Official Dental Journal of Suez Canal University
DENTAL SCIENCE Updates
Print ISSN 2636-3836
Online ISSN 2636-3844
www.dsujournals.ekb.eg
DSU — Vol. 6, No. 2, September (2025) — PP. 275:280

# EVALUATING SOME OF ANTIMICROBIAL PEPTIDES IN SALIVA OF CHILDREN WITH EARLY AND SEVERE EARLY CHILDHOOD CARIES

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*DOI: 10.21608/dsu.2025.311778.1250*
*Manuscript ID: DSU-2408-1250*

***KEYWORDS***

*Antimicrobial peptides (AMPs), Human Cathelicidin (LL-37), Human β-defensin 2 (HβD-2) , Histatin-5 (HTN-5), ELISA test*

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## *ABSTRACT*

**Introduction:** Antimicrobial peptides (AMPs) provide a first line of defense against a wide spectrum of pathogens. Their availability in un-stimulated saliva implies their potential role in protecting tooth structure from bacterially induced caries. So, the presence of AMPs in saliva may be a biological factor that contributes to susceptibility or resistance to caries. **Aim:** This study aimed to evaluate some of AMPs in saliva cathelicidin (LL-37), Human β defensin-2 (HβD-2) and histatin-5 (HTN-5) in three groups of children with caries free, early and severe early childhood caries (S-ECC). **Material and Methods:** A 42 apparently healthy children aged from 3-5 years of both sexes. According to dmfs index the selected children were divided into three groups. Un-stimulated saliva was collected from children for measuring the concentrations of (LL-37), (HβD-2) and (HTN-5) by ELISA test. **Results:** Salivary concentration of HβD-2 and LL-37 did not differ in the three groups. There was a statistically significant difference of HTN-5 in the three groups. Data showed that, dmfs were positive correlated with HβD-2 and LL-37 and negative correlated with HTN-5. **Conclusion:** The lower levels of HTN-5 might associate with S-ECC, but salivary concentrations of HβD-2 and LL-37 might not associate with the severity of dental caries, so it is not possible to conclude that these AMPs levels in saliva can be used as caries risk predictors in children.

## INTRODUCTION

Dental caries is a prevalent chronic oral infectious disease which is featured with progressive destruction of dental hard tissue, as one of the most prevalent infectious diseases worldwide, dental caries endangers human health throughout the life cycle and later, we are even suffering an elevated risk for the incidence of dental caries [1, 2].

One of the main innate defense mechanisms for maintaining oral health and controlling oral infections is the secretion of AMPs in saliva. Identified AMPs and proteins in the whole saliva are secreted by oral epithelial cells, salivary glands, and neutrophils. There are three classes of AMPs in the human mouth include defensins, cathelicidins and histatins. These peptides have various biological effects such as modulating the immune response, promoting wound healing, and broad-spectrum antimicrobial activity that make them popular for the control and treatment of diseases [3].

Human cathelicidin has a broad spectrum of antimicrobial activity against gram-positive and gram-negative microorganisms both in

vitro and in vivo [4]. Human β defensin-2 showed the strongest bactericidal effect on cariogenic microorganisms, primarily S. mutans species, which are most responsible for the development of caries [5].

Histatins may play a role in reducing bacterial colonization on tooth surfaces because it has the ability to incorporate into the acquired pellicle and block the binding site of bacteria on tooth surfaces, reduced S. mutans adhesion, thus inhibit the plaque formation [6, 7].

Some studies have compared and investigated the relationship between the concentration of certain antimicrobial peptides with and without caries in children to define the types of peptides that may indicate a possible risk of caries [8, 9, 10].

There is limited information about the concentration of salivary antimicrobial peptides in children and studies had mainly focused on children with mixed or permanent dentitions. This study hypothesized that variation in salivary levels of AMPs might contribute to caries susceptibility in primary dentition. Salivary levels of peptides could be a new and useful measure to assess caries risk in children. Thus, this study aimed to evaluate and compare the levels of LL-37, HβD-2 and HTN-5 in saliva of caries free (CF), early childhood caries (ECC) and severe early childhood caries (S-ECC) children and to determine the relation of these salivary peptides to the breast feeding duration and the severity of dental caries in children.

