Relationship Between Salivary Alpha-amylase Enzyme Activity, Anthropometric Indices, Dietary Habits, and Early Childhood Dental Caries

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Research Article

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Abstract

Background: Caries risk assessment is of great importance in young children. Although early childhood dental caries (ECC) have the same general etiology as other types of caries, predisposing factors are not well elucidated.

Objectives: To evaluate the effect of salivary alpha-amylase (sAA) activity, body mass index (BMI), dietary habits and oral hygiene on ECC incidence.

Materials and Methods: This study was performed on 38 ECC-affected and 41 caries-free children, aged 36 to 72 months. Upon the parents' consent, 3 mL of non-stimulated saliva was collected from the participants to measure the level of sAA activity through spectrophotometry. Additionally, parents/caretakers completed a structured questionnaire about demographic factors, oral hygiene, and consumption of sugar-containing foods. BMI, BMI z-scores and percentile data were calculated by using an on-line calculator. The independent variables were dichotomized and tested through chi-square test, followed by a backward stepwise regression, by using SPSS software ($\alpha=0.05$).

Results: The sAA activity was significantly higher in caries-free children ($P\leq0.001$). However, the mean BMI was not significantly different between the two groups ($P=0.49$). Brushing and other measured dietary habits were significantly associated with the development of ECC ($P\leq0.001$).

Conclusion: Children with inherently lower levels of sAA activity were more susceptible to dental caries. Improper nutritional habits and poor oral health care could exacerbate the risk of ECC.

Introduction

Predicting caries incidence in young children is becoming increasingly important due to the health care costs and resources constraints [1]. According to the American Academy of Pediatric Dentistry, early childhood caries (ECC) is an important chronic disease, which is progressively caused by the imbalance of various risk and protective factors [2]. This oral health problem is more prevalent among socially-deprived communities [3]. It includes the presence of one or more decayed (non-cavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces of any primary tooth in children ≤ 71 months [2]. The general etiology of ECC is the same as general dental caries (microbiological, dietetic, and environmental risk factors); however, the predisposing factors are poorly identified [4].

Severe ECC is said to be the result of a combination of parents' socioeconomic, psychological, and behavioral features [5]. Its high prevalence is chiefly due to incorrect feeding practices, socioeconomic status of the family, parental illiteracy or inadequate education, and inaccessibility of dental care [3]. However, it can be prevented through establishing healthy eating habits, efficient brushing and flossing, receiving regular preventive dental services, and educating the community with respect to their level [6].

Recently, attentions have been attracted to the possible association between the body composition and dental caries in children [7–12]. Evidence shows that obesity and dental caries hold the two risk factors of dietary features and socioeconomic status in common [13]. However, contrasting reports exist regarding the association between dental caries and body mass index (BMI) as the measurement of body fatness, particularly in younger children [9, 13–16].

On the other hand, the physical features and chemical structure of saliva are reported to have imperative protective mechanisms against dental caries [17], so that some salivary proteins can act as key biomarkers for dental caries [18]. The most abundant enzyme in human saliva is the salivary alpha-amylase (sAA) [19], which hydrolyzes the starch to glucose and maltose, and helps food digestion [20]. This salivary enzyme of the acquired enamel pellicle regulates bacterial colonization and adds glucose for biofilm formation. Moreover, this protein binds to the membrane of cariogenic bacteria and facilitates their elimination from the oral cavity through the salivary clearance, and consequently reduces the risk of dental caries [21].

An association has been reported between amylase gene variation and occurrence of smooth-surface caries [22]. Likewise, sAA concentration was found to be positively related with dental caries [20, 23–26]. Yet, the relationship between salivary proteins and ECC is not well elucidated [21, 27]. Given the inconsistency and restriction of data about the predictors of ECC incidence, this study aimed to investigate how early childhood dental caries could be related with sAA activity, BMI, dietary habits and oral hygiene.

Materials And Methods

The study population

This study was ethically approved by the Ethics Committee of Shiraz School of Dentistry (IR.SUMS.DENTAL.REC.1399.037). All methods were performed in accordance with the relevant guidelines and recognized ethical standards of Shiraz University of Medical Sciences (Ethics Committee) in order to protect the rights, safety, and dignity of all participants. Written informed consent was obtained from the participants' parents/guardians after explaining the aims and methods of the study. A total of 200 children (100 girls and 100 boys, 36 to 72 months old) referring to the Department of Pediatric Dentistry of Shiraz School of Dentistry were examined. The children who were older than 72 months, uncooperative in collecting saliva, unable to expectorate, and those with no teeth, congenital or systemic disease, and a history of dental intervention were excluded. Finally, 38 caries-active (ECC) and 41 caries-free (control) children were selected and matched with respect to the mentioned criteria.

