate: 0.76/10-1.12/18, High: 1.12/18-1.5/24