Sugary Foods and Beverages Relationship to Fungal Colonization and Oral Hygiene in School Children

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Abstract— Sugary foods and beverages are highly consumed by children. It is a substrate for fungal growth and has effects on oral hygiene. The study aimed to determine sugary foods and beverages relationship to oral fungi and hygiene. This cross-sectional study was conducted on 150 students aged 11-12 years old, selected based on inclusive-exclusive criteria. Demographic data and consumption of sugary foods and beverages were recorded in a questionnaire. The oral material was collected and cultured in SDA media. Fungal growth was evaluated microscopically then fungal identification used the Integral System YEASTS Plus (Liofilchem®). OHI was used to assess oral hygiene. Results showed that 72.0% were 12 years olds, the frequency of sugary foods and beverages consumption were more than once per week in 85 children (56.67%); who were mostly girls (30.9%). The fungus found was mostly Candida (55.3%) and 43.3% of children had poor oral hygiene. Statistical analysis showed significant association between the frequency of sugary foods and beverages consumption with fungal colonization (p<0.05), and oral hygiene (p<0.05). The correlation between fungal colonization and oral hygiene was statistically significant (p<0.05). This study provides insight on the relationships between sugary foods and beverages, fungal colonization and oral hygiene in school children.

Keywords— sugary foods and beverages, oral hygiene, fungal colonization

I. INTRODUCTION

Nowadays, people lifestyles tend to adopt an "unhealthy" behavior. In this case, school-age children are an interesting sub-population to study because of the high burden and demand of subjects in primary school and its associated high intense stress levels. This condition further facilitates unhealthy behavior, such as a tendency to overeat and consume foods and beverages high in sugar.[1] More than half of children consumed sugary foods and beverages occurred at home. Thus, the family's food eating environment and parental behavior may influence children's behavior in consuming sugary foods and beverages. [1,2]

Improper eating habits, such as high consumption of sugary drinks can cause many health problems. [3-5] One study found the drinks that are most in-demand and consumed by children and adolescents are those sweetened with sugar. [6]

Sugar-rich foods and drinks are excellent substrates for the microorganisms that inhabit the early parts of digestion, [7] and excess sugar can cause an overproduction of fungi. He B et al., [8] reported that 21.7% of Chinese children received more than one portion of sweet foods and drinks per week, while 9.5% received one or more portion of sugary foods and drinks per day. National nutritional research in Australia showed that daily consumption of sugar had exceeded recommended sugar intake among adolescents. [9,10] In addition, some researchers have reported that consuming excessive sugar decreases pH of oral cavity and changes the microenvironment. [5,11]

The oral cavity is one of the most colonized parts of the human body. [12] The prevalence of fungi in humans continues to increase, estimated to range from 2% to 70% in the immunocompetent population and immunocompromised people about 96%. [13,14] Poor oral hygiene is often considered a predisposing factor for oral candida colonization and the species most frequently isolated is Candida albicans (25-75%). According to Loster et al., [15] and Gacon et al., [16] Candida albicans is a commensal fungus found in the oral cavity of 35-65% of healthy people. Other Candida species, such as C. glabrata, C. tropicalis, C. guilliermondii, C. krusei, and Kluyveromyces marxianus, have rarely been isolated, but their prevalence has increased over the last decade. [17] Candida is an opportunistic-pathogen, commensal and normally found in the oral cavity and found more frequently in younger children because the immune system in children is still in the development stage. [18]

Current research focuses on finding the relationship between consumption of sugary foods/drinks and their impact on nutritional status, cardiovascular system, digestive tract microbiota, obesity and obesity related diseases. There is evidence that the consumption of sugary foods and beverages is related to the oral ecosystem. However, the relation between frequency of sugary foods and beverages consumption and oral microorganisms of school children and early adolescence are still rarely studied. This study objective was to determine the relationships between the frequency of sugary foods and beverages consumption, oral fungal colonization and oral hygiene of school-age children.

II. RESEARCH METHODOLOGY

A. Study Design

The study was cross-sectional and recruitment of subjects used a consecutive sampling technique based on the inclusion criteria. The students who participated in the study were 150 students aged 11-12 years from a public primary school in Gambir sub-district, Central Jakarta. The children must have parental consent in the study. Signed informed consent from parents were requested. This study had ethical clearance from the Medical Research Ethics Commission of Universitas Trisakti that reviewed and approved the research protocol.

