BMC Oral Health

RESEARCH

Open Access

Comparison of salivary statherin and beta-defensin-2 levels, oral health behaviors, and demographic factors in children with and without early childhood caries

Maryam Koopaie[1*†], Faezeh Khajehreza Shahri[1†], Roshanak Montazeri[2], Sajad Kolahdooz[3], Majid Mardani Shahri[4] and Elham Moshkbouy[3]

Abstract

Background Early childhood caries (ECC) is a widespread pediatric dental condition that is influenced by a combination of biological, behavioral, and demographic factors. Salivary biomarkers, including beta-defensin-2 (BD-2) and statherin (STATH), offer potential as non-invasive tools for detecting and assessing the risk of ECC. This study aims to compare the levels of salivary statherin and beta-defensin-2, alongside oral health behaviors and demographic factors, in children both with and without early childhood caries.

Methods This case-control study involved 75 children diagnosed with ECC and 75 age- and gender-matched caries-free controls. Unstimulated saliva samples were obtained and analyzed via ELISA to quantify the levels of beta-defensin-2 and statherin. Demographic and behavioral data were gathered through structured interviews with parents. Statistical analyses included t-tests, logistic regression, and machine learning models to predict the risk of ECC.

Results Salivary beta-defensin-2 levels were significantly higher in children with ECC (9.25 ± 2.89 ng/mL) compared to caries-free controls (6.41 ± 2.45 ng/mL, $p = 0.003$), indicating its potential as a diagnostic biomarker. Statherin levels, although lower in the ECC group, did not differ significantly between groups ($p = 0.08$). Behavioral factors such as regular dental visits and parental education levels were strongly associated with ECC prevalence. Machine learning models demonstrated high accuracy in predicting ECC, with the Gradient Boosting and CatBoost achieving the highest performance.

Conclusions Salivary beta-defensin-2 is a promising ECC risk assessment biomarker, while statherin is less effective as an independent predictor. Behavioral and demographic factors significantly influence ECC prevalence. Machine learning models integrating clinical, demographic, and salivary data provide a robust tool for detection and targeted

†Maryam Koopaie and Faezeh Khajehreza Shahri contributed equally to this work.

*Correspondence:
Maryam Koopaie
mariakoopaie@gmail.com

Full list of author information is available at the end of the article

prevention strategies. Comprehensive approaches combining salivary biomarkers and behavioral interventions are critical to managing ECC, particularly in resource-limited settings.

Keywords Early childhood caries, Saliva, Beta-defensins, Statherin, Biomarkers, Socioeconomic status, Machine learning

Background

Early childhood caries (ECC) is among the most common diseases affecting children globally [1]. The prevalence of ECC varies widely across the globe. It ranges between 30% and 48% in developed countries, while in less-developed nations, it can reach as high as 82% [1–3]. Countries in Africa and Oceania report some of the highest rates of ECC, with a pooled prevalence of 30% in Africa and 82% in Oceania. In the United States, 21.4% of children aged 2 to 5 years are affected by ECC [1, 4]. Shoaee et al. revealed that the prevalence of dental caries in deciduous teeth among Iranian children increased by over 15% from 1990 to 2017, with untreated caries rising by more than 17%, highlighting the urgent need to evaluate oral health policies and implement effective nationwide interventions [5, 6].

Culture, race, ethnicity, socioeconomic status, dietary patterns, lifestyle, oral hygiene habits, and geographic location play a significant role in the prevalence of ECC [7, 8]. During early childhood or pregnancy, the mother is the primary source of cariogenic microorganisms and host-dependent factors [9]. The lowest rate of carbohydrate clearance from the mouth occurs during sleep, and the reduction in salivary flow during this time increases the contact between plaque and teeth, providing an optimal environment for pathogenic bacteria [10]. This situation, combined with frequent nocturnal feeding, particularly breastfeeding, is a major contributor to ECC [11].

Saliva is crucial in the host's defense against dental caries [12]. It helps neutralize acids produced by bacteria, facilitates the clearance of food particles and microorganisms, and acts as a calcium and phosphate reserve to aid enamel remineralization [13]. Research indicates that children typically acquire *Streptococcus mutans* following the eruption of their primary teeth, usually between 7 and 36 months [14]. Infants delivered preterm, with low birth weight, or with hypomineralized teeth require greater attention to oral health care to prevent ECC [15].

Consistent tooth brushing using proper techniques is essential for reducing the risk of ECC [16, 17]. The accessibility of dental care frequently correlates with the child's socioeconomic status [18, 19]. Multiple factors, such as parents' education, employment status, and access to dental insurance, have been identified as affecting factors in the occurrence of ECC [20–22]. Families with a history of dental caries in other members are also at higher risk of ECC [23]. Studies have shown that children whose parents have lower educational levels or are both employed face a higher risk of ECC [24]. ECC not only causes direct harm to a child's oral health but also impacts their overall health. Untreated ECC leads to pain, difficulty in eating, speech issues, and potential orthodontic problems [25].

In addition to demographic and oral health behavioral factors, recent research has pointed to the significant role of salivary biomarkers in predicting the risk of diseases, including ECC [12, 26, 27]. These biomarkers contribute to the body's natural defense against dental caries and have the potential to serve as valuable diagnostic tools for ECC risk assessment. Developing non-invasive monitoring, screening, and diagnostic techniques, including salivary biomarkers, is a promising approach to controlling and managing ECC [12, 26, 28]. The salivary proteome contains over 2000 proteins [29], many of which have antimicrobial properties, and alterations in the salivary protein composition are often linked to oral diseases, including ECC [30, 31]. Identification of specific salivary biomarkers could provide valuable insights into ECC risk. Early childhood caries (ECC) is defined as the presence of one or more decayed (non-cavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a child under the age of 6 years (71 months or younger) [29].

Defensins are antimicrobial peptides critical in maintaining microbial balance in the oral cavity. Beta-defensins, expressed by oral epithelial cells and salivary duct cells, contribute to the innate immune defense of the oral mucosa [32, 33]. Saliva contains both alpha-defensins and beta-defensins. Alpha-defensins are upregulated during acute inflammatory conditions, such as infection, fever, tissue injury, or hemorrhage [34, 35]. Beta-defensins, in addition to their antimicrobial properties, exhibit cytotoxic activity against tumor cells and modulate adaptive immune responses by recruiting immune cells [36]. Beta-defensin expression is induced by pro-inflammatory cytokines, including TNF-α and IL-1β, during infection or tissue damage [37]. While beta-defensins exhibit broad-spectrum activity against bacteria, they show enhanced efficacy against Gram-negative bacteria and are particularly effective against *Streptococcus mutans* [38]. Elevated levels of *Streptococcus mutans* in the oral cavity can trigger increased beta-defensin production as part of the host's antimicrobial response [39].

Statherin, a small acidic salivary peptide, plays a dual role in maintaining oral health by modulating

hydroxyapatite dynamics and inhibiting bacterial colonization [40]. As a key biomolecule in saliva, statherin binds selectively to hydroxyapatite crystals, the primary mineral component of tooth enamel, through its negatively charged domains, stabilizing calcium and phosphate ions at the enamel surface [41, 42]. This interaction prevents spontaneous precipitation of minerals and promotes enamel remineralization by maintaining a supersaturated salivary calcium phosphate state, thereby enhancing early carious lesions' natural repair [43].

Furthermore, statherin exhibits antimicrobial properties by reducing the adherence of *Streptococcus mutans*, a primary cariogenic bacterium, to hydroxyapatite surfaces [44]. This anti-adhesive effect disrupts the formation of pathogenic biofilms, a critical step in dental plaque development and caries progression. Studies suggest that statherin competitively blocks bacterial adhesins from binding to HA, effectively limiting *Streptococcus mutans* colonization and subsequent acidogenic activity [45]. These dual functions underscore statherin's significance as a natural protective agent in the oral cavity, offering potential therapeutic avenues for enhancing remineralization strategies and preventing dental caries. This study introduces integrated approaches that combine demographic and clinical data, including salivary biomarkers (STATH and BD-2), to comprehensively examine the interaction of these factors in the context of ECC. The primary objective of this research is to compare the levels of salivary STATH and BD-2, along with oral health behaviors and demographic variables, in children with and without ECC.

Methods

Ethical statement

This analytical cross-sectional case-control study received ethical approval from the Ethical Committee of Tehran University of Medical Sciences (ethical code: IR.TUMS.DENTISTRY.REC.1400.158) and was conducted in accordance with the Declaration of Helsinki [46]. Participants received written informed consent from a parent or guardian after the study objectives were explained. Children whose parents consented and signed the informed consent form were enrolled. The authors confirm that the relevant guidelines and regulations performed all methods.

