Full Length Research Article
Advancements in Life Sciences – International Quarterly Journal of Biological Sciences

ARTICLE INFO

Date Received: 17/09/2024;
Date Revised: 10/11/2025;
Date Published Online: 28/12/2025;

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How to Cite:
Kareem SJ, Al-Ghurabi BH (2025). Comparative Evaluation of Salivary LL-37 and TNF-α in Children with and without Dental Caries: A Case-Control Study. Adv. Life. Sci. 12 (4): 800-804.

Keywords:
LL-37; TNF-α; Caries, Dental; Antimicrobial peptides (AMPs); Early Childhood Caries

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Comparative Evaluation of Salivary LL-37 and TNF-α in Children with and without Dental Caries: A Case-Control Study

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Abstract

Background: A major social, behavioral, and oral health issue is the prevalence of caries in children's teeth and gums at an early age. Caries arises from a bacterial infection due to several factors, including an unhealthy diet. Natural AMPs serve as a frontline defense against a broad variety of infections, while cytokines are biomarkers for a wide variety of disorders and also serve as essential agents in both immunological and inflammatory reactions. Many researchers have studied and reported the effects of LL-37 and TNF-α in Early Childhood Caries (ECC) among children.

Methods: Eighty kids between the ages of 4 and 5 took part in the research. 40 children were with caries and considered as the patient group, whereas the other children (40) were without caries of similar age and sex as the control subjects. Clinical caries experience is measured through a dental examination and the dmfs index. From all participants, samples were collected. Salivary LL-37 and TNF-α levels were analyzed by the ELISA.

Result: The present investigation demonstrated a substantial rise ($P < 0.01$) in the values of LL-37 and TNF-α in caries children compared to children without caries.

Conclusion: The children with ECC were found to have an elevation in LL-37 and TNF-α. This indicated that decay associated with the elevating LL-37 and TNF-α levels.

Introduction

Advanced demineralization is a hallmark of dental caries, a multifactorial illness of the tooth's hard tissue due to bacteria, and is considered a contagious disease indeed. It is considered to be the most prevalent oral health problem across the world, affecting children and adolescents. It is the leading cause of dental caries in the community and affects both adults and the elderly [1]. Caries, or cavities, are induced by acids created by bacterial fermentation of dietary carbohydrates and the synergistic effects of acids and vulnerable host components, such as saliva and teeth, in young infants [2]. While improvements in nutrition and dental hygiene are effective in preventing ECC [3,4], it remains the most common chronic pediatric illness globally. Antimicrobial peptides (AMPs) are an extensive family of host-defense chemicals that launch an early challenge to invading microbes. These brief cationic peptides play an essential role in the maturation of the body's natural defenses. Salivary glands and the oral epithelium create antimicrobial peptides for defense in the mouth. These peptides possess both direct and indirect antibacterial effects, work as chemokines, and stimulate the production of chemokines [5,6]; they are expressed in the granules of neutrophils and by a wide variety of tissue types. LL-37 is effective against cariogenic and pathogenic bacteria, making it a versatile antibacterial. In response to microbial infection of the oral mucosa, the body produces a plethora of antimicrobial peptides that have both antibacterial and immunomodulatory properties [7,8]. The initial line of defense against infection is nonspecific immunity, which works by recognizing microbe components via toll-like receptors (TLR). For example, Streptococcus mutans' cell wall contains lipoteichoic acid. This triggers the release of many biomarker molecules by activating different cell signaling networks. LL-37 and HNP 1-3 are two examples of biomarkers that are produced as a consequence of the activation of a complex cell signaling network that controls the host immune response [9,10].

Caries is considered one of the various disorders in the mouth that could be detected and examined using cytokines [11]. Proinflammatory cytokines are investigated for their potential part in the detection of caries. Additionally, they were discovered to be signs of harm to the tissues and potential risk factors for subsequent heart problems in subjects who seemed to be in good health.

