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ORIGINAL RESEARCH

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Salivary sIgA, mucin MG1, mucin MG2, lactoferrin and lysozyme related to early childhood caries: a case-control study

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Abstract

Background: Early childhood caries (ECC) is a chronic infectious disease caused by various factors and progresses rapidly, severely affecting children's oral health and overall well-being. Salivary proteins modulate the oral micro-ecological environment by different natural defense mechanisms and prevent dental caries. Aiming to determine whether salivary secretory immunoglobulin A (sIgA), high-molecular-weight mucins (mucin MG1), low-molecular-weight mucins (mucin MG2), lactoferrin (LTF) and lysozyme levels might serve as biomarkers to assess childhood caries risk, the present study investigated the correlations between salivary sIgA, mucin MG1, mucin MG2, lactoferrin (LTF) and lysozyme levels and childhood caries. **Methods**: 51 children aged 3–5 years old were investigated and randomly categorized into three groups (n = 17/group) based on their decayed, missing and filled surfaces (dmfs) index: a caries-free group (dmfs = 0), a low-caries group (dmfs = 1–4), and a high-caries group (dmfs >4). 5 mL of unstimulated saliva was collected from each child, centrifuged to collect the supernatant. Secretory immunoglobulin A (sIgA), mucin MG1, mucin MG2, lactoferrin (LTF) and lysozyme were measured via enzyme-linked immunosorbent assay (ELISA). Receiver operating characteristic (ROC) curves were plotted to evaluate the diagnostic potential of the above salivary proteins as risk indicators for ECC. **Results**: Among the three groups, sIgA, mucin MG1, mucin MG2, LTF and lysozyme expression differed significantly ($p < 0.05$). Pairwise comparisons revealed no significant differences in LTF and lysozyme levels between the low-caries group and the high-caries group. However, statistically significant differences were observed in all other pairwise comparisons ($p < 0.05$). **Conclusions**: As a diagnostic marker, sIgA did not show statistical significance, while the other four salivary proteins did. It appears that LTF, mucin MG1, lysozyme and mucin MG2 could be employed to assess caries risk in children, with LTF demonstrating the most practical significance.

Keywords

Early childhood caries; Salivary proteins; Dental caries susceptibility; Salivary biomarkers

1. Introduction

Dental caries are one of the most serious diseases for children. The World Health Organization (WHO) has identified caries as one of the three major diseases that require prevention and control. Early childhood caries (ECC) is characterized by one or more cavities, loss (caused by decay) or filled surfaces on any deciduous tooth a child has under 6 [1]. Three conditions can indicate severe ECC: smooth surface decay in children under 3 years old, smooth surface decay in upper front teeth of 3–5 years old, or a decayed, lost and filled index of at least 4, 5 and 6 in 3, 4 and 5 years old children, respectively [2]. Untreated ECC can cause pain, impair digestive function, and pose a threat to permanent teeth that can seriously affect a child's physical and mental health [3]. In China, the 4th National Oral Health Epidemiological Survey revealed that deciduous teeth and young permanent teeth caries have significantly increased over the past decade. There was an estimated caries rate of 71.9% for 5-year-old deciduous teeth, which is 5.8% higher than 10 years ago [4] and far from WHO's target of no caries for 90% of 5-year-olds. While significant efforts have been made to prevent caries and educate the public over the past few decades, dental problems remain a major problem in China. Caries is a multifactorial infectious disease. Therefore, early prediction and prevention in high-risk and susceptible children is an effective way to control ECC. With the recent rapid development of proteomics technology, salivary proteins have great potential for disease

prediction and early diagnosis. Salivary proteins employ natural defense mechanisms to control the oral microenvironment and ECC [5]. Caries susceptibility is correlated with salivary protein expression, so it is a potential predictor of dental caries risk [6]. Salivary proteins can also play a crucial role in the defense against ECC by inhibiting cariogenic bacteria' adhesion and colonization [7, 8].

In most previous studies, salivary proteins were compared between children with cavities and those without [9–13] or altered after comprehensive cavity treatment [14]. These studies mainly examined one or two protein components, making systematic comparisons impossible. Therefore, this study subdivided children with dental caries based on the number of decayed, missing and filled surfaces (dmfs) into low-caries (dmfs = 1–4) and high-caries (dmfs >4) groups as well as a control group comprised of children with no dental diseases (dmfs = 0). This study aimed to investigate the correlation between the levels of non-irritating salivary secretory immunoglobulin A (sIgA), mucin MG1, mucin MG2, lactoferrin (LTF) and lysozyme in 3–5-year-old children with free, low, and high dental caries. Additionally, these salivary proteins were tested for their sensitivity and potential to serve as dental caries biomarkers. This study investigated whether changes in the concentrations of the above five salivary proteins influence Early Childhood Caries (ECC) development. By addressing this question, this study aimed to elucidate the role of salivary proteins in oral health and provide direction for future research.