Study design

This study was undertaken on the target population of children aged 48–71 months who met the inclusion and exclusion criteria, including 75 cases of ECC and 75 caries-free (CF) children as a control group. The subjects included in this investigation were randomly chosen from children referred to the Tehran University of Medical Sciences dental clinic for routine oral examinations from April to July 2022. The study's objectives were explained to the parents. Examinations were performed by three pediatric dentists under adequate lighting using a dental mirror, probe, air syringe, disposable gloves, masks, and sterile gauze. Bitewing radiographs were taken if proximal caries were suspected. The case group included individuals with one or more decayed (non-cavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth. The participants were matched according to age and sex. Children with no clinical or radiographic evidence of caries (including white spots) were classified as CF, resulting in a zero decayed, missing, and filled teeth (DMFT) index.

Participants

Inclusion and exclusion criteria

Participants in the case group must exhibit clinical signs of ECC, such as visible cavities, white spot lesions, or active caries in primary teeth, with or without restoration. The study excludes children who meet any of the following conditions: Children with chronic systemic diseases or syndromes, including but not limited to cancer, renal failure, or metabolic disorders, that could interfere with the study's objectives or significantly affect oral health. Children suffering from respiratory infections (pneumonia, influenza, and the common cold) are also excluded. Children with congenital or acquired craniofacial deformities that affect normal oral function or structure, such as cleft lip and palate, which could influence caries development or salivary composition, are excluded from the study. Children diagnosed with significant salivary gland dysfunction or disorders that alter saliva's normal flow and composition, such as sialadenitis or xerostomia, are also excluded.

Furthermore, children who have received invasive dental procedures (dental extractions and extensive fillings) within the past 6 months are excluded. Children currently receiving treatments that affect salivary biomarkers, such as medications known to alter salivation or the microbial composition of the oral cavity, including chemotherapy, antihistamines, or certain antibiotics, are excluded from participation. Children whose parents or legal guardians do not consent to participate, either due to unavailability or refusal, are also excluded. Additionally, children with congenital diseases or conditions that may interfere with the development or evaluation of ECC, such as genetic conditions impacting tooth development or immune function, are excluded. Lastly, children whose parents or guardians refuse consent for participation or who are unable or unwilling to cooperate with study procedures, including providing saliva samples or participating in oral hygiene practices, are excluded.

Data gathering

After conducting the oral examinations of the participants, socioeconomic and demographic characteristics and dietary intake were recorded by filling out a checklist [47], such as birth weight, parents' education, and oral hygiene behaviors. Participants were evaluated using the DMFT index in accordance with the World Health Organization (WHO) Oral Health Surveys Basic Methods [48], and ECC was diagnosed according to the criteria set forth by the American Academy of Pediatric Dentistry [49]. Individuals with a DMFT index of zero were classified as CF. Following a thorough evaluation of the exclusion criteria, 75 individuals were ultimately designated as cases, while 75 were classified as controls (Fig. 1).

Saliva samples

Saliva collection and Preparation

Samples of whole saliva were collected in an unstimulated state, ensuring that the natural composition of saliva was preserved for accurate analysis. Parents were instructed to brush their children's teeth after breakfast, and children were instructed to abstain from food and liquids for half an hour before saliva collection. The saliva collection procedure involved utilizing a minimally invasive suction technique, wherein saliva naturally pooled at the oral cavity floor was gently aspirated using a needleless syringe. By applying controlled low-pressure suction, a vacuum was created to efficiently draw an adequate volume of saliva without causing discomfort to the participant. Samples of saliva were taken between 9:00 am and 11:00 am to minimize variations caused by circadian rhythms. Immediately following the finish of saliva

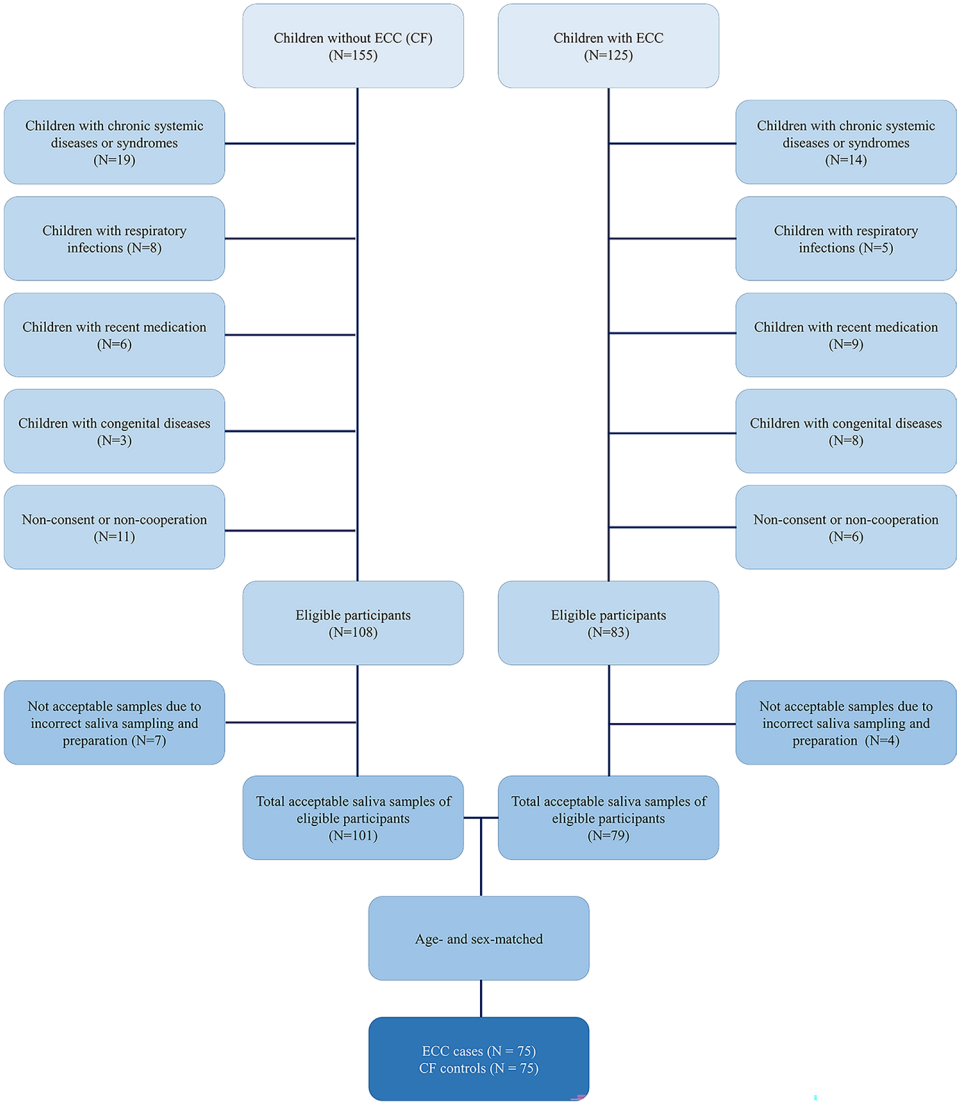


Fig. 1 STARD diagram showing the enrollment of children in the study

collection, a full protease inhibitor cocktail was added. To prepare the unstimulated saliva samples for analysis, they were first centrifugated at 10,000×g for 15 min at 4 °C (DLAB D2012 plus, Beijing, China). This process helps separate the cellular components and particulate matter from the liquid portion of the saliva, resulting in a clearer supernatant for further examination. Following centrifugation, the samples were diluted in saline phosphate buffer at a ratio of 1:10, and 10 mL of the buffer was added to each sample. The diluted samples were centrifuged again for 5 min to remove any remaining debris, ensuring the supernatant was as clean as possible. Saliva was placed in coded microtubes before being stored at -80 °C in ice boxes. Samples were sent to immunology laboratories for analysis.

Determination of salivary Beta-Defensin-2 and Statherin

BD-2 concentrations were determined using human beta-defensin-2 (HBD-2) enzyme-linked immunosorbent assay (ELISA) kit (Human Beta-defensin 2 (DEFB2) ELISA Kit, Cat No: ZB-11936 C-H9648, ZellBio GmbH, Germany) with the detection range of 10-4000 ng/L, and the sensitivity of 5.31 ng/L. Statherin (STATH) level were measured with ELISA kit (Human Statherin (STATH) ELISA Kit, Cat No: ZB-12556 C-H9648, ZellBio GmbH, Germany) with the detection range of 3.12–200 ng/mL, and the sensitivity equal to 1.25 ng/mL. As directed by the manufacturer, the samples were defrosted and analyzed. The samples' absorbance at 450 nm was recorded using the Hyperion ELISA microplate reader. Using spectrometer software and standard curves, the concentrations of STATH and BD-2 were calculated.

