Abstract: *Streptococcus mutans* is important in dental caries. Although the role of cytokines in the pathogenesis of dental caries is not clear, components of *S. mutans* were found to stimulate production of pro-inflammatory cytokines. We examined the associations of interleukin (IL)-1β, IL-1 receptor antagonist (IL-1ra), and IL-10 with dental caries. Unstimulated whole saliva and blood samples were obtained from 108 children aged 6-12 years with high caries (decayed, missing, or filled teeth [dmft/DMFT] index >4, n = 37), moderate caries (dmft/DMFT = 1-4, n = 37), or caries-free (dmft/DMFT = 0, n = 34). *S. mutans* level was classified as low (<10⁵ colony-forming units [CFU]/mL) or high (≥10⁵ CFU/mL). Saliva and serum concentrations of IL-1β, IL-1ra, and IL-10 were determined by ELISA. IL-1β, IL-1ra, and IL-10 gene polymorphisms were genotyped using PCR and restriction fragment length polymorphism analysis. The chi-square, Mann-Whitney U, one-way ANOVA, posthoc, Fisher’s exact, and t tests were used in statistical analysis. Dental caries was not correlated with salivary or serum concentrations of the studied cytokines. *S. mutans* level positively correlated with saliva IL-1β concentration and inversely correlated with saliva IL-1ra concentration. There was no correlation of IL-1β, IL-1ra, or IL-10 gene polymorphisms with dental caries. *S. mutans* is important in stimulating saliva IL-1β and inhibiting IL-1ra. Future studies of associations between cytokines and dental caries should investigate additional cytokines and enroll a larger number of participants. (J Oral Sci 57, 31-36, 2015)

Keywords: dental caries; *Streptococcus mutans*; IL-1β; IL-1ra; IL-10.

Introduction

Dental caries is the most prevalent chronic disease of the oral cavity (1). Although it is very common, the mechanism by which some individuals remain caries-free has attracted the attention of researchers (2). Dental caries is a multifactorial disease and is strongly associated with the presence of cariogenic microorganisms, fermentable carbohydrates, and susceptible teeth, and duration of exposure (3-5). Mutans streptococci have been implicated as causative agents in dental caries (2), and several studies have shown an association between dental caries and *Streptococcus mutans* (6,7).

Numerous studies have evaluated the relation between development of carious lesions and the response of immunocompetent cells (8,9). Cytokines are products of activated monocyte–macrophage cells and are important
mediators of infection, inflammation, and immunologic challenge. Recent evidence suggests that they are important in controlling the inflammatory response to bacterial infection. Interactions between oral microorganisms and the immune system are complex (10,11). Genetic and immunologic differences among individuals may be important in the risk of dental caries (12). Although the role of cytokines in the pathogenesis of dental caries is not clear, components of *S. mutans* were found to stimulate production of proinflammatory cytokines (10-12). This study assessed the associations of interleukin (IL)-1β, IL-1 receptor antagonist (IL-1ra), and IL-10 levels with dental caries.

### Materials and Methods

The participants were 350 children aged 6-12 years. Written informed consent was obtained from all individuals, and the study protocol was approved by the Ethical Committee of Ege University (06-12/3). Children with systemic diseases and those who had used antibiotics during the previous 3-month period were excluded.

Tooth-brushing habits (less than once, once, twice per day), daily dietary sugar exposure (0-2 times, >2 times per day) were ascertained by a structured questionnaire. Dental plaque score was classified according to the Silness and Löe index (13). Children with a dental plaque score of 3 were excluded. Among the 350 children, 108 (53 boys, 55 girls) with similar dental plaque scores, oral hygiene habits, and sugar consumption were included in the study, to eliminate other risk factors for dental caries.

The decayed, missing, or filled teeth (DMFT) index was recorded according to World Health Organization criteria (14), and all children were classified according to DMFT score (15), as follows: group 1, high caries (dmft, DMFT >4, *n* = 37); group 2, moderate caries (dmft, DMFT = 1-4, *n* = 37); and group 3, caries-free (dmft, DMFT = 0, *n* = 34).