Demographic information and consumption of sugary foods and drinks for one week were examined using the modified questionnaire of Neuhouser et al (2009). [19] Oral hygiene status was determined after examination of oral conditions and was assessed using the Simplified Oral Hygiene Index (OHI-S), which summed the Debris Index and Calculus Index, each of which was determined from the amounts of debris and calculus on the six tooth surfaces. [20]

B. Sample collection and Direct microscopic examination

The tools and materials used are sterile distilled water, beaker glass, phosphate buffer solution, solid Sabouraud's Dextrose Agar (SDA) media, object glass, inoculum loop, bunsen burner, agar plate, LPCB, microscope.

Procedure: All the subjects were asked to rinse their mouth with 100 ml distilled water thoroughly to remove any food debris. After 10 min phosphate buffer solution was used as oral rinse method for saliva collection. Samples were obtained by requesting subjects to keep and swirl the solution for 1 min, and then expectorate all saliva into presterilized container without swallowing. All samples were centrifuged at 1700 rpm for 10 min. Now the supernatant was discarded and sediment material was carried with pipette and inoculated in Sabouraud's Dextrose Agar media. The sample was streaked using inoculating loop and incubated in 37°C for 48 hours. The growth appeared in 48 hours as cream/white colored, smooth and pasty colonies. Fungal growth was evaluated using a microscope with LPCB staining, positive culture then was transmitted on solid media Sabouraud's Dextrose Agar (SDA) to obtain pure isolates.

C. Isolation and Species identification

Furthermore, the obtained fungi were identified using a commercial kit (Liofilchem® Integral System YEASTS Plus, cat. no. 7182279822, Italy), which is a 24 reaction well panel containing biochemical substrate and antimycotics for the identification of the most clinically important yeasts. This tool also includes a chromogenic well (no. 13-CHR) which delivers a color-based distinction: green color for Candida albicans, and purple color for Candida tropicalis. The panel is inoculated with the cell suspension and identification results are delivered after incubating at 36°C for 48 hours (Fig.1B). [21] The relationship between consumption of sweetened foods and drinks to the risk of mold in the oral cavity was then determined.

D. Procedur of Oral hygiene examination

The instruments and supplies that are required for oral examination, included plane mouth mirrors; metallic periodontal probes; containers and concentrated disinfecting solution; rubber gloves; wash basin for either water and soap disinfectant solution; cloth or paper hand towels; and gauze. Used instruments should be placed in disinfectant solution, then washed and drained well before sterilization. The area for conducting examinations should be arranged for optimum efficiency. The lighting should be consistent throughout the examination. If necessary, examinations can be carried out outside.

The Oral Hygiene Index is composed of the combined Debris Index and Calculus index, each of these indexes is in turn based on 12 numerical determinations representing the number of debris or calculus found on the buccal and lingual surfaces of each of three segments of each dental arch, namely distal segment to the right cuspid, distal segment to the left cuspid, and mesial segment to the right and left first bicuspids. The maxillary and the mandibular arches are each composed of three segments. Each segment is examined for debris or calculus. From each segment one tooth is used for calculating the individual index, for that particular segment. The tooth used for the calculation must have the greatest area covered by either debris or calculus. The method for scoring calculus is the same as that applied to debris.

Criteria for classifying debris:

0 = No debris or stain present

1 = Soft debris covering not more than one third of the tooth surface, or presence of extrinsic stains without other debris regardless of surface area covered

2 = Soft debris covering more than one third, but not more than two thirds, of the exposed tooth surface.

3 = Soft debris covering more than two thirds of the exposed tooth surface.

Criteria for classifying calculus:

0 = No calculus present

1 = Supragingival calculus covering not more than third of the exposed tooth surface.

2 = Supragingival calculus covering more than one third but not more than two thirds of the exposed tooth surface or the presence of individual flecks of subgingival calculus around the cervical portion of the tooth or both.

3 = Supragingival calculus covering more than two thirds of the exposed tooth surface or a continuous heavy band of subgingival calculus around the cervical portion of the tooth or both.

The index values are calculated after the scores for debris and calculus are recorded. For each individual, the debris scores are totaled and divided by the number of segments scored. The same method is used to obtain the calculus index scores. The average individual or group debris and calculus scores are combined to obtain the Oral Hygiene Index (OHI).

Oral Hygiene Index = Debris Index + Calculus Index

E. Statistical analysis

The data were statistically analyzed using the Chi-square test to identify relationships in the data obtained in the survey and from the fungal identification and OHI test results. Statistical analyzes were performed using SPSS 21.0 for Windows (SPSS Inc., Chicago, IL, USA) software with a 95% confidence level (a = 0.05). The results were considered statistically significant at p value <0.05.