Analysis

Statistical analysis

The statistical analysis for this study was performed utilizing SPSS software (version 22; SPSS Inc., USA) and GraphPad Prism software (version 9.2.1, GraphPad Software, California). The relationship between ECC and various socioeconomic, demographic, and clinical factors was evaluated using statistical methods, including the T-test for comparing group means. The possible contribution of BD-2 and STATH salivary levels and their combination to the detection of ECC was assessed using the receiver operating characteristic (ROC) curve analysis. The correlation between each pair of variables was analyzed using the Spearman correlation coefficient. Multiple logistic regression models were used to identify the factors and their effects associated with ECC. The findings are shown as mean ± standard deviation (SD), and a p-value of less than 0.05 is considered statistically significant.

Machine learning analysis

Different supervised machine learning techniques were used to assess ECC's salivary biomarkers and other clinical and demographic factors. For this purpose, Python Software (version 3.8) and the open-source deep learning package Keras [50] were used. The dataset was divided into training and testing sets, with 80% designated for training and 20% for testing the models. The data was standardized using Standard Scaler from scikit-learn to normalize all features to the same scale, which improved model performance, especially for neural networks. To ensure the robustness of the machine learning models, k-fold cross-validation was applied during the training process. Additionally, using grid search to optimize the models' performance, hyperparameter tuning was performed. This approach allowed for fine-tuning of the model parameters instead of using the default hyperparameters. These steps were crucial in improving the accuracy and reliability of the model predictions, ensuring that the results obtained were robust and generalizable. All available clinical and demographic variables were included in the machine learning models. Subsequently, several machine learning models were selected for this study, including Neural Networks, XGBoost, Random Forest, Support Vector Machine (SVM), Logistic Regression, AdaBoost, CatBoost, Gradient Boosting, and K-Nearest Neighbors (KNN). Initially, a neural network model was constructed using the Keras library, consisting of two hidden layers with ReLU activation functions and one output layer with a sigmoid activation function. The binary cross-entropy loss function and Adam optimizer then trained the model. Next, the XGBoost model was implemented using the xgboost library to perform classification tasks. A Random Forest model was created using scikit-learn's Random Forest Classifier and trained on the data, while the SVM model was implemented using scikit-learn's SVC class with probability prediction enabled.

Logistic regression, AdaBoost, CatBoost, Gradient Boosting, and KNN algorithms were employed to diagnose and predict ECC. Their performance was evaluated based on two key metrics, accuracy and area under the ROC curve (AUC). Predictions were made for each model, sensitivity and specificity, and AUC were computed for performance evaluation and comparison (Supplementary File 1, Machine learning details). To assess feature importance across the models, we employed different methods tailored to the characteristics of each model. The built-in feature importance method was utilized for tree-based models, including Random Forest, AdaBoost, Gradient Boosting, XGBoost, CatBoost, and KNN. For Neural Networks and SVM, we applied permutation importance, where the impact of each feature is measured by randomly shuffling its values and observing

changes in model performance. In Logistic Regression, feature importance was determined by the absolute magnitude of the model's coefficients, with larger coefficients indicating greater significance of the corresponding features.

Results

Participants characteristics

Table 1 shows the clinical and demographic features of 150 sex- and age-matched participants from the CF and ECC groups (75 participants from each group). There

Table 1 Demographic and clinical characteristics of children with ECC and CF

Characteristics	Control group (CF) ($n=75$)	children with ECC (ECC) ($n=75$)	*P* value (*p*)
Age of participants, months *	4.67 ± 0.87	4.79 ± 0.88	0.40
Sex			0.63
Male	34 (45.33%)	38 (50.67%)	
Female	41 (56.67%)	37 (49.33%)	
Age of participants, year *	4.67 ± 0.87	4.79 ± 0.88	0.40
Mother's age, years *	35.46 ± 4.47	33.92 ± 5.32	0.78
Father's age, years *	37.79 ± 5.19	37.08 ± 5.91	0.44
Mother's education level (based on ISCED 2011** [51])			0.005
Post-secondary non-tertiary education and other lower educational levels (ISCED 4 and lower levels)	1 (1.33%)	13 (17.33%)	
Short-cycle tertiary education (ISCED 5)	17 (22.67%)	18 (24.00%)	
Bachelor or equivalent (ISCED 6)	52 (69.33%)	42 (56.00%)	
Master or equivalent (ISCED 7)	5 (6.67%)	2 (2.67%)	
Father's education level (based on ISCED 2011** [51])			0.26
Post-secondary non-tertiary education (ISCED 4) and other lower educational levels	8 (2.67%)	11 (14.67%)	
Short-cycle tertiary education (ISCED 5)	21 (28.00%)	27 (36.00%)	
Bachelor or equivalent (ISCED 6)	28 (37.33%)	31 (41.33%)	
Master or equivalent (ISCED 7)	19 (32.00%)	6 (8.00%)	
Toothbrush in a day			0.80
0 (Did not brush teeth daily)	9 (12.00%)	3 (4.00%)	
1 (Brushed teeth once daily)	34 (45.33%)	54 (72.00%)	
2 (Brushed teeth twice daily)	31 (41.33%)	13 (17.33%)	
3 (Brushed teeth three or more times daily)	1 (1.33%)	5 (6.67%)	
The use of fluoride toothpaste			0.21
Yes	51 (68.00%)	56 (74.67%)	
No	24 (32.00%)	19 (25.33%)	
Toothbrushing by the child or the child's parents			0.01
Parents	8 (10.67%)	13 (17.33%)	
child	67 (89.33%)	62 (82.67%)	
The consumption of vitamin supplements			0.002
Yes	7 (9.33%)	18 (24.00%)	
No	68 (90.67%)	57 (76.00%)	
The daily frequency of consuming sweets and snacks			0.04
0 (Did not consume daily)	4 (5.33%)	23 (30.67%)	
1 (Consumed once daily)	41 (54.67%)	27 (36.00%)	
2 (consumed twice daily)	29 (38.67%)	22 (29.33%)	
3 (consumed at least three times daily)	1 (1.33%)	3 (4.00%)	
Routine dental checkups (at least every six months)			< 0.001
Yes	49 (65.33%)	3 (4.00%)	
No	26 (34.67%)	72 (96.00%)	
Birth weight	3.21 ± 0.33	3.18 ± 0.29	0.61
Current weight	16.46 ± 3.15	17.48 ± 3.16	0.69
Salivary statherin *, ng/ml	222.31 ± 44.47	206.32 ± 64.21	0.080
Salivary beta-defensin-2 *, ng/ml	6.41 ± 2.45	9.25 ± 2.89	0.003

* Value presented as mean ± SD

** ISCED: International standard classification of education

was a significant difference in mothers' education levels between the two groups ($p = 0.005$). Mothers in the control group had higher education, while a higher percentage of mothers in the ECC group had lower educational levels. Vitamin supplement consumption was significantly higher in the ECC group (24.00%) compared to the control group (9.33%) ($p = 0.002$).

A significant difference was noted in the daily consumption of sweets and snacks ($p = 0.04$). In the ECC group, a higher percentage of children did not consume snacks daily, with 30.67% compared to just 5.33% in the control group. There was a significant difference in the frequency of regular dental visits ($p < 0.001$): only 4.00% of children in the ECC group made regular dental visits, whereas 65.33% of children in the control group did. Salivary BD-2 levels were significantly higher in the ECC group (9.25 ± 2.89 ng/mL) compared to the CF group (6.41 ± 2.45 ng/mL) ($p = 0.003$). The mean level of STATH in the ECC group was 206.32 ± 64.21 (ng/ml), and in the CF was 222.31 ± 44.47 (ng/ml). Although salivary STATH levels in children with ECC were lower than those in the control group, the T-test analysis showed no statistically significant difference in salivary STATH levels between the ECC and CF groups (Table 1).

The salivary levels of STATH measured indicate that salivary STATH levels alone may not serve as a strong or independent biomarker for distinguishing between CF children and those with ECC (Fig. 2-A). The salivary STATH and BD-2 cut-off value was defined considering Youden's index [52, 53]. Taking the value of 8.205 ng/ml as a cut-off point, the sensitivity and specificity of salivary BD-2 in ECC diagnosis were 76% (Fig. 2-B). Multiple logistic regression combined the salivary levels of STATH and BD-2 to diagnose ECC. The sensitivity of the multiple logistic regression model using salivary levels of STATH and BD-2 in ECC diagnosis was 76%, and its specificity was 79% (Fig. 2-C).