Saliva and blood samples were obtained from all participants. Unstimulated whole saliva was collected for 10 min by the spitting method. Saliva samples from the children were collected between 9:00 AM and 11:00 AM. The CRT Bacteria test (Ivoclar-Vivadent, Schaan, Liechtenstein) was used to determine *S. mutans* count in 1 mL of saliva. After removing the agar carrier from the test vial, a sodium bicarbonate (NaHCO₃) tablet was placed at the bottom of the vial. The protective foils were removed from the two agar surfaces, and the collected saliva samples were transferred to the test vial, using a pipette from the test kit, and spread on both sides of the plastic vial. The agar carrier was inserted back into the vial, and the vial was closed. The test vial was placed upright in the incubator and incubated at 37°C for 48 h. After removing the vial from the incubator, colonies of *S. mutans* were counted with the aid of a chart provided by the manufacturer. The *S. mutans* level was classified as low (<10⁵; *n* = 42) or high (≥10⁵; *n* = 66) (16).

Saliva samples for determination of cytokine concentrations were homogenized and clarified by centrifugation at 10,000 g for 15 min at 4°C. The aliquots of clarified supernatants were stored at −70°C until cytokine measurement. The concentrations of saliva and serum IL-1β, IL-1ra, and IL-10 were assessed with an enzyme-linked immunosorbent assay (ELISA; BioSource, Nivelles, Belgium), according to the manufacturer’s instructions.

Genomic DNA was isolated from peripheral blood using standard techniques. Polymerase chain reaction (PCR)-based genotyping of IL-1β –511 C→T, IL-1ra VNTR (variable number of tandem repeats in intron 2), and IL-10 –1082 G/A was performed as previously described (17,18). Specific primers and PCR conditions are shown in Table 1. The PCR products were then visualized by electrophoresis on 2% agarose gel.

### Table 1 Specific primers and PCR conditions for analysis of interleukin (IL)-1β, IL-1 receptor antagonist (IL-1ra), and IL-10

<table>
<thead>
<tr>
<th>Specific primers</th>
<th>PCR conditions</th>
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<tr>
<td><strong>IL-1β</strong>&lt;br&gt;- 511&lt;br&gt;T&gt;C</td>
<td>Denaturation: 94°C, 2 min&lt;br&gt;30 cycles, 94°C, 30 s&lt;br&gt;60°C, 30 s&lt;br&gt;Final extension: 72°C, 5 min</td>
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<tr>
<td><strong>IL-1ra</strong>&lt;br&gt;VNTR</td>
<td>Denaturation: 94°C, 2 min&lt;br&gt;30 cycles, 94°C, 30 s&lt;br&gt;60°C, 30 s&lt;br&gt;Final extension: 72°C, 5 min</td>
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<tr>
<td><strong>IL-10</strong>&lt;br&gt;1082&lt;br&gt;G/A</td>
<td>Denaturation: 95°C, 1 min&lt;br&gt;30 cycles, 95°C, 15 s&lt;br&gt;65°C, 50 s&lt;br&gt;72°C, 50 s&lt;br&gt;Final extension: 72°C, 5 min</td>
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All data were analyzed by using the Statistical Package for the Social Sciences (SPSS) for Windows (version 15.0; SPSS Inc., Chicago, IL, USA). Associations of saliva and serum concentrations of IL-1β, IL-1α, and IL-10 with dental caries and S. mutans level were evaluated with the Mann-Whitney U test. Associations between allele frequencies, genotype frequencies, and carriage rates of alleles in all groups were compared by using Fisher’s exact test. The t test and ANOVA were used to compare numeric variables within groups. The Hardy–Weinberg equilibrium at individual loci was assessed by the chi-square test, and clinical association was determined by using the paired t test. A P value of less than 0.05 was considered to indicate statistical significance.

Results

The mean (±SD) age of the 108 children was 10.07 ± 1.63 years. The mean DMFT and dmft scores of the 108 children were 1.02 ± 0.87 (group 1: 2.53 ± 1.07; group 2: 0.44 ± 0.16; group 3: 0) and 3.58 ± 2.42 (group 1: 4.82 ± 2.70; group 2: 0.42 ± 0.21; group 3: 0), respectively. The mean overall dental plaque score was 1.23 ± 0.17.

Among the 350 children, 108 with similar dental plaque scores, oral hygiene habits, and sugar consumption were included in the study, to eliminate other risk factors for dental caries. DMFT score was not correlated with caries risk factors (P > 0.05, chi-square test). Only 30% of children regularly brushed their teeth twice a day. Sugar was consumed more than twice per day in 24% of the children. Dental caries was not significantly correlated with salivary or serum concentrations of the studied cytokines (Table 2). Salivary S. mutans level was positively correlated with saliva IL-1β level (P = 0.01) and inversely correlated with IL-1α level (P = 0.01) (Table 3). Serum concentrations of the studied cytokines were not correlated with dental caries or saliva S. mutans levels (P > 0.05). In addition, salivary and serum concentrations of the studied cytokines were not correlated (P > 0.05).