TNF-alpha is a cytokine with pleiotropic function, which was discovered as a molecule with necrotizing events in animal tumors. Certain tumor cells are both cytostatically and cytotoxically affected by TNF-α, but it also affects the evolution, discrimination, and/or function of each cell type searched thus far. Furthermore, TNF-α is considered a component of a network of communicated signals that coordinates immune and inflammatory responses [12-14]. Saliva, as a host-derived factor, plays an essential role in preserving dental health and preventing dental caries. Cationic peptides, immunoglobulins, lysozyme, and lactoferrin are a few examples of antimicrobial proteins and peptides that are produced in the saliva and have a protective function [15,16].

Methods

Subjects and Sample Size

In all, eighty kids between the ages of four and five took part in the research. The age was calculated using the most recent birthday. The children were split into a study group of 40 with ECC and a control group of 40 who were caries-free, as the control group, and whose age and sex match with study group. The following criteria excluded kids from participating in the study: lack of consent, taking antibiotics or any other medicine, eating within an hour of saliva collection, having the flu or any other systemic sickness, and uncooperative children.

The sample size was determined with Franz-Faul's (KielUniversity, Germany) G power 3.1.9.7. Eighty people were surveyed, forty from each group, Effect size is: Small = 0.1, medium = 0.25, large = 0.4. An independent T-test with two samples was used for the statistical analysis, with a power of 85% and a 0.05 alpha probability error.

Ethical Approval

The subjects had a comprehensive explanation of the study's purpose with methods. Their permission was gained by a document vetted via the dentistry school's ethical committee at the University of Baghdad.

Clinical examination

The gingival index was used to assess dental health according to [17]. In 1987, tooth decay assessment was documented as dmfs using WHO standards.

Salivary sampling

The drooling technique was used to obtained, 3 ml of unstimulated saliva sample. The specimens were transferred to the icebox after being labeled with a unique number for each child, which corresponds to the case report number. The specimen was centrifuged at 3000 rpm for 10 minutes. The supernatant was gathered and kept at -20°C for analysis later on.

Assessment of the levels of LL-37 and TNF-α

The level of LL-37 and TNF-α (MyBioSource, USA) in saliva was detected by the ELISA kit.

The statistical analysis

The T-tests and chi-squares were utilized to identify significant variations between the groups. To examine the relationship between biomarkers, this study used

the Pearson correlation coefficient. The threshold of significance used was $P < 0.05$.

Results

In the ECC group, the LL-37 level (21.87 ng/ml) was significantly increased ($P < 0.01$), according to the current results as compared to control children (10.03 ng/ml), as demonstrated in Table 1. Furthermore, as shown in Table 2, in the ECC group, the results revealed that LL-37 was not significantly associated with any clinical factors.

LL-37	Caries group	Control group	P-value
Minimum	16.44	8.58	< 0.000*
Maximum	34.42	12.37	
Mean	21.87	10.03	
SD	3.77	0.85	

Table 1: The average LL-37 levels ng/ml in the two groups that were investigated.
Significant elevation in the mean of LL-37 among the ECC group, as compared to the caries-free group

LL-37	Caries group	
	R	P-value
GI	0.069	> 0.05
Ds	-0.125	> 0.05
ms	0.055	> 0.05
Fs	-0.090	> 0.05
Dmfs	-0.022	> 0.05

Table 2: Pearson's correlation coefficient between clinical factors and LL-37 level in the study group.
The ECC group does not show a statistically significant relationship between LL-37 and clinical factors

The values of TNF-α cytokine in patients and control groups were observed in Table 3. The level of TNF-α in ECC (188.38 pg/ml), was elevated as compared to the control group ($P < 0.05$). Moreover, the present finding found a positive relationship ($r = 0.544$, $P = 0.017$) between TNF-α and GI, but no correlation with caries experience (Table 4).

TNF-α	Caries group	Control group	P-value
Minimum	132.29	59.12	< 0.000*
Maximum	242.51	114.63	
Mean	188.38	81.08	
SD	27.54	15.94	

Table 3: The levels of TNF-α pg/ml in saliva of studied groups.
Salivary TNF-α levels were greater in children with ECC as compared to those without caries

TNF-α	Caries group	
	R	P
GI	0.544	< 0.01*
Ds	-0199	> 0.05
ms	0.047	> 0.05
Fs	-0.054	> 0.05
dmfs	-0.072	> 0.05

Table 4: Pearson correlation between TNF-α and clinical variables in the study group.
*The level of TNF-α is positively and significantly associated with GI .