Experimental design

Dental caries was diagnosed and confirmed through visual inspection according to the World Health Organization criteria. A single calibrated examiner dried the teeth by using a sterile gauze and explored caries with dental mouth mirror under artificial light. Saliva samples were collected on a different day, for which the regular oral hygiene procedure was supposed to be performed after breakfast and the children were prevented from eating or drinking until sample collection.

To collect 3 mL of non-stimulated saliva through spiting, the child was asked to sit on a chair and lean forward while dropping down the head. Since the level of salivary secretion might be different during the day, all samples were collected between 9 to 11 a.m. within a maximum procedure of 30 minutes. The saliva samples were stored at 4 °C and then transferred to the laboratory.

Alpha-amylase activity assay

The saliva samples were centrifuged and stored at -20 °C for further analysis. The level of sAA activity was measured through spectrophotometry and salivary protein activity was measured by using the enzyme-linked immunosorbent assay (ELISA) (ZellBio GmbH kit, Veltlinerweg, Germany).

Dietary sugar intake and oral hygiene care

A self-administered structured questionnaire was completed by parents/caretakers about the demographic factors and oral hygiene care (frequency of tooth brushing per day), as well as the sweet snacks frequency (> 5 times: high risk, 3 to 5 times: moderate risk, < 3 times: low risk), stickiness, and time (between the main meals or along with the main meals) of consuming.

BMI

The participants' barefoot height and weight was measured with a digital scale and a portable stadiometer, respectively. The BMI was calculated through the standard formula: weight (kg) divided by height (m^2). In order to appropriately compare the subjects of different ages and sexes, the BMI was converted to BMI z-scores and percentile data by using an on-line calculator (Health Watch Pro software; Version 3.1). Based on the input information of age, sex, weight, height and race, the participants were grouped as:

a. Underweight: BMI-for-age < 5th percentile
b. Normal: 5th percentile ≤ BMI-for-age < 85th percentile
c. At risk of being overweight and overweight: 85th percentile ≤ BMI-for-age < 95th percentile, BMI-for-age > 95th percentile

Statistical analysis

For the statistical analysis, dental caries was considered as the dependent variable; while, the maternal working status (working or non-working), parental educational level, oral hygiene status (tooth brushing per day), snacking frequency, time and stickiness, BMI and sAA activity were the independent variables. Quantitative data like sAA activity was expressed as mean and standard deviation. Normal distribution of variables was tested with Kolmogorov-Smirnov test. To analyze the questionnaire responses, descriptive statistics were calculated for all variables, and the independent variables were dichotomized and tested with chi-square test to investigate possible associations between the dependent and independent variables. Finally, a backward stepwise regression was performed to identify the predictors of ECC development. Logistic regression analysis and relative risk were expressed as odds ratios with their respective 95% confidence intervals. The statistical analyses were done by using SPSS software (version16.0, SPSS Inc., IL, Chicago, USA) ($\alpha = 0.05$ in all tests).

Results

In this study, 38 cases of ECC were compared with 41 caries-free controls. Table 1 displays the distribution of the participants' general characteristics. Among the demographic characteristics, only mothers' education level was significantly associated with the occurrence of ECC ($P = 0.033$) (Table 2). Besides, ECC was found to be significantly correlated with brushing ($P \leq 0.001$) and all measured dietary habits ($P \leq 0.001$) (Table 3). The mean BMI was comparable between the ECC (22.12 ± 4.59) and control group (21.39 ± 9.05) with no statistically significant difference ($P = 0.49$) (Table 4).