To guide our study design and data analysis, we propose the following null hypothesis (H_0) and alternative hypothesis (Ha):

Null Hypothesis (H_0): There is no significant association between changes in the concentrations of specific salivary proteins and the incidence of ECC.

Alternative Hypothesis (Ha): There is a significant association between changes in the concentrations of specific salivary proteins and the incidence of ECC.

2. Materials and methods

The study flow diagram of the experimental process of this study is shown in Fig. 1.

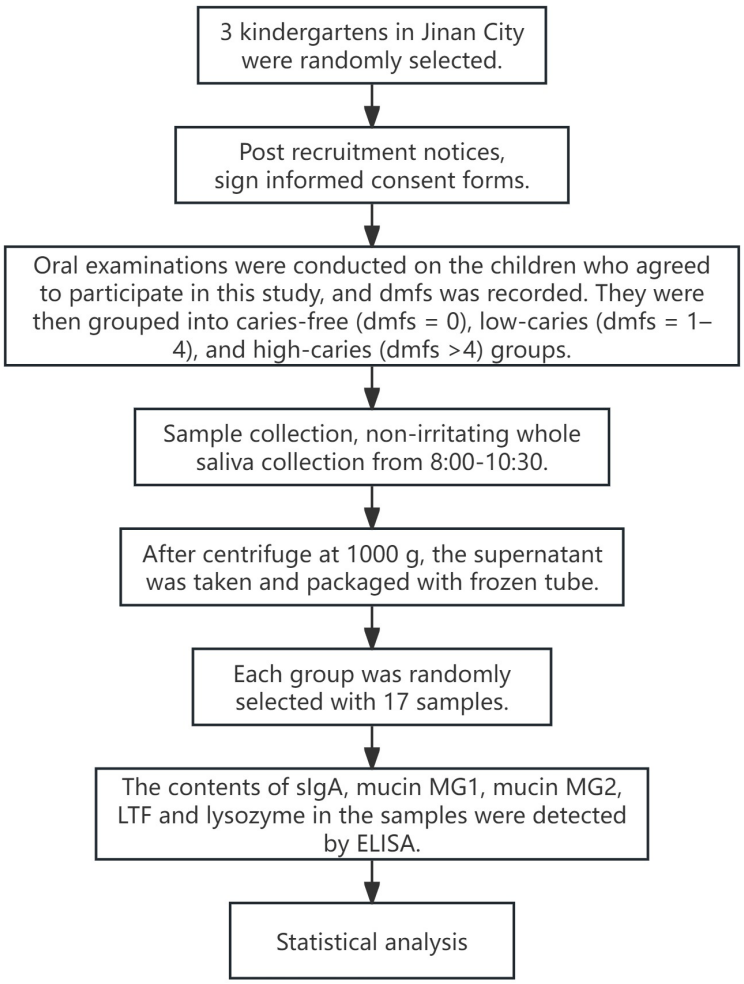

FIGURE 1. Study flow diagram. dmfs: decayed, missing and filled surfaces; sIgA: secretory immunoglobulin A; LTF: lactoferrin; ELISA: enzyme-linked immunosorbent assay; mucin MG1: high-molecular-weight mucins; mucin MG2: low-molecular-weight mucins.

2.1 Sample size calculation

The sample size was estimated during the pilot study phase between 06 November and 20 November 2023. As this study involved multiple group comparisons and the evaluation of diagnostic indicators for dental caries, sample size estimations were conducted during the pilot phase for two different study designs. Details were:

First, an independent multi-group design was employed, and one-way analysis of variance (ANOVA) to compare the levels of sIgA, mucin MG1 and mucin MG2 among three groups: the control group, the low-caries group and the high-caries group. The pilot study included 10 participants in each group. Mean values and overall standard deviations of sIgA, mucin MG1 and mucin MG2 for the three groups are shown in Table 1. Assuming a significance level (α) of 0.05, a statistical power of 80% (β = 0.2), and a two-sided test, pairwise comparisons among groups were conducted using the All-Pairs Tukey-Kramer method. Based on calculations from PASS 2021 (NCSS, LLC., Kaysville, UT, USA), the largest calculated sample size for each group was determined to be N1= N2 = N3 = 17.

Second, this study aimed to evaluate whether sIgA, mucin MG1 and mucin MG2 could serve as effective diagnostic biomarkers for dental caries, enabling differentiation between caries patients and healthy controls. Preliminary pilot experiments revealed that the areas under the ROC curve (AUC) for sIgA, mucin MG1 and mucin MG2, as diagnostic biomarkers for caries patients, were 0.711, 0.958 and 0.868, respectively. Assuming AUC values >0.5, with a significance level (α) of 0.05 (one-sided), a statistical power of 90%, and a ratio of caries patients (N2) to healthy controls (N1) of 2:1, the required sample sizes were calculated using PASS 2021. Results are summarized in Table 2.