Significant correlations between several variables were found in the correlation analysis of the different ECC-related components. The connection between ECC and routine dental checkups was significantly negative (-0.66) when considering ECC's occurrence. This suggests that frequent dental checkups play a significant role in lowering the incidence of ECC. Additionally, a moderate

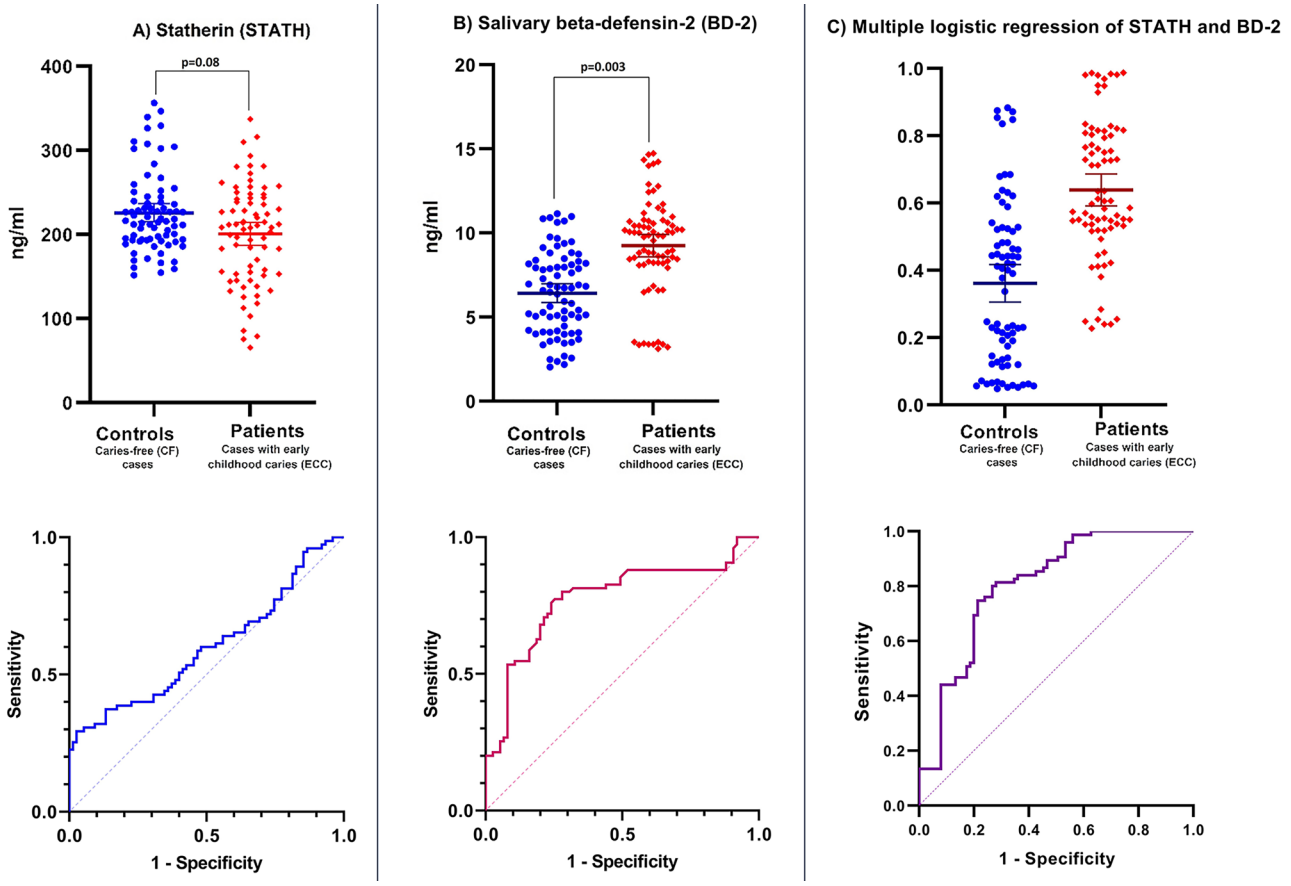


Fig. 2 Comparison of salivary biomarkers between caries-free (CF) children and those with early childhood caries (ECC). **A**: STATH levels show no significant difference ($p = 0.08$). **B**: BD-2 salivary levels are significantly higher in ECC than CF ($p = 0.003$). **C**: Multiple logistic regression of STATH and BD-2 shown by ROC curves

correlation (0.48) was observed between salivary BD-2 levels and ECC, indicating that elevated levels of this salivary marker may be linked to a greater risk of ECC. Examining the association between ECC and parental education, there was a somewhat negative correlation between ECC and the mother's and father's education (-0.22 and −0.29, respectively). These findings highlight how crucial parental knowledge and education are for lowering the risk of ECC (Fig. 3).

The Gradient Boosting and CatBoost models demonstrated the highest Area Under the Curve (AUC) of 1.000, followed closely by the Random Forest and AdaBoost models with AUC values of 0.987 and 0.982, respectively (Fig. 4). These results reflect the exceptional ability of these models to discriminate between classes, making them highly effective for identifying true positives. Furthermore, the Random Forest, Neural Network, Gradient Boosting, CatBoost, and AdaBoost models achieved perfect sensitivity, signifying their capability to accurately detect all true positive cases.

On the other hand, the K-Nearest Neighbors model exhibited the lowest sensitivity at 0.687, indicating challenges in identifying true positive cases. Despite this, specificity was high across all models, with the K-Nearest Neighbors model achieving perfect specificity (1.000), meaning it showed no false positives. However, the Neural Network model displayed the lowest specificity (0.857), suggesting a higher tendency for false positives than the other models. The highest accuracy (0.964) was observed in the Gradient Boosting, CatBoost, Random Forest, and AdaBoost models. In contrast, the XGBoost model had the lowest accuracy (0.8345), indicating relatively lower overall performance in classifying instances accurately.

The AUC and accuracy metrics should be considered together to evaluate model performance comprehensively. AUC measures the model's ability to correctly distinguish between classes, while accuracy provides a holistic view of correct classifications. The models with the highest AUC values, such as Gradient Boosting (1.000), CatBoost (1.000), and Random Forest (0.987), also maintained high accuracy, demonstrating a strong balance between discriminative power and classification success. These results underscore the models' effectiveness in distinguishing ECC cases from non-cases while ensuring high accuracy. Conversely, although models like XGBoost (AUC = 0.951) and K-Nearest Neighbors (AUC = 0.978) showed moderate AUC values, their lower accuracy scores (0.8345 and 0.8435, respectively) suggest a relatively weaker performance, potentially due to errors in classifying more complex instances (Fig. 4). The details of the machine learning models are provided at the following address: https://github.com/sajadbior/Salivary-biomarker-of-Early-childhood-caries-ECC-.

