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Salivary concentration of the antimicrobial peptide LL-37 in children

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ABSTRACT

Objective: Antimicrobial peptides are important components of innate immunity, especially in the unique environment of the oral cavity. Lack of the human cathelicidin LL-37 has been implicated in severe periodontitis, whilst high salivary levels of LL-37 seem to increase caries resistance. Limited data exists about the concentration of LL-37 in saliva of young children. In this study, the salivary concentration of LL-37 was examined in relation to age, gender, type of dentition (primary, mixed or permanent) and caries experience of children.

Design: Unstimulated whole saliva was collected from 49 systemically healthy and gingivitis free children aged 2–18 years old. Their caries activity was recorded. The salivary LL-37 concentration was determined by enzyme linked immunosorbent assay (ELISA).

Results: LL-37 was detected in all saliva samples. Its concentration varied widely, with girls exhibiting higher peptide levels than boys. A positive correlation of the LL-37 concentration was observed with age. Children with primary dentition had significantly lower peptide concentration than those with mixed or permanent dentition. Significantly lower concentrations of LL-37 were also found in children with high caries activity, compared to caries free children or to children with low to moderate caries activity.

Conclusions: Our results reinforce the belief that LL-37 is an important molecule of immunity in the oral environment and it seems to play a protective role against caries.

1. Introduction

Antimicrobial peptides are essential components of innate immunity, participating in first line defence reactions at various body sites.[1–4] Innate immunity plays an important role in wound healing and in the maintenance of the tissue health, particularly in environments, like the oral cavity, colonized by a microbial plethora.[5] Amongst the antimicrobial peptides found in the oral environment are α- and β-defensins, LL-37 antimicrobial peptide and histatins.[6–8] Besides their direct bactericidal activity, these peptides have other distinct and overlapping properties, such as chemotactic activity or induction of cytokine release.[9]

The LL-37 antimicrobial peptide is the proteolytically processed extracellular form of human cationic antimicrobial protein of 18 kDa (hCAP-18), the only known member of cathelicidins in humans,[10] found in the secondary granules of neutrophils and various other cells.[11] LL-37 is present in the pulmonary[12,13] and the digestive system[14] and it has also been

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detected in plasma,[15,16] sweat,[17] skin,[18,19] and human milk.[20] Regarding the oral cavity, LL-37 has been detected in saliva,[21] whilst the peptide itself or its mRNA or both have been detected in salivary glands,[21,22] in lingual epithelium and palatal mucosa.[23] Following inflammatory stimulation, LL-37 is released at the inflamed sites mainly by neutrophils that migrate through the junctional epithelium.[24,25] This pattern of expression implies a possible protective role of the peptide on both hard and soft oral tissues.[6]

The aetiology of periodontal diseases and dental caries is the microbial plaque accumulated on dental surfaces.[26–28] Controlling microbial dental plaque is of primary importance in preventing and treating these diseases. LL-37 has a broad antimicrobial activity against both cariogenic and periopathogenic bacteria[29–32] and its role in maintaining oral health has recently been suggested.[6] Inheritable neutrophil defects leading to a lack of LL-37 have been associated to severe periodontitis,[33,34] whilst low salivary levels of LL-37 were frequently detected in caries susceptible subjects.[35] Recently, further studies give new insight in the role of the LL-37 antimicrobial peptide in periodontal disease.[36–38]

Limited information exists on the salivary antimicrobial peptide concentration in adults and children.[35,39] Moreover, the concentration of LL-37 was determined by semi-quantitative methods in the study dealing with children.[35] Thus, we aimed to examine the LL-37 concentration in saliva of children and its possible correlation to age, gender, type of dentition and caries experience using a quantitative method.

2. Materials and methods

2.1. Study design

The study was approved by the Ethics Committee of the Dental School in Aristotle University of Thessaloniki. The purpose and procedures were fully explained and informed consents, in accordance with Helsinki Declaration, were signed by the parents or guardians of the children before enrolled in the study.

The study group consisted of 49 children, 21 boys and 28 girls, 2–18 years old (mean age 8.7 years). All children were examined by trained pedodontists using standardized clinical procedures. The examination included caries registration recorded as decayed teeth (DT scores) and existence of inflammation in periodontal (GI index)[40] and other oral soft tissues. Information about the health history of the children was given by their parents. To be included in the study the children had to be in good general health, with no history of major illness or disease, or intake of medication. In addition, they should have healthy soft oral tissues with no clinical sign of inflammation.

A sample of unstimulated whole saliva was obtained from each child and kept at −75 °C till analyzed. Sampling was performed at the same time of the day, around 6.00 p.m., to avoid a possible diurnal variation in peptide concentration as observed with other saliva constituents.[41] The spitting method was used for saliva collection for 47 children. About 1 ml of unstimulated whole saliva was spitted in a clean plastic container, this procedure lasting 2–3 min. From two children 2 and 3 years old, saliva was collected by the absorption method using the collecting devise Salivette®,[42] because the spitting method could not be applied. Initial experiments revealed no influence on the quantitative estimation of the peptide, of the collection method, spitting or absorption.

2.2. Analysis of saliva LL-37 peptide levels

The saliva samples were thawed and cleared by centrifugation at 10,000 × *g* for 5 min, as previously described.[39,43,44] The concentration of LL-37 in the saliva samples was determined by an enzyme-linked immunosorbent assay (ELISA) using a commercially available analysis kit, specific for LL-37 peptide (HyCult Biotechnology, Uden, The Netherlands) and following the manufacturer's instructions.

Each sample or a 5 times dilution of it was analyzed in duplicate and the mean value of the measurements was calculated. When individual absorbance values differed by more than 15% from the corresponding mean value the analysis was repeated.

The equation of regression analysis, obtained with standard peptide solutions (concentration range 0.1–100 ng/ml) was used for calculation of LL-37 concentration in the samples.

2.3. Statistical analysis

Data were summarized by means of descriptive statistical indices (min, max, median values and mean values ± standstandard deviation). Statistical comparisons between groups of children were performed with Mann–Whitney tests. The correlation between children's age and LL-37 concentration in saliva was examined using the Spearman's ρ coefficient. Non-parametric statistical methods were preferred because the normality of LL-37 concentration in saliva was not assumed or confirmed. In all hypotheses testing procedures the observed significance level (*P*-value) was computed by the Monte-Carlo simulation method.[45] This approach leads to valid inferences even in cases where the methodological presuppositions of the non-parametric tests are not satisfied. All analyses were performed with SPSS v15.0 statistical package enhanced with the module Exact Tests. The significance level of all statistical tests was predetermined at $P < 0.05$.

3. Results

Out of 49 children, 30 were caries free (DT score 0), 9 had low to moderate caries activity (DT score 1–3) and 10 exhibited high caries activity (DT score ≥4). The data is presented in Table 1.

The LL-37 antimicrobial peptide was detected in all saliva samples. Its concentration considerably varied from 0.22 to 275 ng/ml in the samples. The median value was 22 ng/ml. Younger children mostly had low LL-37 concentration (Fig. 1) and a positive significant correlation was observed between peptide concentration and age ($\rho = 0.413$, $P < 0.05$). Though not significant ($P = 0.063$), girls exhibited higher levels of salivary LL-37 than boys.

The mean LL-37 concentration in children with primary dentition was significantly lower than in children with mixed