Table 1
Distribution of general characteristics of the case and control groups

Groups **Variables**		**ECC (n = 38)**	**Control (n = 41)**	**P value**
Sex	Male	16 (50%)	16 (50%)	0.451 [a]
	Female	22 (46.8%)	25 (53.2%)	
Age (mean ± SD [months])		56.5 ± 10.3	53.1 ± 11.9	0.186 [b]
a: chi-square test				
b: Mann-Whitney test				

Table 2
Demographic characteristics associated with ECC

Groups Variables			ECC (n = 38)	Control (n = 41)	P value
Maternal working status		Working	21 (51.2%)	20 (44.8%)	0.363
		Non-working	17 (44.7%)	21 (55.3%)	
Education level	Paternal	Academic	18 (40.9%)	26 (59.1%)	0.113
		≤High school	20 (59.1%)	15 (42.9%)	
	Maternal	Academic	15 (36.6%)	26 (63.4%)	0.028
		≤High school	23 (60.5%)	15 (39.5%)	

Table 3
Health and dietary habits associated with ECC (Chi-square test)

Groups Variables			ECC (n = 38)	Control (n = 41)	P value
Oral Hygiene (brushing per day)		Once or less	32 (68.1%)	15 (31.9%)	≤ 0.001
		Twice	6 (18.8%)	26 (81.3%)	
Snacking	Time	With main meals(scheduled)	4 (13.8%)	25 (86.2%)	≤ 0.001
		Between main meals(Unscheduled)	34 (68%)	16 (32%)	
	Stickiness	Fruit juice and sugar liquids	1 (4.8%)	20 (95.2%)	≤ 0.001
		Cake and biscuit	17 (54.8%)	14 (45.2%)	
		Pastel and toffee	20 (74.1%)	7 (25.9%)	
	Frequency (per day)	< 3 times	5 (13.2%)	33 (86.8%)	≤ 0.001
		3–5 times	20 (74.1%)	7 (25.9%)	
		> 5 times	13 (92.9%)	1 (7.1%)	

Table 4
Comparison of BMI between caries free and ECC group

Groups BMI	ECC (n = 41)	Control (n = 38)
Underweight (< 5th percentile)	6 (40%)	9 (60%)
Normal (5th < 85th percentile)	12 (60%)	8 (40%)
At risk of overweight (85th to < 95th percentile), overweight (≥ 95th percentile)	19 (48.7%)	20 (51.3%)

Meanwhile, the mean sAA activity was significantly higher in the control group (P ≤ 0.001), indicating it to be inversely related with caries incidence. Moreover, a logistic regression analysis was done to control the variables and identify predictors of ECC. According to the 8 steps of backward stepwise regression analysis, the strongest predictors were sAA activity (P ≤ 0.001), snacking time (P = 0.093), and snack stickiness (P = 0.117 for low and P = 0.805 for medium stickiness, respectively) (Tables 5 and 6).

Table 5
The mean ± SD salivary α-amylase activity between the groups (independent t-test)

Groups Variable	ECC (n = 38)	Control (n = 41)	P value
Salivary α-amylase activity (U/L)	222.8 ± 65.7	363.6 ± 69.6	≤ 0.001

Table 6
Logistic regressions identifying predictors of ECC

Predictor variables		Control vs. ECC (reference category)	
		Odds ratio (95% confidence interval)	P value
α-amylase		0.968 (0.950–0.985)	≤ 0.0001
stickiness	Low stickiness (juice and sugar liquids)	71.095 (0.341-14802.27)	0.117
	Medium stickiness (cake and biscuit)	131.360 (0.513-33667.21)	0.805
time	With meals vs. between meals	6.367 (0.732–55.347)	0.093

Discussion

The present study tried to find if ECC is related with sAA enzyme activity, BMI, nutritional and oral hygiene related variables. The sAA activity was found to be significantly higher in caries-free children, indicating an inverse relationship between the ECC and sAA; which was consistent with Borghi *et al.*'s findings [21]. Similarly, Scannapieco *et al.*[28] noted that sAA could attach to cariogenic bacteria and facilitate their elimination from the oral cavity, and consequently reduce the incidence of ECC. In contrast, another study reported the mean amylase activity, total protein concentrations, and total IgM to be similar in caries-free and ECC groups. However, this could be due to their limited sample size (20 in each group), which precluded any significant difference [29]. Sitaru *et al.*[30] also detected that caries-active children had higher levels of salivary enzyme activity compared with caries-free groups, and pronounced sAA as a predictive biomarker in preventive dentistry.

Controversies also exist about the concentration of this protein in saliva. Compared with caries-free controls, higher concentrations of sAA were detected not only in children with ECC [19, 24, 31], but also in caries-susceptible young adults, particularly overweight adolescent girls [10, 25, 32]. Seemingly, excessive amounts of sAA contribute to hydrolysis of starch and acid release by cariogenic bacteria, and thereby raise the risks of dental caries. In contrast, Mojarad *et al.*[26] concluded that ECC might also be developed in case of decreased sAA concentration. Such a controversy can be justified by the multicomponent nature of human saliva (water, several electrolytes, mucus, glycoproteins, enzymes, and antibacterial compounds), which incorporates a confounding effect that does not allow assessing the effect of a single component in such a media [26].