Based on the sample size estimation results from the aforementioned research design, the final sample size for each group in this study was determined to be 17.

2.2 Study subjects and grouping

This study randomly selected three kindergartens in Jinan City and issued recruitment notices. Parents of children who agreed to participate signed informed consent forms. Dental caries status was assessed and recorded using the dmfs index for children who consented to participate in the study. Participants were categorized into three groups based on their dmfs scores: caries-free group (dmfs = 0), low-caries group (dmfs = 1–4) and high-caries group (dmfs >4), with 17 children randomly selected from each group. 51 children (31 females and 20 males) aged 3–5 years were randomly selected. A blinded design was not feasible in this study because there were no interventions for dental caries. Participants and researchers were both aware of the group assignments.

Inclusion criteria: Children ① Aged 3–5 years old; ② In good general health with no systemic diseases; ③ Provided signed informed consent by parents or guardians.

Exclusion criteria: Children ① Taking antibiotics or had fluoride treatment within the past 2 weeks; ② Did not complete an oral examination and sample collection [15].

2.3 Research methods

2.3.1 Oral examination

All oral examinations were conducted by two experienced pediatric dentists. The standard consistency test of Kappa value between the examiners was 0.84. Briefly children were placed in a semi-reclining position under natural light. An oral examination was performed using a disposable plane mirror and community periodontal index probe (CPI probe) with ball end. Deciduous teeth dmfs were recorded. Based on the 2013 Basic Methods for Oral Health Surveys recommended by the World Health Organization, diagnostic criteria for dental caries were developed. Caries were diagnosed when the teeth indicated obvious cavities in the pits and fissures or smooth surfaces, clear subsurface enamel destruction or detectable softened lesion bottoms or walls by probing. Moreover, teeth with temporary fillings, as well as with pit and fissure sealants

TABLE 1. Sample size calculation for multi-group comparison of pre-experimental indices.

Variable	Caries-Free Group	Low-Caries Group	High-Caries Group	Variance1	Variance2	Variance3	SD	N1 = N2 = N3
sIgA	46.22	56.73	31.96	10.51	14.26	24.77	5.6	17
Mucin MG1	50.66	38.09	26.88	12.57	21.78	11.21	4.62	11
Mucin MG2	28.53	39.76	52.78	11.23	24.25	13.02	6.09	16

sIgA: secretory immunoglobulin A; Mucin MG1: high-molecular-weight mucins; Mucin MG2: low-molecular-weight mucins; SD: standard deviation.

TABLE 2. Sample size calculation for evaluating the pre-experimental index as a diagnostic indicator of dental caries.

Variable	Pre-test AUC	N1	N2
sIgA	0.711	16	32
Mucin MG1	0.958	3	6
Mucin MG2	0.868	5	10

AUC: areas under the curve; sIgA: secretory immunoglobulin A; Mucin MG1: high-molecular-weight mucins; Mucin MG2: low-molecular-weight mucins.

combined with caries were considered carious [16].

2.3.2 Saliva sample collection

Following the oral examination, a sample collection was conducted. Children were instructed not to brush their teeth on the morning of sampling and to refrain from drinking or eating for at least 90 minutes before sample collection. During the same period (8:00–10:30 AM), the subjects naturally spat about 5 mL of saliva slowly into an enzyme-free and sterile high-speed centrifuge tube. After sealing the tube caps, they were placed in an insulated container with ice packs and transported to the laboratory at 0 °C. Saliva samples were centrifuged at room temperature (1000 g) for 20 mins to collect the supernatant, which was aliquoted and stored at −80 °C for future use [17]. Thawed saliva samples were not reused to avoid false results due to protein degradation.

2.3.3 Determination of salivary protein levels

An enzyme-linked immunosorbent assay (ELISA) kit (Shanghai Enzyme-Linked Bio-Tech Co., Ltd., Shanghai, China): Human sIgA ELISA Kit (E20240515-10580B), Human MUC1 ELISA Kit (E20240515-11732B), Human MUC2 ELISA Kit (E20240515-19308B), Human LTF ELISA Kit (E20240515-11233B), Human LZM ELISA Kit (E20240515-11222B) was employed to measure the salivary expression of sIgA, mucin MG1, mucin MG2, LTF and lysozyme proteins. Each sample was analyzed in duplicates, and an average value was taken. Protein levels were determined at 450 nm on a multi-functional ELISA reader (Thermo Fisher, Thermo Multiskan FC, Shanghai, China).

As per the instructions, the procedure is as follows: (1) Prepare all reagents before starting the assay procedure. All standards and samples should be added to the microtiter plate in duplicate. (2) Add 50 μL of standard or sample to the appropriate wells. Blank wells are not added. (3) Add 100 μL of enzyme-conjugate to standard wells and sample wells except the blank well, cover with an adhesive strip and incubate for 60 minutes at 37 °C. (4) Wash the microtiter plate 4 times. (5) Add 50 μL substrate A and 50 μL substrate B to each well. Gently mix and incubate for 15 minutes at 37 °C. Protect from light. (6) Add 50 μL stop solution to each well. The well color should change from blue to yellow. Tap the plate gently to ensure thorough mixing if the well color is green or the color change is not uniform. (7) Read the Optical Density (OD) at 450 nm using a microtiter plate reader within 15 minutes.