Discussion

Inappropriate eating, prolonged exposure to carbohydrates, and other social factors all combine to cause early childhood caries, making it a complex illness with several causes. To aid in the prevention and treatment of ECC, a closer examination of its risk factors is usually useful. It may be able to predict and track the likelihood of onset and course of such dental ailment or an oral illness by analyzing an assortment of elements (like proteins and peptides) found in the mouth [18,19].

The antibacterial properties of saliva, such as the aggregation and removal of microorganisms, immunological monitoring, and oral health [20,21], being a component of the body's innate immune system, perform a crucial function in preserving the health of the mouth and gums. The current investigation collected unstimulated saliva for the test, which is consistent with the results of Ribeiro *et al.* (2013) that the children were recalcitrant when their saliva was stimulated [22,23].

As a possible reaction to bacterial stimulation and the presence of an inflammatory process in dental caries, the current investigation found that the concentration of LL-37 was significantly higher in salivary samples from the caries group compared to the healthy group. This observation was consistent with those reported by Ribeiro *et al.,* (2013). Culture-positive streptococci were reported to have greater concentrations of LL-37 than culture-negative children in a separate investigation comparing AMP levels in children aged 1-3 years [24]. However, previous studies reported by Malcolm *et al.,* (2014) and Colombo *et al.,* (2016) found that there were non-significant differences in the level of cathelicidin in both studied groups [25,26]. On the other hand, Davidopoulou (2012) and Al-Ali G (2021) compared patients of a comparable age who exhibit typical caries activity; children with a high caries score exhibit reduced expression of LL-37 in unstimulated saliva [7,27]. Genetic factors may explain why children's salivary LL-37 levels fluctuate, according to research by Prasad (2019) [28,29]. Non-stimulated whole saliva LL-37 levels were estimated in children and adolescents aged 2-18, and those with high caries activity had lower LL-37 levels than those who had mild and intermediate caries activity [30].

In addition, the results found that there is no important association between LL-37 with caries experience and GI in the ECC group. Consistent with this outcome, other studies also failed to observe the correlation between caries experience and AMP levels [31]. Furthermore, Al-Ali showed a non-significant correlation between GI and LL-37 levels; LL-37 has an inverse relationship with the severity of caries [27].

Prior studies have shown that children with ECC have substantially greater salivary TNF-α levels compared to those without caries [32-35]. Previous research

discovered that people whose teeth have decayed had greater concentrations of the cytokines TNF-α and IL-6 in their saliva, indicating that the immune system may react with inflammation when decay occurs, leading to the generation of TNF-α [36-38]. However, Kurtis *et al.,* (2005) have shown that elevated TNF-α levels in caries patients lead to a decrease in osteoblasts and fibroblasts, which exacerbates demineralization of teeth and the advancement of dental caries [39]. IL-6 and other factors boost chemokine production and initiate bone resorption [40]. Created locally by osteoclasts, TNF-α is a crucial element controlling differentiation of these cells and participating in resorption, as shown by Tani-Ischii [41].

In addition, a significant connection between TNF-α level and GI was found in the present research, but caries experience with TNF-α is not correlated [42]. An increase in cytokines in children has been linked to an accumulation of plaque, irritation of the gums, and inadequate plaque treatment. The results of this investigation showed that LL-37 and TNF-α were significantly and positively correlated. Consistent with earlier research, this finding reported that defensin and LL-37, through their chemotactic activity, bridge the gap between innate and adaptive immunity by inducing cytokine synthesis, dendritic cell activation, and T lymphocyte polarization. AMP is a component of the regulation of innate immunity over adaptive immunity when it acts in a specific biological setting [43].
Children diagnosed with ECC had elevated levels of LL-37 and TNF-α, this indicated that decay associated with elevating LL-37 and TNF-α levels.

Author Contributions

BHA contributed to conceptualization and supervision. SJ collected the patients, samples, and provided clinical information. BHA and SJ performed the experiments and contributed to the statistical analysis, reviewing the work. The work was finalized after the auothers reviewed it and gave their approval.

Conflict of Interest

Authors have not indicated any potential monetary or other conflicts of interest.

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