Carbohydrates and sugar are extensively approved as the chief dietary elements that account for the incidence of dental caries [33–36]. Similarly, the current findings confirmed that dental caries are significantly associated with brushing and all dietary habits, particularly the amount and frequency of sugar consumption. The frequency of sugar intake (restricted to main meals or between meals) is reported to play an important role in both dental caries and childhood malnutrition [8]. Moreover, improper feeding practices, lack of parental education, and poor oral hygiene are known to raise the risk of ECC. Oral health is imperative for children to maintain the oral functions such as eating and speech, as well as developing a positive self-image [3].

Pediatric growth disorders have always been a multidisciplinary clinical concern for the specialists, dentists included. Recently, more investigations have been focused on the metabolic effects of obesity on oral health like higher risks of caries and periodontal diseases [12]. Although the relationship between ECC and BMI has been formerly evaluated, the mean BMI, BMI percentiles, or mean weight have been assessed in populations of different ages and mixed sexes [9]. However, in the present study, adjusting the z-scores for both age and sex by Health Watch Pro software yielded more logical report of means.

In line with some previous studies, the current findings revealed the dental caries to be more prevalent among underweight children; however, this was not statistically significant. Kumar et al. [11] noted that the socio-economic level affected the association between BMI and dental caries. Accordingly, overweight children of high socio-economic families had less dental caries than the normal-weight children.

Conflicting results have been obtained regarding the relationship between dental caries and BMI in children [7]. While some studies rejected any association between dental caries and obesity [7, 35, 37, 38], Hooley *et al.*'s recent systematic review provided document that proved dental caries is relatable to both low and high BMI [39]. Unlike the present study, some studies noted the dental caries to be more frequent in obese children (BMI > 30) than those with normal body weight (BMI < 25) [12, 17, 40, 41]. Pannunzio *et al.* [42] attributed the higher prevalence of caries in obese children to the decreased activity of salivary peroxidase enzyme, which accounts for the antibacterial and antioxidant features of saliva.

It is recommended to emphasize the importance of oral hygiene provide nutritional counselling, and take appropriate preventive measures for the children with growth disorders. Both malnutrition and dental caries can have lifelong negative repercussions for children. An interdisciplinary approach between the pedodontists and primary health care providers or pediatricians can offer a good opportunity to prevent chronic oral diseases and treat these childhood diseases [43].

In the present study, the regression analysis identified the ECC risk factors that were associated with the interaction of sAA activity, snacking time and stickiness. It implies that in caries-active children with genetically lower levels of sAA activity, incorrect dietary habits can exacerbate the susceptibility to dental caries formation [26].

Due to the small sample size and difficulty of sample collection especially in caries-free group, it was impossible in this study to match the variables such as oral hygiene and nutritional habits between the case and control groups. However, they were almost similar regarding the general characteristics such as sex, age and parents' education level. Further studies are suggested to match the confounding variables such as oral hygiene, nutritional habits, salivary immunoglobulins, and stress level to assess the correlation between sAA and ECC more precisely. Larger sample size and evaluating more clinical and biochemical parameters are recommended to predict the potential factors that affect the initiation and development of dental caries.

Conclusion

With respect to the present findings, it can be concluded that children with inherently lower levels of salivary alpha-amylase activity are more susceptible to dental caries. Additionally, development of dental caries has a strong inverse relationship with both the amount and frequency of sugar consumption. However, no correlation was detected between dental caries and BMI in the present study.

Declarations

Ethics approval and consent to participate

All procedures performed in this study were in accordance with the ethical standards of the local ethics committee of Shiraz University of Medical Sciences (IR.SUMS.DENTAL.REC.1399.037) and written informed consent was obtained from the participants' parents/guardians after explaining the aims and methods of the study.

Consent for publication

“Not applicable”

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

The authors declare no conflict of interest.

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Authors Contributions

Z.P. and N.M. did the methodology, software, literature review, formal analysis, writing – original draft preparation, supervision, project administration and funding acquisition.

N.A. and Z.P. did the experimental work and specimens’ preparation.

P.R. did the validation, resources, data curation, writing – review & editing.

All authors have read and approved the manuscript.

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