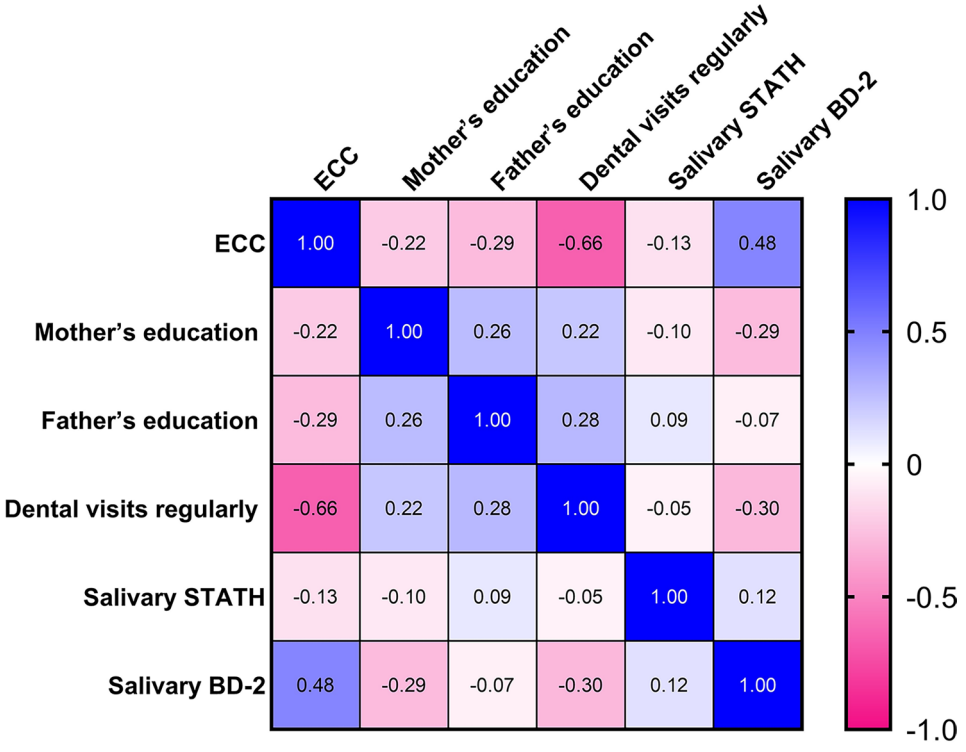
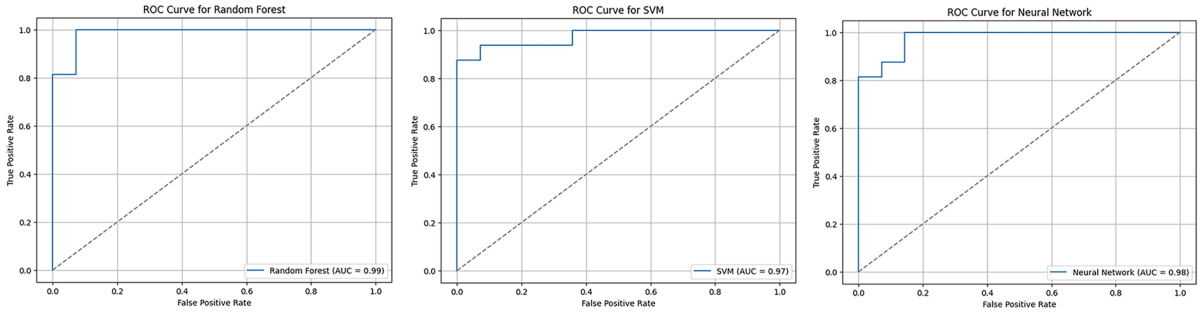
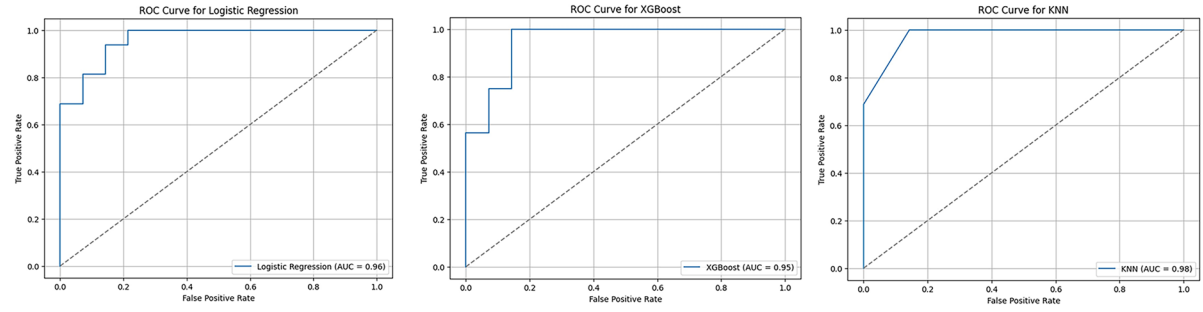


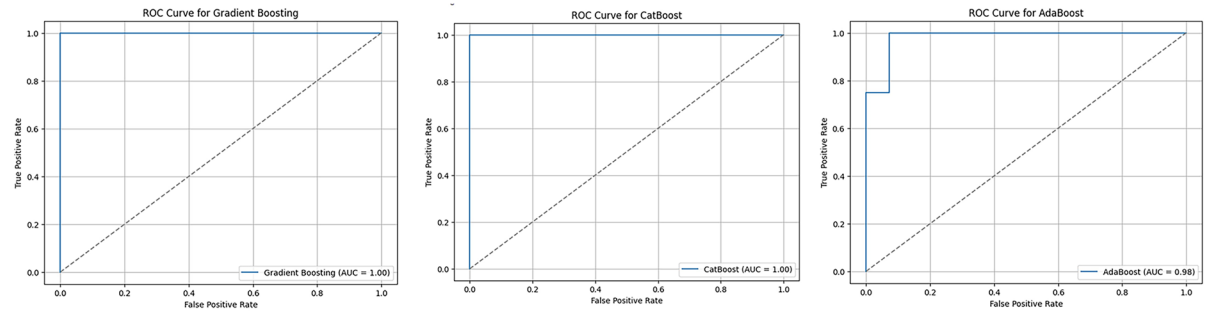
Fig. 3 Correlation matrix of factors related to ECC, parents' education levels, regular dental visits, and salivary markers (STATH and BD-2)



Model	Random Forest	Support Vector Machine	Neural Network
AUC	0.987	0.973	0.978
Sensitivity	1.000	0.937	1.000
Specificity	0.929	0.929	0.857



Model	Logistic Regression	XGBoost	K-Nearest Neighbors
AUC	0.960	0.951	0.978
Sensitivity	0.937	0.812	0.687
Specificity	0.958	0.857	1.000



Model	Gradient Boosting	CatBoost	AdaBoost
AUC	1.000	1.000	0.982
Sensitivity	1.000	1.000	1.000
Specificity	0.928	0.928	0.928

Fig. 4 Performance comparison of machine learning models for ECC diagnosis, including SVM, AdaBoost, Random Forest, Neural Network, Logistic Regression, KNN, CatBoost, and Gradient Boosting

Discussion

The results demonstrated that levels of BD-2 in the saliva of children with ECC were significantly higher than those in the control group. BD-2 is an antimicrobial protein in saliva that is crucial in defending the teeth and oral cavity against microbial infections and inflammation [54, 55]. These results align with studies demonstrating that elevated BD-2 levels can act as an inflammatory marker in response to dental caries-causing microbes and gingival inflammation [56–58]. The increase in BD-2 levels in saliva, particularly in inflammation and cariogenic bacterial activity, appears logical, as this protein is known to act as an antimicrobial agent against *Streptococcus mutans*, a key contributor to dental caries [59, 60]. These

findings are consistent with previous research, which associates changes in BD-2 levels with an increased risk of dental caries and oral inflammation [55, 61]. Therefore, BD-2 levels in saliva may be a reliable tool for identifying children at risk for ECC.

Furthermore, STATH levels in the saliva of children with ECC showed no significant difference compared to the control group. STATH is a salivary protein known to play an essential role in maintaining the calcium and phosphate balance in the mouth and preventing the deposition of minerals [62]. Although some studies have reported a correlation between changes in STATH levels and dental caries [63, 64], our study demonstrated that STATH levels alone are insufficient to differentiate between children with ECC and those who are caries-free, likely due to the multifactorial nature of ECC [65–67]. These findings are consistent with studies indicating that changes in STATH levels may be influenced by factors other than dental caries, such as the oral microbiome [68].

Cationic antimicrobial peptides (CAMPs) represent a crucial component of the oral innate immune system, offering natural protection against dental caries through multiple mechanisms [69, 70]. While the classification of salivary proteins can be complex, STATH and BD-2 contribute to oral health maintenance through antimicrobial and remineralization properties [12, 71]. Cationic antimicrobial peptides are generally characterized by their positive charge, amphipathic structure, and ability to interact with microbial membranes [72]. In the oral cavity, these peptides form part of the host defense system against pathogenic microorganisms. While STATH is primarily described as an acidic salivary peptide rather than a typical CAMP, it shares important functional properties with this group, particularly its antimicrobial activity [73]. The C-terminal fragment of STATH inhibits the growth of anaerobic bacteria in the oral cavity. This antimicrobial activity occurs through binding to bacterial fimbriae via recognition receptors when STATH is adsorbed to hydroxyapatite on the mineral surface [74]. The peptide undergoes a structural transition or folding upon adsorption to hydroxyapatite, which may explain the structural basis for its biological functioning [75].

STATH plays a critical role in the remineralization process and prevention of demineralization, which are essential for caries control. It inhibits hydroxyapatite crystallization and spontaneous calcium phosphate precipitation in vivo [76, 77]. Remarkably, STATH is the only salivary protein that inhibits the spontaneous precipitation of calcium phosphate salts from supersaturated saliva and functions as a very potent inhibitor of crystal growth compared to other salivary proteins [43]. It inhibits both primary and secondary precipitation of calcium phosphate salts. Beta defensins belong to the family of true cationic antimicrobial peptides. Beta defensins are produced by epithelial cells, including those in the oral cavity, and possess direct antimicrobial activity against a broad spectrum of oral pathogens [78, 79].