2.4 Data analysis

Data analysis was performed using SPSS 22.0 (IBM, Armonk, NY, USA), with a two-sided test and an alpha level of 0.05. Kolmogorov-Smirnov test and Shapiro-Wilk test were used to determine whether the data fit the normal distribution. Normally distributed quantitative data was presented as mean ± standard deviation. One-way Analysis of Variance (ANOVA) was performed to compare the sIgA, Mucin MG1, Mucin MG2, LTF and lysozyme levels between the three groups. For pairwise comparisons, the Bonferroni method was used. Moreover, receiver operating characteristic (ROC) curves were generated to investigate the diagnostic efficacy of the above salivary proteins as diagnostic markers for childhood caries.

3. Results

Statistically significant differences were found between caries-free, low-caries and high-caries groups for sIgA, mucin MG1, mucin MG2, LTF and lysozyme levels ($p < 0.05$). Comparing low caries and high caries groups revealed no significant differences in LTF and lysozyme levels. However, all other pairwise comparisons were statistically significant ($p < 0.05$). The sIgA value decreased successively among the low-caries group, caries-free group and high-caries group in saliva. The mucin MG1 value decreased successively in the caries-free, low-caries and high-caries group in saliva, whereas the mucin MG2, LTF and lysozyme values increased successively. Figs. 2,3 illustrate more intuitive results.

Caries were detected in children using ROC curves based on five salivary proteins. Participants were categorized into a control group and a caries group. Except for sIgA, the other four salivary proteins can serve as diagnostic indicators of childhood caries (Table 3). As a diagnostic indicator, mucin MG1 had an area under the curve (AUC) of 0.993, whereas its sensitivity, specificity and Youden index were 91.2%, 100% and 91.2%, respectively. AUC of mucin MG2 was 0.946, sensitivity was 76.5%, specificity was 100% and Youden index was 76.5%. AUC of LTF was 1.00, 100% sensitivity, 100% specificity and 100% Youden index. Moreover, AUC of lysozyme was 0.964, 88.2% sensitivity, 94.1% specificity and 82.3% Youden index. Based on these results, LTF demonstrated the best diagnostic efficiency. Fig. 4 illustrate diagnostic ROC curves.

4. Discussion

Saliva is crucially involved in various physiological mechanisms in the human body. It has diverse and critical functions such as food pre-digestion, oral tissue lubrication and oral health maintenance. It contains a complex composition of salivary proteins, which allow a wide range of physiological functions. Histatins in saliva inhibit calcium phosphate dissolution, maintaining tooth surface integrity [9–11, 18]. Moreover, immunoglobulins (Igs), lysozymes, LTF and other salivary proteins exhibit significant antibacterial activity, provides a robust defense for the oral cavity [19]. Salivary proteins regulate bacterial colonization and adhesion on tooth surfaces, promoting dental plaque microecological structure formation, which is the predominant bacterial cause of caries. Caries is characterized by the demineralization of inorganic substances and the breakdown of organic materials, with dental plaque as the key factor. The occurrence and development of dental caries is closely related to the properties of salivary proteins, including inhibition of calcium phosphate dissolution, regulation of bacterial adhesion and antibacterial activity. In addition, saliva reflects the complex balance between systemic and oral environments and is often used for the auxiliary diagnosis of oral and systemic diseases [20, 21]. Since salivary proteins play a critical role in preventing and controlling dental caries through various mechanisms, this study investigated