## MATERIAL AND METHODS

This study was conducted in the Department of Pediatric Dentistry Clinic, Faculty of Dentistry, Suez Canal University and had been approved by the Research Ethics Committee (REC) with code No: (245/2019). This study included 42 apparently healthy children aged from 3-5 years with complete deciduous dentition of both sexes, who did not suffer from any systemic diseases or syndromes, Positive and definitely positive children were included according to wright's behavior scale and informed consent was taken before the beginning of the procedures. All children were examined clinically and radiographically (bitewing radiograph) to ensure eligibility criteria [11]. Children using long term medication or antibiotics less than one month before the examination and children with periodontitis or mucosal lesions were excluded [11].

**Sample Size Calculation: [12]**

Total sample size N =

$$\frac{(Z_\alpha)^2 * (S)^2}{(d)^2} = \frac{(\mathbf{1.96})^2\mathbf{x}(\mathbf{6.61})^2}{(\mathbf{2})^2} =$$

41.962 = 42 patients.

**Children Grouping:**

According to dmfs index the selected children were divided into three groups: Group I: 14 children with caries free (dmfs = zero). Group II: 14 children with ECC, had dmfs = 1-3. [13, 14] Group III: 14 children with S-ECC had dmfs ≥ 4 (age 3 years),≥ 5 (age 4 years), or ≥6 (age 5 years) surfaces [14].

**Data collection:**

Assessment charts were given to the parents and included personal history, breast feeding duration, medical history and an intraoral examination that included bilateral bitewing radiographs for diagnosis of proximal caries and caries assessment by counting decayed, missed and filled tooth surfaces for each child.

**Saliva Sample Collection:**

Un-stimulated saliva samples were collected in a 15 ml falcon tube in the morning and at least 1hour after eating or drinking. Children were seated in the dental chair, asked to rinse their mouth with clear water before collection of samples, instructed to keep their heads slightly forward and tilted

downward so that the saliva could flow without any stimulus until 5 ml of saliva was obtained, placed in ice box and transported to the laboratory at Biochemistry Department, Faculty of Medicine, Suez Canal University and processed within 1hour. Saliva was clarified by centrifugation at 10000 rpm for 10 minutes and frozen at the Biochemistry Department at –70 °C to preserve the samples until measuring AMPs levels by ELISA kits.

### Salivary assessment methods:

Measuring the AMPs levels of (LL-37, HβD-2 and HTN-5) was done by using LL-37 Elisa Kit (cat.no: E-02406hu), HβD-2 Elisa kit (cat. no: E-02405hu) and HTN-5 Elisa Kit (cat.no: E-00776hu). (1304 Langham Creek Dr, Suite 226, HoustonTx77084, USA), according to method described by ***Zainab* et al** [15].

### Statistical Analysis:

Qualitative data represented as frequencies (n) and percentages (%). The Chi-square test was used to test significance of association between categorical variables. For quantitative data we tested the normal distribution using the Kolmogorov-Smirnov test and then One-way ANOVA with Duncan's post hoc tests was performed for the evaluation of statistical significances among the groups.

## RESULTS

There was no significant difference between groups for the levels of HβD-2 and LL-37, but it was significant difference for HTN-5 levels (Fig 1). There was a statistically significant difference in duration of breast feeding in the three groups. dmfs were positive correlated with breast feeding duration and HβD-2 and LL-37 and negative correlated with HTN-5. Duration of breast feeding was positive correlated with LL-37 and negative correlated with HβD-2 and HTN-5. HβD-2 was positive correlated with LL-37 and HTN-5, but HTN-5 was negative with LL-37 (Table 1).

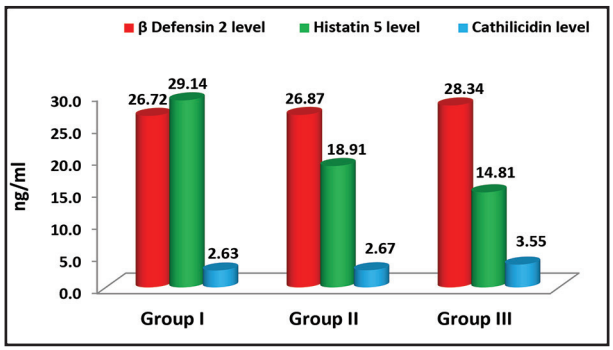


Fig. (1) Bar chart representing the comparison between HβD-2, HTN-5 and LL-37 levels in the three groups.