One of the key findings of this research was the relationship between oral hygiene behaviors and parental education level with the occurrence of ECC. The analysis revealed that parents with higher levels of education were less likely to have children affected by ECC. Our results are consistent with studies showing that parental awareness and education on oral hygiene and access to dental care are directly associated with lower dental caries in children [80–82]. This study found that irregular dentist visits significantly contributed to the ECC, which aligns with other research [83, 84]. Furthermore, Aliakbari et al. have shown that proper brushing can considerably reduce the risk of developing dental caries [85].

One of this work's breakthroughs is using machine learning algorithms to forecast ECC risk using clinical and demographic data. In this study, machine learning models such as Gradient Boosting, CatBoost, and Random Forest could accurately predict ECC. These models suggested that combining biological information (BD-2 and STATH levels) with demographic characteristics is a useful method for determining the risk of ECC. Other research studies have reported results that utilized machine learning techniques to predict ECC [26, 86–88]. Salivary markers, particularly BDF2, consistently rank among the top three in nearly every model, indicating their strong association with ECC. Salivary biomarkers, alongside other variables like Routine dental checkups and STATH, highlight the importance of biological and behavioral factors in determining the risk for ECC. This suggests that preventive measures such as regular dental visits are equally significant in predicting ECC. Additionally, parental education levels are important predictors in several models, especially in Logistic Regression and Support Vector Machines (Supplementary File 2, Variable importance plots). This aligns with existing literature that suggests socioeconomic factors and access to dental care impact the likelihood of ECC.

One of limitation of this study is the sample size, which, while sufficient for the analyses conducted, may not fully represent the diversity of children across different geographical regions, socio-economic backgrounds, and access to healthcare. Additionally, the study relied on parental self-reporting of children's behaviors and oral health habits, which may introduce bias due to recall errors or inaccuracies in reporting. Parental involvement and the reliability of their responses could impact the data collected regarding oral hygiene practices, dietary habits, and other relevant variables. This study's lack of longitudinal follow-up further limits the ability to assess how changes in salivary biomarkers and oral health status

may evolve over time. A longitudinal design would allow for better evaluation of how variations in these factors influence the progression or prevention of ECC. Future studies are recommended to include larger and more diverse samples, employ longitudinal designs, and utilize advanced methods to understand the multifactorial nature of ECC better.

Conclusion

This study investigated the role of salivary biomarkers, oral health behavioral factors, and demographic variables in understanding the complex etiology of ECC. The findings indicate that elevated salivary levels of BD-2 in children with ECC suggest its potential role as a biomarker for early diagnosis. Additionally, while STATH levels in the saliva of children with ECC were lower than in CF cases, no significant difference was observed. The study further highlights the importance of behavioral and demographic factors, such as parental education, in mitigating the risk of ECC. Machine learning techniques were highly effective in predicting ECC with accuracy. The study advocates for a multidisciplinary approach, integrating salivary biomarkers, oral health behavioral factors, demographic variables, and machine learning to provide a comprehensive and tailored strategy for understanding the risk factors of ECC.

Abbreviations

AUC	Area under the ROC curve
BD-2	Beta-defensin-2
CF	Caries-free
CAMPs	Cationic antimicrobial peptides
DMFT	Decayed, missing, and filled teeth
ECC	Early childhood caries
ELISA	Enzyme-linked immunosorbent assay
HBD-2	Human beta-defensin-2
ISCED	International standard classification of education
KNN	K-Nearest Neighbors
ROC	Receiver operating characteristic
SD	Standard deviation
STATH	Statherin
SVM	Support Vector Machine
WHO	World Health Organization

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12903-025-06252-3.

Supplementary Material 1

Supplementary Material 2

Acknowledgements

Not applicable.

Author contributions

MK and FKSh conceived the study idea and led data collection. MK, FKSh, and RM created the study protocol and wrote the original draft. MK, FKSh, and SK contributed to data analysis / interpretation and preparation of the manuscript. MK, EM, and RM led the writing review and editing. SK and MMSh performed machine learning. MK, FKSh, and SK interpreted the results. All authors read and approved the final manuscript.

Funding

There is no source of financial support or funding.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. The details of the machine learning models are provided at the following address: https://github.com/sajadbior/Salivary-biomarker-of-Early-childhood-caries-ECC-.

Declarations

Ethics approval and consent to participate

This analytical cross-sectional case-control study received ethical approval from the Ethical Committee of Tehran University of Medical Sciences (ethical code: IR.TUMS.DENTISTRY.REC.1400.158) and conducted by the Declaration of Helsinki [46]. Participants were provided with written informed consent obtained from a parent or guardian after the study objectives were explained. Children whose parents consented and signed the informed consent form were enrolled. The authors confirm that the relevant guidelines and regulations performed all methods.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Oral Medicine, School of Dentistry, Tehran University of Medical Sciences, P.O.BOX:14395 -433, North Kargar St, Tehran 14399-55991, Iran
[2]Department of Pediatric Dentistry, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran
[3]Universal Scientific Education and Research Network (USERN), Tehran, Iran
[4]Department of Industrial and Systems Engineering, Faculty of Engineering, Ferdowsi University of Mashhad, Mashhad, Iran

Received: 5 February 2025 / Accepted: 23 May 2025
Published online: 31 May 2025

References

1. Uribe SE, Innes N, Maldupa I. The global prevalence of early childhood caries: a systematic review with meta-analysis using the WHO diagnostic criteria. Int J Pediatr Dent. 2021;31(6):817–30.
2. Kazeminia M, Abdi A, Shohaimi S, Jalali R, Vaisi-Raygani A, Salari N, Mohammadi M. Dental caries in primary and permanent teeth in children's worldwide, 1995 to 2019: a systematic review and meta-analysis. Head Face Med. 2020;16:1–21.
3. Thakur S, Sharma R, Singhal P, Chauhan D. Prevalence and determinants of early childhood caries among preschool children in district Shimla, North India: a cross -sectional study. BMC Oral Health. 2025;25(1):168.
4. Kotha A, Vemulapalli A, Mandapati SR, Aryal S. Prevalence, trends, and severity of early childhood caries in the united States: National health and nutritional examination survey data 2013 to 2018. Pediatr Dent. 2022;44(4):261–8.
5. Shoaee S, Saeedi Moghaddam S, Masinaei M, Sofi-Mahmudi A, Hessari H, Heydari M-H, Shamsoddin E, Parsaeian M, Ghasemian A, Larijani B. Trends in dental caries of deciduous teeth in Iran: a systematic analysis of the National and sub-national data from 1990 to 2017. BMC Oral Health. 2022;22(1):634.
6. El Tantawi M, Attia D, Virtanen JI, Feldens CA, Schroth RJ, Al-Batayneh OB, Arheiam A. Foláyan MnO: A scoping review of early childhood caries, poverty and the first sustainable development goal. BMC Oral Health. 2024;24(1):1029.
7. Anil S, Anand PS. Early childhood caries: prevalence, risk factors, and prevention. Front Pead. 2017;5:157.