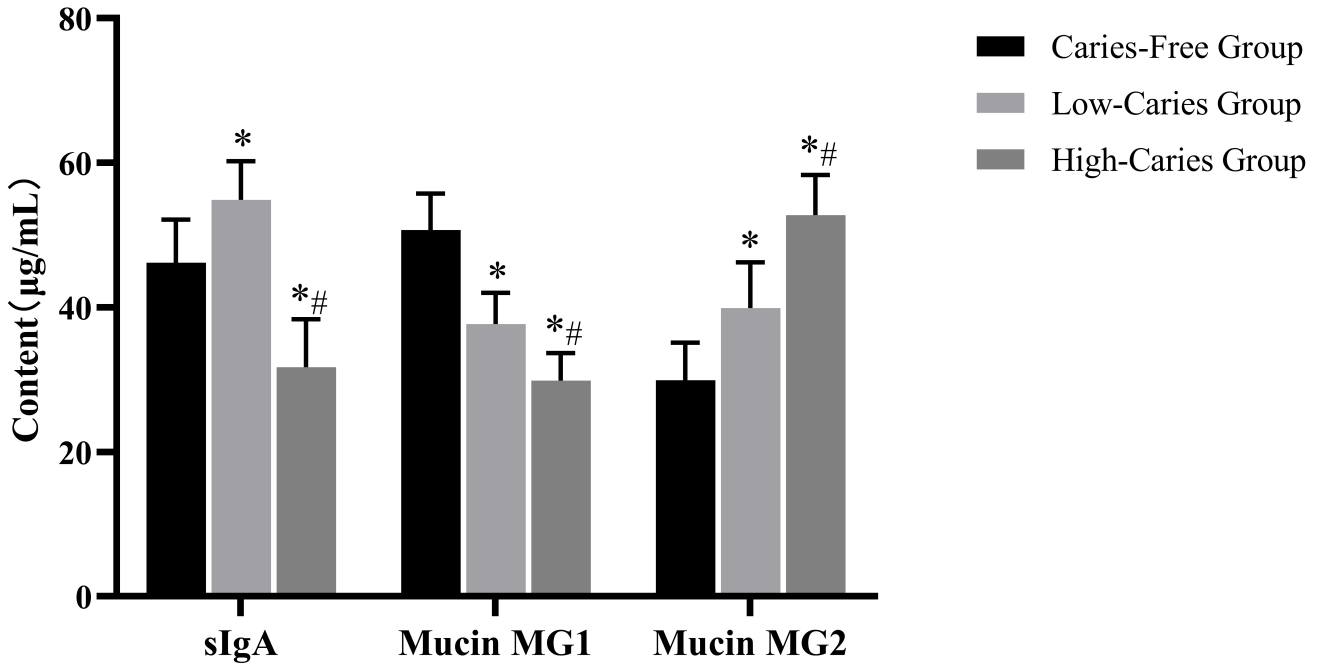

FIGURE 2. Comparison of sIgA, mucin MG1, and mucin MG2 content between the three groups. *$p < 0.05$ compared to the caries-free group; #$p < 0.05$ compared to the low-caries group. sIgA: secretory immunoglobulin A; Mucin MG1: high-molecular-weight mucins; Mucin MG2: low-molecular-weight mucins.

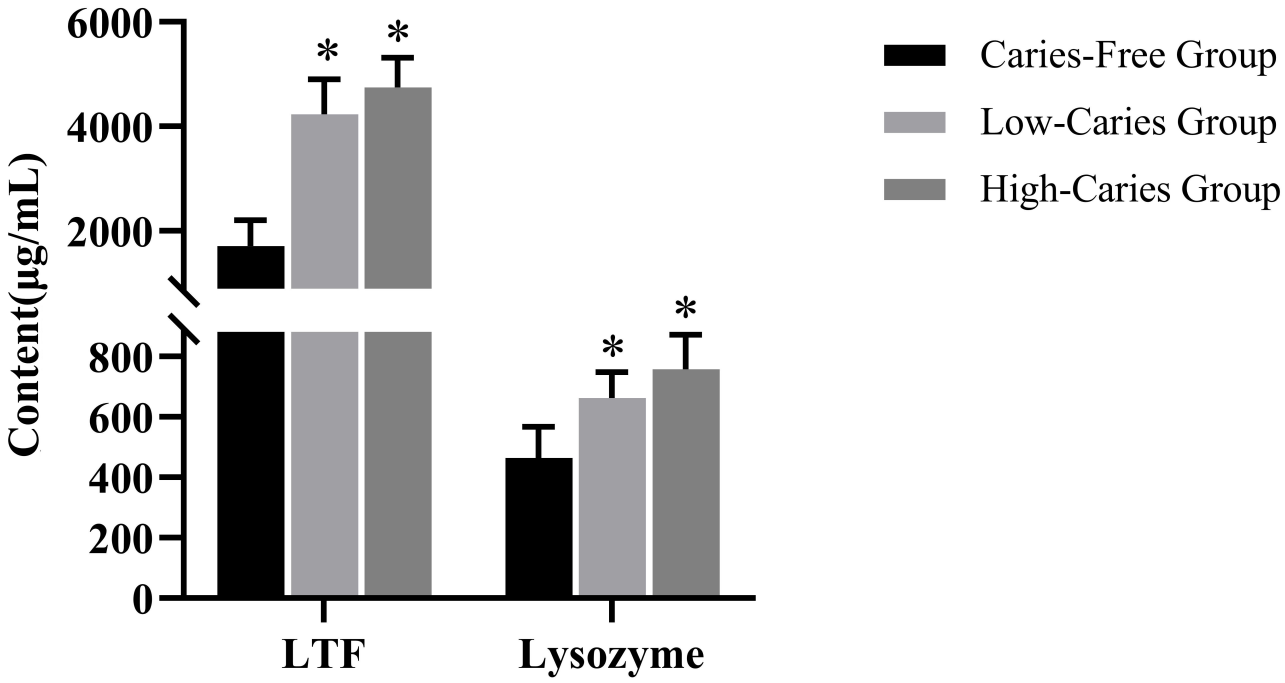

FIGURE 3. Comparison of lactoferrin (LTF) and lysozyme content between the three groups. *$p < 0.05$ compared to the caries-free group.