III. RESULT AND DISCUSSION

The frequency of sugar intake is responsible for shifting the balance of the microorganism's population. Thus, the acidity of the oral cavity is a prerequisite for caries formation and the acidogenic microorganisms plays an important role in oral hygiene. [22-23]

The result of this study showed that 56.67% (85/150) of respondents consumed sugary foods and drink more than once per week ($\geqslant$1 time per week) and 65/150 (43.33%) had consumed sugary foods and drink less than once per week (<

1 time per week). The number of respondents' gender in this study was not much different, namely 54.0% (81/150) girls and 46.0% (69/150) boys. Most of the respondents age was 12 years (108/150; 72.0%) and the rest was aged 11 years (42/150; 28.0% - Table 1).

TABLE I. CHARACTERISTICS OF RESPONDENTS, SWEET FOODS AND DRINK

Category	Frequency	Sugary Foods and Drinks Consumption		p-Value
	n (%)	*<1x/week (%), n=65*	*≥1x/week (%), n=85*	
Age				
11 yo	42 (28.0)	31 (20.7)	12 (8.0)	0.000[a]
12 yo	108 (72.0)	20 (13.3)	87 (**58.0**)	
Gender				
Male	69 (46.0)	26 (17.1)	38 (25.0)	0.000[a]
Female	81 (**54.0**)	39 (25.7)	47 (**30.9**)	
Colony				
Candida	83 (55.3)	41(27.3)	42(28.0)	0.000[a]
Unidentified	67 (44.7)	23 (15.3)	44 (29.4)	
OHI				
Good	85 (56.7)	39(26.0)	46(30.7)	0.000[a]
Poor	65 (43.3)	26 (17.3)	39 (26.0)	

[a]. Chi-square test, $p < 0.05$

All collected samples were directly streaked on SDA. Of the 150 samples; 55.33% were yeasts in 83 isolates and 44.67% were unidentified in 67 isolates (Table 1). The colonies growth were cream/white colored colonies, protruding from the surface of the medium, the surface of the colony was smooth and pasty (Fig.1A). Candida species identification used Integral System YEASTS Plus that showed a chromogenic well (no. 13-CHR) changing color into green indicating Candida albicans (Fig.1B). The proportion of respondents who had good Oral Hygiene Index (OHI) were 56.7% and 43.3% had poor OHI.

This study found that respondents aged 12 years who consume sugary foods and beverages more than once per week was 58.0% (87 children) and 30.9% (47 children) were girls. Statistical analysis showed that age and gender of respondents were significantly associated with sugary foods and beverages consumption ($p < 0.05$, Chi-square test).

It is known that the consumption of high amounts of simple carbohydrates can increase the risk of dental caries because the cariogenic microorganisms requires sugar for its nutrition [24-26]. Vinke et al., [27] found 50.3% of 1257 respondents were boys, and Song et al., [26] also reported the highest frequency of consuming sugary drinks two or more times per week (36.8%) and mostly in boys (p for trend < 0.05). He et al., [8] reported that age and gender were associated significantly with frequency of sugary foods and beverages consumption. In a study involving 2,032 respondents consisting of 1,019 girls (50.15%) and 1,013 boys (49.85%), with the age of respondents ranging from 6 to 12 years as many as 69.0% consumed sugary drinks. They also found that 34.7% of respondents did not consume sugary drinks and 21.6% consumed sugary drinks of more than 120 ml per day (equivalent to more than 3 times per week), and mostly in girls.

Chen et al., [6] in a study involving 180 sch10-age children found 102 girls (56.7%) and 78 boys (43.3%) with an age range of 10 to 12 years. The study found that 16.7% of respondents consumed sugary beverages once a day or more per week and they were boys. Another study in China reported that 9.6% of respondents consumed sugary beverages more than 7 portions per week, [29] and in Germany found high daily consumption of sugary beverages more than 4 times in girls aged 3 – 17 years and more than 6 times in boys. [30] Based on previous studies, it is known that children aged 11-12 years often consume sugary foods and more in boys, while in this study was more in girls. This may be related to various factors, e.g. parenting patterns and puberty period in some girls start at aged 11-12 years, therefore they started to pay attention to their body image and restricted food intake.

