

Evaluation of Relationship between *Streptococcus mutans*, Dental Caries and IL-1 α and IL-6

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Abstract

Background and aims. *Streptococcus mutans* is an important species in oral microflora and its components have been found to stimulate production of proinflammatory cytokines in dental caries. The aim of this study was to evaluate proinflammatory cytokines (IL-1 α and IL-6) in patients with *S. mutans*.

Materials and methods. Seventy samples were selected during pulpectomy and investigated for the presence of IL-1 α and IL-6 by ELISA. The results were analyzed by t-test ($\alpha = 0.05$).

Results. The results showed higher mean concentrations of IL-6 and IL-1 α in inflamed pulpal tissues in subjects with dental caries associated with *S. mutans*, compared with intact pulpal tissue samples; these higher means were statistically significant in all cases ($P < 0.05$).

Conclusion. The results of this study suggested relations between the production of IL-1 α and IL-6 in dental caries caused by *S. mutans*.

Key words: Dental caries, interleukin-6, interleukin-1 α , inflammation, *Streptococcus mutans*.

Introduction

The most dominant chronic disease of the oral cavity is dental caries.¹ Dental caries is a multifactorial disease and is powerfully linked with the presence of cariogenic microorganisms, fermentable carbohydrates, sensitive teeth and duration of exposure.²⁻⁴ *Mutans streptococci* are the etiologic factor for dental caries,⁵ and numerous studies have revealed a relationship between dental caries and *Streptococcus mutans*.⁵ Also, many previous studies

have assessed the relationship between progress of carious lesions and the response of immunocompetent cells.⁶ During inflammation and infections, cytokines are important mediators, in addition to their role in controlling the inflammatory response to bacterial infection. Although the role of cytokines in the pathogenesis of dental caries is not distinct, proinflammatory cytokine production is induced by components of *S. mutans*.^{7,8} The aim of this study was to assess association between IL-1 α and IL-6 levels and dental caries, especially in *S. mutans* infections.

Materials and Methods

In this study, 70 patients with dental caries were selected, who referred to the Department of Oral and Maxillary Surgery, Faculty of Dentistry, Tabriz University of Medical Sciences. Tissue samples (2mm) were obtained from the intact and inflamed pulp regions. Healthy dental pulp samples and irreversible dental pulp samples were achieved from third molars and carious molars, respectively, during pulpectomy procedures (Figure 1). The Hanks' balanced salt solution was used to transfer samples to the Immunology Laboratory. Informed consent was obtained from the patients (20–40 years of age). The samples were extracted under aseptic conditions and kept for identifying bacterial infections, especially *S. mutans*. Two media, Cavex ZOE and Golchai ZOE, were used for determination of growth of *S. mutans*. The tissue samples were stained with the H&E method.⁹ The tissue samples (1 mm) were homogenized by phosphate buffer saline (pH=7) and clarified by centrifugation at 10,000 g for 15 min at 4°C for determination of cytokine concentrations. The aliquots of clarified supernatants were stored at -70°C until cytokine measurements. The concentrations of IL-6 and IL-1 α were evaluated with an enzyme-linked immunosorbent assay (ELISA; BioSource, Nivelles, Belgium), according to the manufacturer's instructions. Data were analyzed with SPSS 17. T-test was used for statistical analysis. Statistical significance was set at $P < 0.05$.

Results

In this study, *S. mutans* infection was detected in 40 patients (57.1%). This bacterium had better growth and persisted in Cavex ZOE media compared with Golchai media (Figure 2). Staining by H&E showed higher lymphocyte levels in inflamed tissue samples. Means (SD) of IL-1 α and IL-6 levels are presented in Table 1 and Figure 3. The results showed that the means of the cytokines were not significantly differ-



Figure 1. Tissue sample during pulpectomy.

ent between female and male subjects ($P = 0.391$). But IL-1 α and IL-6 levels were higher in inflamed tissues compared with intact tissues ($P < 0.05$).

Discussion

In this study, IL-1 α and IL-6 levels exhibited statistically significant differences in inflamed tissues associated with *S. mutans* ($P < 0.05$).