TABLE 3. Diagnostic performance of salivary proteins as dental caries indicators in children.

Indicator	AUC	95% CI for AUC	*p*-value	Cut-off Value	Sensitivity	Specificity	Youden Index
sIgA	0.664	0.399, 0.709	0.536	39.9	47.1%	88.2%	35.3%
Mucins MG1	0.993	0.979, 1.000	<0.001	42.38	91.2%	100%	91.2%
Mucins MG2	0.946	0.890, 1.000	<0.001	39.81	76.5%	100%	76.5%
LTF	1.000	1.000, 1.000	<0.001	2930.255	100%	100%	100%
Lysozyme	0.964	0.920, 1.000	<0.001	605.8025	88.2%	94.1%	82.3%

sIgA: secretory immunoglobulin A; LTF: lactoferrin; AUC: area under the curve; CI: confidence interval; Mucin MG1: high-molecular-weight mucins; Mucin MG2: low-molecular-weight mucins.

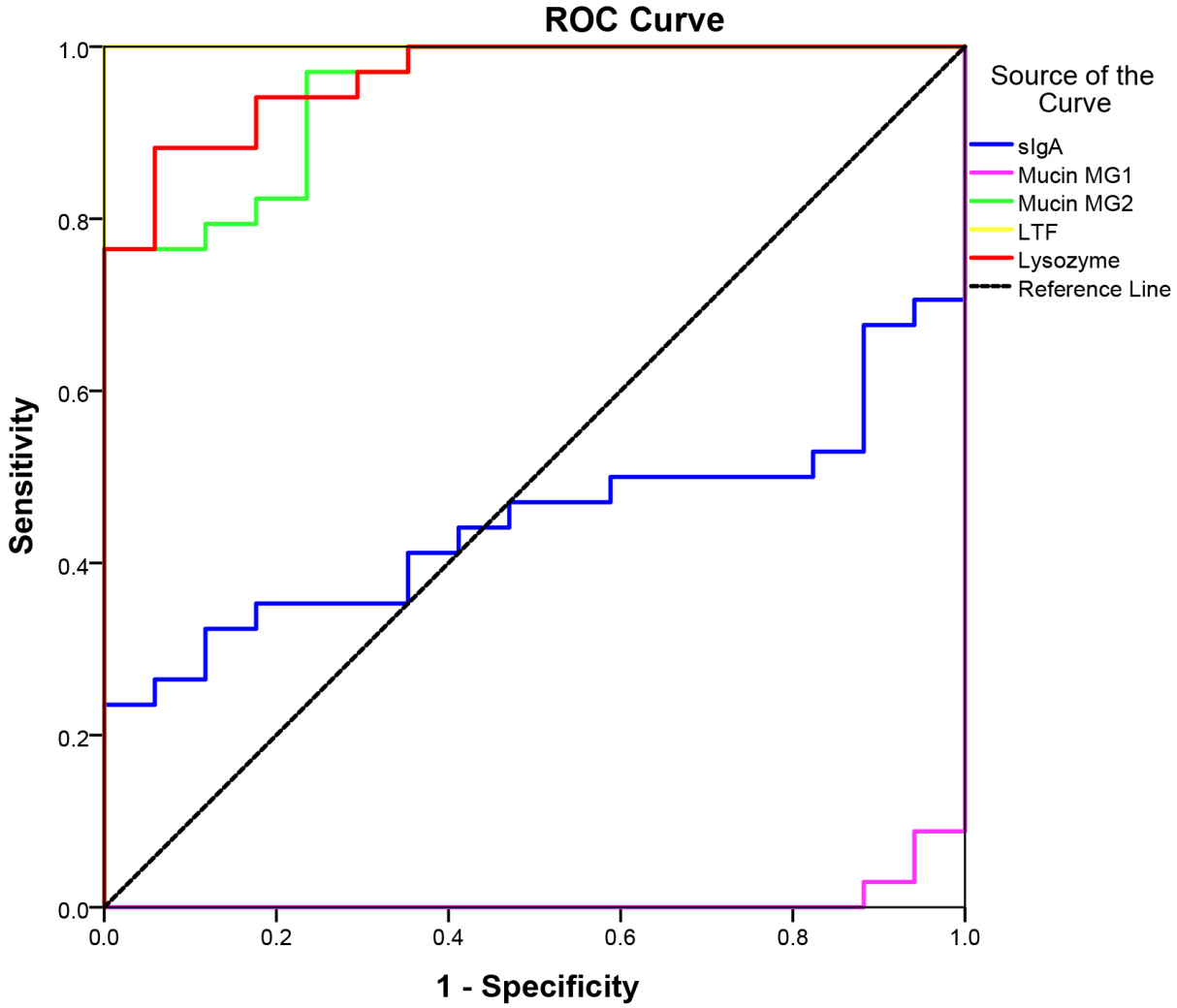

FIGURE 4. ROC curve for salivary sIgA, mucin MG1, mucin MG2, LTF and lysozyme levels. LTF showed the best diagnostic effect, with a diagnostic AUC value of 1.00, 100% diagnostic sensitivity, 100% specificity and 100% Youden index. ROC: Receiver operating characteristic; sIgA: secretory immunoglobulin A; LTF: lactoferrin; Mucin MG1: high-molecular-weight mucins; Mucin MG2: low-molecular-weight mucins.