8. Abbass MMS, Mahmoud SA, El Moshy S, Rady D, AbuBakr N, Radwan IA, Ahmed A, Abdou A, Al Jawaldeh A. The prevalence of dental caries among Egyptian children and adolescences and its association with age, socioeconomic status, dietary habits and other risk factors. A cross-sectional study. *F1000Research* 2019, 8:8.
9. Jevtić M, Pantelinac J, Jovanović-Ilić T, Petrović V, Grgić O, Blažić L. The role of nutrition in caries prevention and maintenance of oral health during pregnancy. Medicinski Pregled. 2015;68(11–12):387–93.
10. Ganesh A, Muthu M, Padmanabhan R, Nuvvula S. Association of sleep-time feeding practices with early childhood caries: A case-control study. Caries Res. 2022;56(4):399–406.
11. Branger B, Camelot F, Droz D, Houbiers B, Marchalot A, Bruel H, Laczny E, Clement C. Breastfeeding and early childhood caries. Review of the literature, recommendations, and prevention. Archives De Pédiatrie. 2019;26(8):497–503.
12. Hemadi AS, Huang R, Zhou Y, Zou J. Salivary proteins and microbiota as biomarkers for early childhood caries risk assessment. Int J Oral Sci. 2017;9(11):e1–1.
13. Ravikumar D, Ramani P, Gayathri R, Hemashree K, Prabhakaran P. Physical and chemical properties of saliva and its role in early childhood caries–A systematic review and meta-analysis. J Oral Biology Craniofac Res. 2023;13(5):527–38.
14. Ghazal TS, Levy SM, Childers NK, Carter KD, Caplan DJ, Warren JJ, Cavanaugh JE, Kolker J. Mutans Streptococci and dental caries: A new statistical modeling approach. Caries Res. 2018;52(3):246–52.
15. Xu S, Zhao C, Jia L, Ma Z, Zhang X, Shi H. Relationship between preterm, low birth weight, and development defects of enamel in the primary dentition: A meta-analysis. Front Pead. 2022;10:975340.
16. Li Y, Zhang Y, Yang R, Zhang Q, Zou J, Kang D. Associations of social and behavioural factors with early childhood caries in Xiamen City in China. Int J Pediatr Dent. 2011;21(2):103–11.
17. Simancas-Pallares MA, Ginnis J, Vann WF Jr, Ferreira Zandona AG, Shrestha P, Preisser JS, Divaris K. Children's oral health-related behaviours and early childhood caries: A latent class analysis. Commun Dent Oral Epidemiol. 2022;50(3):147–55.
18. Souza JGS, Sampaio AA, Costa Oliveira BE, Jones KM, Martins AMEDBL. Socioeconomic inequalities in the use of dental care services during early childhood: an epidemiological survey. Int J Pediatr Dent. 2018;28(4):400–9.
19. Gunasinghe KAMM, Wijesinghe MSD, Ratnayake NC. Socio-behavioural associates of early childhood caries among preschool children aged three to four years in Gampaha district, Sri Lanka: a cross sectional study. BMC Oral Health. 2024;24(1):1293.
20. Rai NK, Tiwari T. Parental factors influencing the development of early childhood caries in developing nations: a systematic review. Front Public Health. 2018;6:64.
21. Pierce A, Singh S, Lee J, Grant C, Cruz de Jesus V, Schroth RJ. The burden of early childhood caries in Canadian children and associated risk factors. Front Public Health. 2019;7:328.
22. Liu S-M, Xin Y-M, Wang F, Lin P-C, Huang H-L. Parental health belief model constructs associated with oral health behaviors, dental caries, and quality of life among preschool children in China: a cross-sectional study. BMC Oral Health. 2024;24(1):1497.
23. Ghazal T, Levy SM, Childers NK, Broffitt B, Cutter GR, Wiener HW, Kempf MC, Warren J, Cavanaugh JE. Factors associated with early childhood caries incidence among high caries-risk children. Commun Dent Oral Epidemiol. 2015;43(4):366–74.
24. Zhou Y, Yang J, Lo E, Lin H. The contribution of life course determinants to early childhood caries: a 2-year cohort study. Caries Res. 2012;46(2):87–94.
25. Losso EM, Tavares MCR, da Silva JY, Urban CA. Severe early childhood caries: an integral approach. Jornal De Pediatria. 2009;85:295–300.
26. Koopaie M, Salamati M, Montazeri R, Davoudi M, Kolahdooz S. Salivary Cystatin S levels in children with early childhood caries in comparison with caries-free children; statistical analysis and machine learning. BMC Oral Health. 2021;21(1):650.
27. Koopaie M, Mortazavi H, Khatami A, Khodashenas Z. Salivary and serum antidesmoglein 1 and 3 ELISA and indirect Immunofluorescence in pemphigus vulgaris: correlations with serum ELISA, indirect Immunofluorescence and disease severity. Acta Dermatovenerol Croat. 2018;26(2):91–91.
28. Zhou X, Li H, Zhu C, Yuan C, Meng C, Feng S, Sun X, Zheng S. Analysis of salivary proteomic biomarkers for the surveillance of changes in high-risk status of early childhood caries. BMC Oral Health. 2021;21:1–10.
29. Policy on Early Childhood Caries (ECC). Classifications, consequences, and preventive strategies. Pediatr Dent. 2016;38(6):52–4.
30. Huang S, Tian W, Tian J, Tang H, Qin M, Zhao B, Wang J, Chen Y, Xu H. Deep saliva proteomics elucidating the pathogenesis of early childhood caries and identifying biomarkers for early prediction. J Proteome Res 2024.
31. Afsaneh Abadi P, Koopaie M, Montazeri R. Comparison of salivary nitric oxide and oral health in diabetic patients with and without Xerostomia. Diabetes Metabolic Syndrome. 2020;14(1):11–5.
32. Chen M, Hu Z, Shi J, Xie Z. Human β-defensins and their synthetic analogs: natural defenders and prospective new drugs of oral health. Life Sci. 2024;346:122591.
33. Dommisch H, Jepsen S. Diverse functions of defensins and other antimicrobial peptides in periodontal tissues. Periodontol 2000. 2015;69(1):96–110.
34. Basingab F, Alsaiary A, Almontashri S, Alrofaidi A, Alharbi M, Azhari S, Algothmi K, Alhazmi S. Alterations in Immune-Related defensin alpha 4 (DEFA4) gene expression in health and disease. Int J Inflamm. 2022;2022(1):9099136.
35. Yuan J, Yan Y, Zhang J, Wang B, Feng J. Diagnostic accuracy of alpha-defensin in periprosthetic joint infection: a systematic review and meta-analysis. Int Orthop. 2017;41(12):2447–55.
36. Fesahat F, Firouzabadi AM, Zare-Zardini H, Imani M. Roles of different β-Defensins in the human reproductive system: A review study. Am J Men's Health. 2023;17(3):15579883231182673.
37. Zaga-Clavellina V, Martha RVM, Flores-Espinosa P. In vitro secretion profile of pro-inflammatory cytokines IL-1β, TNF-α, IL-6, and of human Beta-Defensins (HBD)-1, HBD-2, and HBD-3 from human chorioamniotic membranes after selective stimulation with gardnerella vaginalis. Am J Reprod Immunol. 2012;67(1):34–43.
38. Ahn KB, Kim AR, Kum K-Y, Yun C-H, Han SH. The synthetic human beta-defensin-3 C15 peptide exhibits antimicrobial activity against Streptococcus mutans, both alone and in combination with dental disinfectants. J Microbiol. 2017;55:830–6.
39. Hatipoğlu Ö, Saydam F. Association between rs11362 polymorphism in the beta-defensin 1 (DEFB1) gene and dental caries: A meta-analysis. J Oral Biosci. 2020;62(3):272–9.
40. Ferrari CR, de Oliveira TE, Buzalaf MAR, Netz PA. Interaction of Statherin derived peptide with the surface of hydroxyapatite: perspectives based on molecular dynamics simulations. Caries Res 2024.
41. Xiao Y, Karttunen M, Jalkanen J, Mussi M, Liao Y, Grohe B, Lagugné-Labarthet F, Siqueira W. Hydroxyapatite growth Inhibition effect of pellicle Statherin peptides. J Dent Res. 2015;94(8):1106–12.
42. Steinbauer P, Rohatschek A, Andriotis O, Bouropoulos N, Liska R, Thurner PJ, Baudis S. Single-Molecule force spectroscopy reveals Adhesion-by-Demand in Statherin at the Protein–Hydroxyapatite interface. Langmuir. 2020;36(44):13292–300.
43. Pateel DGS, Gunjal S, Math SY, Murugeshappa DG, Nair SM. Correlation of salivary Statherin and calcium levels with dental calculus formation: a preliminary study. Int J Dent 2017, 2017(1):2857629.
44. Araujo TT, Debortolli ALB, Carvalho TS, Rodrigues CMVBF, Dionizio A, de Souza BM, Vertuan M, Ventura TM, Grizzo LT, Marchetto R. Paving the way for the use of Statherin-Derived peptide (StN15) to control caries through acquired pellicle and biofilm Microbiome engineering: Proof-of-concept in vitro/in vivo studies. Arch Oral Biol. 2025;171:106159.
45. Shimotoyodome A, Kobayashi H, Tokimitsu I, Matsukubo T, Takaesu Y. Statherin and Histatin 1 reduce Parotid saliva-promoted Streptococcus mutans strain MT8148 adhesion to hydroxyapatite surfaces. Caries Res. 2006;40(5):403–11.
46. Association WM. World medical association declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191–4.
47. Naidu R, Nunn J, Kelly A. Socio-behavioural factors and early childhood caries: a cross-sectional study of preschool children in central Trinidad. BMC Oral Health. 2013;13(1):1–7.
48. Organization WH. Oral health surveys: basic methods. World Health Organization; 2013.
49. Pediatrics AAo. Policy on early childhood caries (ECC): classifications, consequences, and preventive strategies. Pediatr Dent. 2008;30(7 Suppl):40–3.
50. Chollet F. keras. In.; 2015.
51. Statistics Ulf. International standard classification of education: ISCED 2011. UNESCO Institute for Statistics Montreal; 2012.