IL-1 is one of the main mediators of immune and inflammatory responses.^{10,11} Different agents, including microorganisms, microbial metabolites, inflammatory causes or antigens could induce IL-1 production. The activity of IL-1 is controlled via IL-1ra existing in the immune system by means of binding with high affinity to the similar receptors as IL-1 β .¹² In addition to IL-1, IL-6, IL-8 and TNF- α are associated with inflammatory responses that are related with dental caries.¹³⁻¹⁵

S. mutans is the major factor responsible for dental caries.¹⁶⁻¹⁹ The cell surface protein antigens of this bacterium (Pac, Ag I/II, PI, and B) help colonization of tooth surfaces.^{7,20,21} After colonizing the oral cavity, the inflammation process begins. Then, due to this lesions, innate and adaptive host immune re-

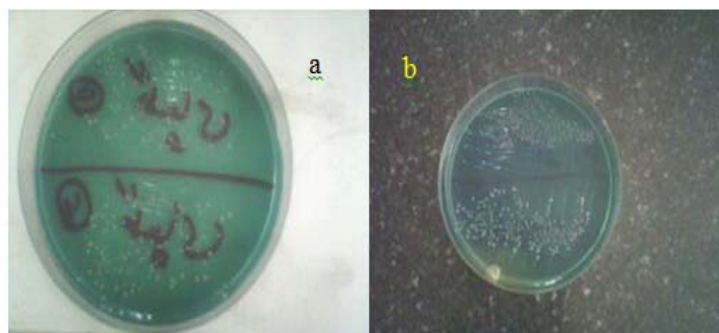


Figure 2. Growth of *S. mutans* in the two media (a: Golchai ZOE; B: Cavex ZOE).

Table 1. Cytokine concentrations (pg/mL) in terms of *S. mutans* infections

	IL-1 α			IL-6		
	Female	Male	Total	Female	Male	Total
Intact tissue	34.28 \pm 13.25	71.84 \pm 36.97	53.06 \pm 19.59	16.49 \pm 6.4	45.83 \pm 25.96	31.16 \pm 13.44
Inflamed tissue	164.9 \pm 43.37	231.97 \pm 70.88	198.44 \pm 41.02	118.14 \pm 31.9	171.68 \pm 58.17	144.91 \pm 32.89
P-value	0.001*	0.008*	0.000*	0.000*	0.008*	0.000*

*P-values less than 0.05 were considered as significant.

sponses are induced.²²

In a research on Swedish children, chlorhexidine was used to prevent *S. mutans* colonization; development of caries took a mean of three years, while titers of lactobacilli and other virulent oral bacteria were undetermined.²³ Meiers et al²⁴ analyzed the water spray of high-speed drills for restoring both carious and non-carious lesions and concluded that *S. mutans* was the only predominant bacterium in carious lesions compared to caries-free individuals.

S. mutans is an effective initiator of caries since there is a diversity of virulence factors unique to the bacterium that have been identified to play a role in caries formation. Firstly, *S. mutans* is categorized as anaerobic bacteria that produce lactic acid. Secondly, *S. mutans* can bind to tooth surfaces in the presence of sucrose. Also, the most essential virulence factor is the acidophilicity of *S. mutans*. Unlike common oral microorganisms, *S. mutans* grows well under acidic conditions and is the main bacterium in cultures with permanently reduced pH.²⁵

Conclusion

The presented data are founded on a very small group and the results propose a link between IL-6 and IL-1 α in dental caries associated with *Streptococcus mutans*.

Conflict of interests

“No potential conflict of interests relevant to this article is reported”.

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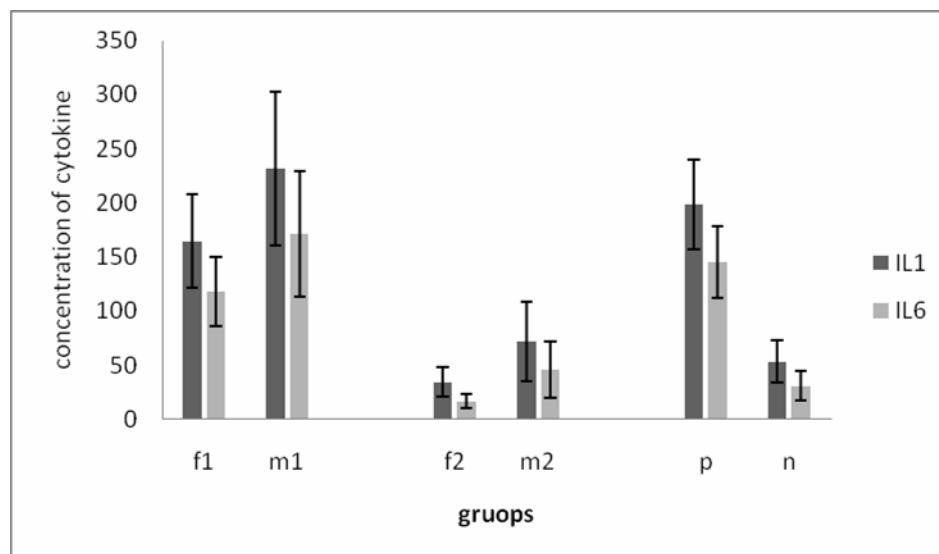


Figure 3. Mean concentrations of IL-1 α and IL-6 (pg/mL) in groups (f: female; m: male; 1: inflamed tissue; 2: intact tissue; p: all patients; n: normal tissue).

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