whether salivary concentrations of sIgA, mucin MG1, mucin MG2, lactoferrin (LTF) and lysozyme differed among children in the caries-free, low-caries and high-caries groups. Three groups showed significant differences in salivary protein concentrations. As a result, the null hypothesis (H_0) was rejected: that there is no significant association between changes in specific salivary protein concentrations and the incidence of early childhood caries (ECC). Compared with blood collection, saliva sample collection uses noninvasive procedures, making it a good bodily fluid to study biomarkers of disease. Salivary glands are primarily functional when unstimulated, so this study selected unstimulated whole saliva [17] as the sample.

Igs in saliva primarily comprise IgA and IgG, as well as a small amount of IgM, IgD and IgE. Among these, sIgA is a

critical local immune defense system against the oral cavity, forming the first line of defense against pathogens. sIgA is predominantly secreted by plasma cells in the oral cavity and is found on the oral mucosal surfaces, in saliva and gingival crevicular fluid. Moreover, it promotes an immune response against specific pathogens, protecting oral health. Also, sIgA can prevent plaque formation and reduce dental caries by inhibiting bacterial aggregation on tooth surfaces. It inhibits the toxin-induced erosion of teeth by binding to bacterial toxins. Additionally, sIgA activates the complement system and works in synergy with other oral defense mechanisms to combat pathogens [22]. Here in, children with low caries had significantly higher saliva sIgA concentrations than children without caries. However, saliva sIgA levels were lower in the caries-free group compared with the high caries group. This is in line with previous research [23–25]. There might be two main reasons for these altered sIgA levels: Firstly, after dental caries onset, the cariogenic bacteria stimulate the body to produce a large amount of sIgA against invading pathogens; however, as sIgA binds to Streptococcus mutans glucosyltransferase, its concentration decreases. Secondly, Streptococcus mutans secretes IgA protease to cleave sIgA. Besides total sIgA, some researchers have demonstrated that sIgA targeting Streptococcus mutans glucan-binding protein B (GbpB) has greater biological relevance. sIgA levels specific to GbpB declined, but not those specific to antigen I/II (AgI/II) and glucosyltransferase (GTF). This phenomenon is speculated to result from relatively low concentrations of specific sIgA in saliva, making the changes less pronounced [6, 22]. Based on the ROC curve analysis, sIgA had an AUC of 0.664, 47.1% diagnostic sensitivity, 88.2% specificity, and 35.3% Youden index. According to these findings, salivary sIgA levels do not have statistical significance in predicting dental caries in children since they were associated with the altered sIgA level curve in the caries-free, low caries and high caries groups.

Mucins are primarily secreted by the submandibular, sublingual and minor salivary glands. Mucins in saliva come in two basic types: the high molecular weight mucin MG1, encoded by the muc5b gene, and the low molecular weight mucin MG2, encoded by the muc7 gene. Both mucin types play crucial roles in plaque formation and bacterial adhesion. MG1 is primarily involved in the formation of the acquired pellicle. MG2 acts as an antimicrobial protein that induces bacterial aggregation, promoting their clearance from the oral cavity. Literature [16, 26] reported reduced salivary MG1 levels, while salivary MG2 levels were increased in the low-caries group. Furthermore, MG1 levels decreased as the ECC progressed in both low- and high-caries groups. According to the findings of the current study, both low-caries and high-caries groups showed gradual increases in saliva MG2 levels as the ECC progressed. Angwaravong *et al*. [27] reported that school-aged children with low caries had higher MG1 and lower MG2 levels in their saliva than children with high caries. However, neither mucin level showed a significant difference between preschoolers with caries and those without. The present study showed that as a diagnostic marker, mucin MG1 had an AUC of 0.993, diagnostic sensitivity of 91.2%, specificity of 100% and Youden index of 91.2%. While mucin MG2 indicated an AUC of 0.946, diagnostic sensitivity of 76.5%, specificity of 100% and Youden index of 76.5%. Both mucins can serve as diagnostic indicators for dental caries in children. Overall, these results indicate that the two components of mucin had significant functional and expression differences during ECC progression, where MG1 levels decrease while MG2 levels increase. In this way, salivary levels of MG1 and MG2 may be useful for evaluating dental caries progression [28].