52. Ruopp MD, Perkins NJ, Whitcomb BW, Schisterman EF. Youden index and optimal cut-point estimated from observations affected by a lower limit of detection. Biometrical Journal: J Math Methods Biosci. 2008;50(3):419–30.
53. Smits N. A note on Youden's J and its cost ratio. BMC Med Res Methodol. 2010;10(1):1–4.
54. Pereira AL, Franco GC, Cortelli SC, Aquino DR, Costa FO, Raslan SA, Cortelli JR. Influence of periodontal status and periodontopathogens on levels of oral human β-defensin-2 in saliva. J Periodontol. 2013;84(10):1445–53.
55. Salem A, Almahmoudi R, Hagström J, Stark H, Nordström D, Salo T, Eklund KK. Human β-defensin 2 expression in oral epithelium: potential therapeutic targets in oral lichen planus. Int J Mol Sci. 2019;20(7):1780.
56. Öztürk A, Kurt-Bayrakdar S, Avci B. Comparison of gingival crevicular fluid and serum human beta-defensin-2 levels between periodontal health and disease. Oral Dis. 2021;27(4):993–1000.
57. Ayoub CG, Aminoshariae A, Bakkar M, Ghosh S, Bonfield T, Demko C, Montagnese TA, Mickel AK. Comparison of IL-1β, TNF-α, hBD-2, and hBD-3 expression in the dental pulp of smokers versus nonsmokers. J Endod 2017, 43(12):2009–13.
58. Cieślik M, Bagińska N, Górski A, Jończyk-Matysiak E. Human β-defensin 2 and its postulated role in modulation of the immune response. Cells. 2021;10(11):2991.
59. Kusumaningsih T, Subijanto M, Indrawati R, Devijanti RR. The level of beta defensin-2 in saliva and its expression in Parotid gland epithelial cells after probiotic (Lactobacillus reuteri) induction to inhibit Streptococcus mutans in caries. Eur J Dentistry. 2016;10(04):556–60.
60. Koopaie M, Fatahzadeh M, Jahangir S, Bakhtiari R. Comparison of the effect of regular and probiotic cake (Bacillus coagulans) on salivary pH and Streptococcus mutans count. Dent Med Probl. 2019;56(1):33–8.
61. Farges J-C, Alliot-Licht B, Renard E, Ducret M, Gaudin A, Smith AJ, Cooper PR. Dental pulp defence and repair mechanisms in dental caries. Mediat Inflamm. 2015;2015(1):230251.
62. de Sousa-Pereira P, Amado F, Abrantes J, Ferreira R, Esteves PJ, Vitorino R. An evolutionary perspective of mammal salivary peptide families: cystatins, histatins, Statherin and PRPs. Arch Oral Biol. 2013;58(5):451–8.
63. Pappa E, Vougas K, Zoidakis J, Papaioannou W, Rahiotis C, Vastardis H. Downregulation of salivary proteins, protective against dental caries, in type 1 diabetes. Proteomes. 2021;9(3):33.
64. Nussrat RL, Aldhaher ZA. Evaluation of Statherin and ß-Defensin-3 in saliva of children with dental caries: case control study. J Emerg Med Trauma Acute Care. 2024;2024(2):7.
65. Blanco-Victorio DJ, López-Luján NA, Bernaola-Silva W, Vicuña-Huaqui LA, Cacñahuaray-Palomino R, Diaz-Campos JS, Anicama-Barrios YA, Rojas-Apaza Z, Castañeda-Sarmiento S, Vidigal EA. Sociodemographic and clinical factors associated with early childhood caries in Peruvian pre-schoolers. BMC Oral Health. 2025;25(1):125.
66. Zou J, Du Q, Ge L, Wang J, Wang X, Li Y, Song G, Zhao W, Chen X, Jiang B. Expert consensus on early childhood caries management. Int J Oral Sci. 2022;14(1):35.
67. Lund IO, Hannigan LJ, Ask H, Askelund AD, Hegemann L, Corfield EC, Wootton RE, Ahmadzadeh YI, Davey Smith G, McAdams TA, et al. Prenatal maternal stress: triangulating evidence for intrauterine exposure effects on birth and early childhood outcomes across multiple approaches. BMC Med. 2025;23(1):18.
68. Dipalma G, Inchingolo A, Inchingolo F, Charitos I, Di Cosola M, Cazzolla A. Focus on the cariogenic process: microbial and biochemical interactions with teeth and oral environment. J Biol Regul Homeost Agents. 2021;35(1023812):20–747.
69. LaRock CN, Nizet V. Cationic antimicrobial peptide resistance mechanisms of Streptococcal pathogens. Biochim Et Biophys Acta (BBA)-Biomembranes. 2015;1848(11):3047–54.
70. Jourdain ML, Velard F, Pierrard L, Sergheraert J, Gangloff SC, Braux J. Cationic antimicrobial peptides and periodontal physiopathology: A systematic review. J Periodontal Res. 2019;54(6):589–600.
71. Zafar MS, Fareed MA, Riaz S, Latif M, Habib SR, Khurshid Z. Customized therapeutic surface coatings for dental implants. Coatings. 2020;10(6):568.
72. Bechinger B. Rationalizing the membrane interactions of cationic amphipathic antimicrobial peptides by their molecular shape. Curr Opin Colloid Interface Sci. 2009;14(5):349–55.
73. Trindade F, Amado F, da Costa JP, Ferreira R, Maia C, Henriques I, Colaço B, Vitorino R. Salivary peptidomic as a tool to disclose new potential antimicrobial peptides. J Proteom. 2015;115:49–57.
74. Lei J, Sun L, Huang S, Zhu C, Li P, He J, Mackey V, Coy DH, He Q. The antimicrobial peptides and their potential clinical applications. Am J Translational Res. 2019;11(7):3919–31.
75. Rimola A, Aschi M, Orlando R, Ugliengo P. Does adsorption at hydroxyapatite surfaces induce peptide folding? Insights from large-scale B3LYP calculations. J Am Chem Soc. 2012;134(26):10899–910.
76. Zhang OL, Niu JY, Yu OY, Mei ML, Jakubovics NS, Chu CH. Peptide designs for use in caries management: A systematic review. Int J Mol Sci 2023, 24(4).
77. Lopez Cascales JJ, Zenak S, García de La Torre J, Lezama OG, Garro A, Enriz RD. Small cationic peptides: influence of charge on their antimicrobial activity. ACS Omega. 2018;3(5):5390–8.
78. Diamond G, Ryan L. Beta-defensins: what are they REALLY doing in the oral cavity? Oral Dis. 2011;17(7):628–35.
79. Atalay N, Balci N, Gürsoy M, Gürsoy UK. Systemic factors affecting human Beta-Defensins in oral cavity. Pathogens. 2024;13(8):654.
80. Saldūnaitė K, Bendoraitienė EA, Slabšinskienė E, Vasiliauskienė I, Andruškevičienė V, Zūbienė J. The role of parental education and socio-economic status in dental caries prevention among Lithuanian children. Medicina. 2014;50(3):156–61.
81. Ardérius A, Veiga N, Godinho M, Ribeiro C. The influence of parents' educational level in children's oral health behavior. Public Health Res. 2015;5(1):28–31.
82. Chen L, Hong J, Xiong D, Zhang L, Li Y, Huang S, Hua F. Are parents' education levels associated with either their oral health knowledge or their children's oral health behaviors? A survey of 8446 families in Wuhan. BMC Oral Health. 2020;20:1–12.
83. Mallineni SK, Alassaf A, Almulhim B, Alghamdi S. Influence of tooth brushing and previous dental visits on dental caries status among Saudi Arabian children. Children. 2023;10(3):471.
84. Li J, Fan W, Zhou Y, Wu L, Liu W, Huang S. The status and associated factors of early childhood caries among 3-to 5-year-old children in Guangdong, Southern China: a provincial cross-sectional survey. BMC Oral Health. 2020;20:1–8.
85. Aliakbari E, Gray-Burrows KA, Vinall-Collier KA, Edwebi S, Marshman Z, McEachan RR, Day PF. Home-based toothbrushing interventions for parents of young children to reduce dental caries: A systematic review. Int J Pediatr Dent. 2021;31(1):37–79.
86. Park Y-H, Kim S-H, Choi Y-Y. Prediction models of early childhood caries based on machine learning algorithms. Int J Environ Res Public Health. 2021;18(16):8613.
87. Qu X, Zhang C, Houser SH, Zhang J, Zou J, Zhang W, Zhang Q. Prediction model for early childhood caries risk based on behavioral determinants using a machine learning algorithm. Comput Methods Programs Biomed. 2022;227:107221.
88. Hasan F, Tantawi ME, Haque F, Foláyan MO, Virtanen JI. Early childhood caries risk prediction using machine learning approaches in Bangladesh. BMC Oral Health. 2025;25(1):49.

Publisher's note