Assessment of Relationship between *Streptococcus mutans*, Dental Caries and TGF-β

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ABSTRACT :
Streptococcus mutans is an important species in oral microflora and its components have been found to stimulate production of cytokines in dental caries. The aim of this study was to evaluate TGF-β in patients with *S. mutans*. Seventy samples were selected during pulpectomy and investigated for the presence of TGF-β by ELISA. The results were analyzed by t-test (α = 0.05). The results showed higher mean concentrations of TGF-β 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). The results of this study suggested relations between the production of TGF-β in dental caries caused by *S. mutans*.

Keywords: Dental caries, TGF-β , 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*. 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, some cytokine production is induced by components of *S. mutans* [7,8]. The aim of this study was to assess association between transforming growth factor beta (TGF-β) 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 (2 mm) 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 TGF-β 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 accepted at P < 0.05.

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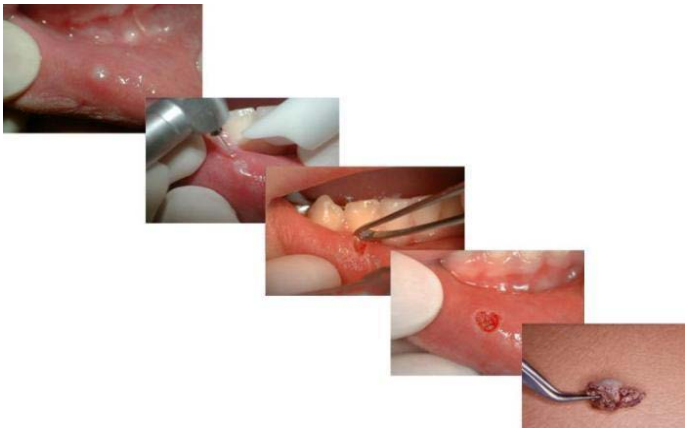

Figure 1. Tissue sample during pulpectomy.

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 TGF-β level are presented in Table 1 and Figure 3. The results showed that the means of the cytokines were significantly different between female and male subjects (P = 0.000). But TGF-β level were higher in inflamed tissues compared with intact tissues (P < 0.05).

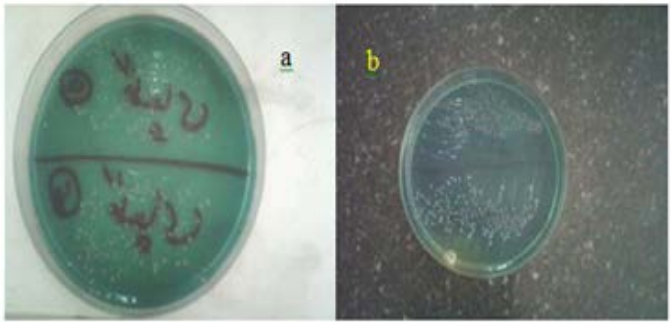

Figure 2. Growth of *S. mutans* in the two media (a: Golchai ZOE; B: Cavex ZOE).

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

	IL-1α		
	Female	**Male**	**Total**
Intact tissue	27.83 ±8.28	14.77 ± 4.27	21.30 ± 4.71
Inflamed tissue	148.24± 24.4	105.81± 18.59	127.02 ± 15.51
P-value	0.000*		

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

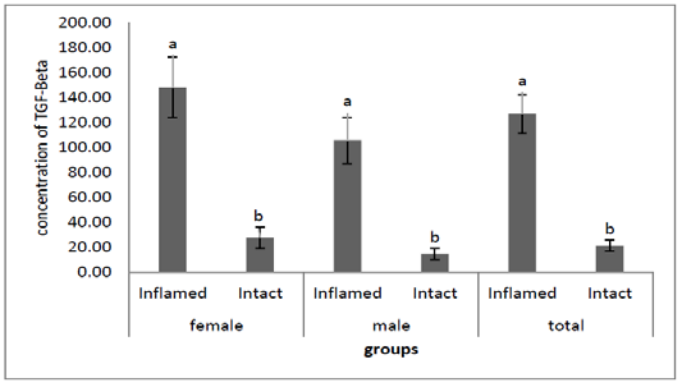

Figure 3. Mean concentrations of TGF-β (pg/mL) in groups

Discussion

In this study, TGF-β level exhibited statistically significant differences in inflamed tissues associated with *S. mutans* (P < 0.05).

S. mutans is the major factor responsible for dental caries [10-13]. The cell surface protein antigens of this bacterium (Pac, Ag I/II, PI, and B) help colonization of tooth surfaces [7,14,15]. After colonizing the oral cavity, the inflammation process begins. Then, due to this lesions, innate and adaptive host immune responses are induced [16].

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 [17]. Meiers et al[24] 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[18]. Growth factors facilitate several functions associated with the turnover, healing, and restoration of tissues in periodontal diseases[19]. Transforming growth factor (TGF)-β1 acts in lesion healing, and in addition two character , anti-inflammatory and proinflammatory, causes alteration of native microbial environment [20,21]. MMP-1 activity was inhibited by TGF-β1, and this anti-inflammatory role of TGF-β1 demonstrates tight controlling of the MMP/tissue inhibitor of MMP equilibrium[22]. Stein et al. examined GCF TGF-β1 levels in two groups (smokers and nonsmokers subjects) with chronic periodontitis prior and later of periodontal treatment. According to the baseline`s results concluded that smokers had a high concentration of GCF TGF-β1 in compared with non-smokers[20]. On the other hand, IL-1 is one of the main mediators of immune and inflammatory responses[23,24]. Different agents, including microorganisms and metabolites, inflammatory causes, or antigens could induce IL-1 production. The activity of IL-1 was controlled via IL-1ra presents in the immune system due to high affinity to analogous receptors as IL-1β [25]. In addition to IL-1 , IL6 , IL-8 and TNF-a have relevant to inflammation response that was found to have links with dental caries diseases[26-28]. Solan *et al.* concluded that the odontoblasts and pulp cells expressed the TGF-β receptors (I & II) , which some level of its expression may be involved in the tissue reaction to wound [29].

Conclusion

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

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