Lactoferrin is an unique glycoprotein with exceptional iron-chelating ability. It interacts with iron ions to create an iron-deficient environment around pathogens, thereby reducing microbial absorption of this essential element and inhibiting microbial growth. Due to this characteristic, LTF has significant antibacterial properties in the oral cavity, particularly against cariogenic bacteria. Furthermore, LTF has antibacterial activity against both aerobic and anaerobic bacteria. It has bactericidal and bacteriostatic effects on Streptococcus mutans. Combined with calcium and phosphate, LTF promotes the remineralization of early carious lesions and prevents bacterial-induced dental erosion to provide anti-caries protection. Several studies [29, 30] indicated that compared to the caries-free group, salivary LTF levels in children with low and high caries were significantly higher; however, they decreased after treatment [31]. Yan Guowei *et al*. [30] performed electrospray ionization-tandem mass spectrometry analysis, which revealed that salivary LTF levels in children with high caries were significantly higher than in children without caries, and increased as ECC progressed. Similarly, the salivary LTF levels increase progressively in caries-free, low caries, and high caries groups, consistent with the present study. However, Moslemi *et al*. [32] found lower lactoferrin levels in children with dental caries compared to children without caries. The saliva samples were collected with disposable needle-free syringes from the buccal and labial vestibules, both of which could have influenced the results. A compensatory mechanism could be underlying fluctuations in LTF levels, but the specific conditions underlying the mechanism remain unclear. Abbasoğlu *et al*. [33] found that genetic polymorphisms of LTF were associated with lower caries rates in Turkish children. LTF levels indicated an increasing trend as ECC progressed. Here, LTF as a diagnostic marker indicated an AUC of 1.00, with 100% diagnostic sensitivity, specificity, and Youden index. The LTF, therefore, is considered the best diagnostic indicator of dental caries risk in children and should be used in clinical prediction of ECC risk.

Lysozyme is a key component of human saliva's innate defense mechanism, possessing multiple antibacterial properties. It inhibits direct bacterial killing and bacterial agglutination by binding with Gram-negative bacteria lipopolysaccharides. It disrupts bacterial cell walls, causing bacterial lysis, clearing pathogens. Moreover, lysozyme can inhibit biofilm formation, which prevents harmful bacteria such as Streptococcus mutans from adhering to the tooth surface, maintaining oral health. Ruan Wenhua *et al*. [34] have indicated that salivary lysozyme levels in children with ECC were higher than in caries-free children, which is consistent with this study. However, Moslemi *et al*. [32] found that salivary lysozyme levels in the ECC group were lower than in the caries-free group. Conversely, Hertel *et al*. [35] observed no significant difference in lysozyme levels between the ECC and caries-

free groups. It appears that lysozyme levels in ECC patients vary complexly. A study on preschool children in Thailand [36] showed that salivary lysozyme levels and activity were significantly increased in the high caries group, suggesting that lysozyme may play an immune defense role during ECC progression. Here, lysozyme levels increased as ECC progressed. Lysozyme had an AUC of 0.964, while the diagnostic sensitivity, specificity, and Youden index were 88.2%, 94.1% and 82.3%, respectively. Therefore, lysozyme can serve as a diagnostic indicator for dental caries in children, where its elevation may indicate ECC progression. The findings provide insight into the pathogenesis and prevention of ECC; however, further study is required to uncover the underlying mechanisms.

Dental caries have a complex etiology and progress rapidly. Furthermore, saliva has diverse components and their composition and concentration are influenced by various factors as well as by their interaction with one another. Moreover, salivary microbial components also play a role in caries onset and progression. Though the methodology was simple and feasible, the study's conclusions were limited by a limited number of participants and lack of independent replication. The study also has limitations due to the lack of control over confounding factors such as oral hygiene practices, diet quality, and socioeconomic status. To identify more sensitive biomarkers for dental caries, more comprehensive research is needed with an integrated analysis of salivary components.

5. Conclusions

ECC occurrence and progression are associated with changes in salivary sIgA, mucin MG1, mucin MG2, LTF and lysozyme levels. Based on the AUC of the ROC curve and other comprehensive indicators, it appears that sIgA lacks statistical significance as a diagnostic marker, whereas LTF, mucin MG1, lysozyme and mucin MG2 can all be used to predict caries in children. Moreover, LTF was found to be the most practical indicator for diagnosing cavities in children. For this conclusion to be confirmed, larger sample sizes are required in further studies.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

LXZ—designed the research study. CMZ—performed the research; analyzed the data. KGL and YKS—provided help and advice on data organization. CMZ and LXZ—wrote the manuscript. All authors contributed to the editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All procedures performed in this study were in accordance with the Declaration of Helsinki (1964) and it was obtained approval from the Ethics Committee of Jinan Stomatological Hospital (JNSKQYY-2023-011). Informed consent was obtained from a parent or guardian; assent was obtained from the minor. Participants could withdraw from the study at any time.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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