**Table (1)** *Correlation coefficient between dmfs with breast feeding duration, β defensin-2 level, histatin-5 level and cathelicidin level*

| Variables | | Breast feeding duration | HβD-2 level | HTN-5 level | LL-37 level |
|---|---|---|---|---|---|
| **dmfs** | R | 0.385* | .084 | -.330-* | .162 |
| | Sig | .012 | .595 | .033 | .306 |
| **Breast feeding duration** | R | | -.020 | -.080 | .080 |
| | Sig | | .902 | .615 | .616 |
| **HβD-2 level** | R | | | .250 | .070 |
| | Sig | | | .110 | .659 |
| **HTN-5 level** | R | | | | -.268 |
| | Sig | | | | .087 |

**; Correlation is significant at the 0.05 level*

## DISCUSSION

Regarding the inclusion criteria, the children were free from any systemic disease because chemoradiation and numerous medications may affect salivary gland function, volume, composition of saliva and may influence the diagnostic usefulness of many salivary constituents and impaired of host defenses and levels of salivary AMPs [16, 17, 18].

The selected age ranged from three to five years, this may be due to lack of awareness about health caring and retaining of primary teeth as well as the parental attitude that the primary teeth are exchangeable by permanent teeth and are not important [19].

Regarding the exclusion criteria, children using long term medications or antibiotics less than one month before examination because the rate of saliva reduction increases by regular intake of medication, the reduction is due to parenchyma of the minor salivary glands being slowly replaced by connective tissue and fat [20].

Regarding the precautions taken before saliva collection, not eating or drinking anything at least 1hour to avoid contamination with non salivary components [21]. The saliva samples were collected in the morning in order to avoid a fluctuation in peptide levels because of circadian rhythm [15]

With regards to the duration of breast feeding, there was significant difference between groups. In agreement with ***Azevedo* et al** [22] reported that breast feeding after 1 year of age was associated with ECC.

Regarding the HβD-2 level there was no significant difference between groups. This result in agreement with ***Colombo* et al** [11] who did not find significant difference of HβD-2 level between CF, ECC and S-ECC groups.

As for the HTN-5 level this study showed that, the highest levels were in CF group with significant difference between groups. On contrast, ***Jurczak* et al** [10] who studied on 82 pediatric patients with average age $=5 \pm 1.5$ found the salivary HTN-5 level was significantly higher in severe caries group compared to caries free group. This difference may be due to the sample size was approximately twice the number of children in this study.

With regard to LL-37 level, there was no significant difference between groups. On contrast, ***Davidopoulou* et al** [8] found that the concentration of LL-37 was lower in children with high caries activity in the primary dentition compared with caries-free children.

Regarding the correlation of dmfs, it was positive with breast feeding duration with significant difference and with HβD-2 level and LL-37 level, while it was negative with HTN-5 level with significant difference. These results in consistent with some studies [23, 24] which found a positive association between dental caries and breastfeeding for >10 months, ≥ 18 months and >19 months. In agreement with ***Colombo* et al** [11] who observed an association between HβD-2 and LL-37 levels and caries extent (dmfs), which was not found for HTN-5. These results suggest that innate immune system of children, represented by antimicrobial peptides, reacted to the presence of S. Mutans and their production is increased in the presence of dental caries.

Concerning to the correlation between breastfeeding duration and AMPs, the correlation coefficient was positive with LL-37 level, negative with HTN-5 and HβD-2 level. This may be due to the secretion of LL-37 in human milk, so with an increase in the duration of breastfeeding the level of LL-37 will be increased in saliva and contribute to anti infectious properties of human milk and saliva as reported by ***Murakami* et al** [25].

Regarding the correlation between AMPs with each other, HβD-2 level had positive correlation with HTN-5 level and LL-37 level, while HTN-5 level had negative correlation with LL-37 level. In agreement with ***Colombo*** **et al** [(11)] who showed positive correlations between HβD-2, LL-37 and HTN-5 (except for the combination of HTN-5 and LL-37), suggested there might be a combined action of these peptides in the host response possibly to caries pathogens since the children studied were healthy except for having dental caries.

## CONCLUSIONS

Prolonged breast feeding and the lower levels of HTN-5 might associate with S-ECC. Salivary concentrations of HβD-2 and LL-37 might not associate with the severity of dental caries. It is not possible to conclude that these AMPs levels in saliva can be used as caries risk predictors in children.

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