Fig. 1. Colonies of Candida on solid media Sabouraud's Dextrose Agar (A). Identification of Candida albicans with Integral System YEASTS Plus (B).

Consumption of sugary foods and drinks is a predisposing factor for fungal colonization and Candida species are opportunistic pathogens that are common inhabitants of the normal oral microorganisms. [24] Fungal colonization was identified in respondents with consumption frequency of sugary foods and beverages more than once a week (28.0%) and less than once a week (27.3%) had a similar proportion (Table 1). Furthermore, fungus grew on all culture media and most were Candida species, due to the limitations of the commercial kit used in this study, 67 fungus isolates could not be identified.

Candida species, that were found in respondents who consume sugary foods and beverages more than once a week, consisted of Candida albicans (24.09%), Candida glabrata (10.84%), Candida tropicalis (8.43%), Candida krusei (6.02%), and 44 isolates (53.01%) were unidentified

(Fig.2). Fungal colonization was significantly increased in respondents who consumed sugary foods and beverages ($p < 0.05$, Table 1).

Previous study reported that fungi incidence in oral cavity was statistically significant ($p = 0.004$) in those adding sugar to beverages. The fungi were isolated from 68.1% (± 4.95) respondents and Candida albicans was the most dominant species 49%, while Candida glabrata and Candida krusei were found to be 2-10%. [31]

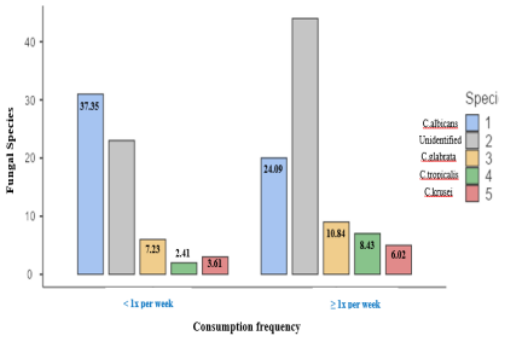

Fig. 2. Prevalence of oral fungal species to frequency of sweetened foods and beverages consumption.

The acidity condition of the oral cavity and the microorganism in the oral cavity are factors of caries formation which play an important role in oral hygiene. Respondents who had frequency of sugary foods and beverages consumption more than once a week and had good OHI were 30.7% and 26.0% had poor OHI. Oppositely, 26.0% respondents with frequency of sugary foods and beverages consumption less than once a week had good OHI and 17.3% had poor OHI. In this study, the Oral Hygiene Index was significantly associated in respondents who consumed sugary foods and beverages ($p < 0.05$, Table 1).

TABLE II. CHARACTERISTICS OF RESPONDENTS, SWEET FOODS AND DRINK

Fungal colony	Oral Hygiene Index				p-Value
	Good		*Poor*		
	n	%	*n*	%	
Candida spp	49	32.67	34	22.67	
Unidentified	36	24.00	31	20.66	
Total	85	56.67	65	43.33	0.044[a]

[a] McNemar test, $p < 0.05$

Table 2 showed that Candida species were found on 34/65 (22.67%) of respondents with poor oral hygiene, and respondents with good oral hygiene was 49/85 (32.67%). Based on Table 2, it could be concluded that association between Oral Hygiene Index and oral fungal colonization were statistically significant ($p = 0.044$).

Farhanaz et al., [32] reported that identified Candida albicans ranged from 12% to 93% in children with caries. The study of Wu et al., [33] showed the decrease of salivary s-IgA level correlated with caries in children was statistically significant. In contrast to Hemadi et al., [34] found a week association between Candida spp. in oral cavity and caries levels in children. In addition to dietary habit, consuming sugary foods and beverages has a role in regulating the ecological balance of oral microorganisms, thus being responsible for changes in oral hygiene. [23,33]

CONCLUSION

The result of this study provides additional insight into the relationship between sugary foods and beverages consumption with fungal colonization and oral hygiene in primary school children.

This study showed that sugary foods and beverages consumption has a strong association with demographic factors (age and gender). Our findings that high intake of sugary foods and beverages consumption was associated with oral fungal colonization. Furthermore, sugary foods and beverages consumption can be one of the factors that play a role in oral hygiene, due to its significant relationship with fungal colonization ($p<0.05$) and OHI ($p< 0.05$).

The consumption of sugary food and beverages in children should be controlled by promoting a healthy lifestyle in school. In addition, counseling is highly recommended to change the lifestyle of the families, especially high-risk groups, which may help to improve dental and oral health in children.

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