IL-1β, IL-1α and IL-10 gene polymorphisms were not correlated with dental caries (P > 0.05). The allele and genotype frequency distributions of the studied cytokine polymorphisms in the patients are shown in Fig. 1.
**Discussion**

Dental caries is one of the most common bacterial infections in humans (2). Several factors—including genetic, immunologic, microbial, behavioral, and environmental factors—are involved in its pathogenesis and progression (5). Dental plaque score, oral hygiene habits, and sugar consumption are generally considered the primary factors in evaluating the risk of dental caries (1-5). In the present study we recorded DMFT/dmft scores and plaque index in 350 children and designed and distributed a questionnaire survey. Ultimately, 108 children with similar dental plaque scores, oral hygiene habits, and sugar consumption were included. DMFT/dmft scores were not correlated with risk factors for dental caries ($P > 0.05$).

The familial nature and genetic transmission of dental caries have been confirmed in genetic association studies, genome-wide linkage scans, and expression profiles (10-12). Cytokines are important in immunity and inflammation (11); however, their role in the pathogenesis of dental caries is not clear. Cytokines are closely associated with the pathogenesis of inflammation in soft tissues (19-22), and evidence indicates that they contribute to the initiation and progression of dental caries (23,24). Elevated levels of the cytokines IL-6, tumor necrosis factor α, and IL-8 were found in caries-active saliva (25). However, dental caries was not significantly correlated with salivary or serum concentrations of IL-1β, IL-1ra, or IL-10 in the present study.

IL-1 is an important mediator of immune and inflammatory responses (19,22). IL-1 production is induced by microorganisms, microbial products, inflammatory agents, or antigens. IL-1ra exists in the immune system to control IL-1 activity. It acts by binding with high affinity to the same receptors as IL-1β, without inducing any of the biological effects of IL-1β. The effect of IL-1ra was confirmed in studies showing that a subpopulation of human monocytes responded differently to another cytokine, i.e., granulocyte-macrophage colony-stimulating factor (20,21).

*S. mutans* was reported to be the primary factor responsible for dental caries (6,7). The cell surface protein antigens of *S. mutans*—Pac, Ag I/II, Pl, and B—assist in bacterial colonization of tooth surfaces and interact with the salivary pellicle that coats the enamel (10). Bacteria colonize the oral cavity, a process that triggers inflammation. These lesions induce innate and adaptive host immune responses. Antigen I/II of *S. mutans*, an adhesin for saliva-coated surfaces, interacts directly with human monocytes or epithelial cells through lectin-like binding and stimulates production of pro-inflammatory cytokines such as IL-1 (12).

The cell wall-anchored protein I/II of *S. mutans* has an important role in the colonization of dental structures and induces synthesis of pro-inflammatory cytokines. It is possible that fragments of the cell wall from *S. mutans* permeate through dental tubules during early caries, thereby stimulating pulpal dendritic cells and macrophages. The metabolic activity of *S. mutans* results in the creation of acids. Secreted products are recognized as pathogenic factors and agents that promote immunomodulation by stimulating release of pro-inflammatory cytokines. Components of *S. mutans* were reported to stimulate production of proinflammatory cytokines, such as IL-1β, from monocytes (10,11). In contrast, IL-1ra was shown to inhibit IL-1β activity (26). *S. mutans* stimulated in vitro proliferation of peripheral blood mononuclear cells, including IL-10 (27). In the present study, *S. mutans* level was positively correlated with saliva IL-1β concentration and inversely correlated with saliva IL-1ra concentration.

One study found that the response to *S. mutans* was a Th1 response, which increased IL-10 concentration (28). IL-10 is produced by monocytes, macrophages, and dendritic cells in response to microbes (29). It stimulates B-cell maturation and antibody production. Production and secretion of IL-10 from human monocytes in response to Gram-negative bacteria probably depend on bacterial lipopolysaccharide content, which efficiently triggers IL-10 production (30). Proinflammatory cytokines are reported to be the first cytokines produced in response to pathogenic bacteria (31). IL-10 produced by macrophages and lymphocytes can inhibit IL-1β production (32). In the present study, salivary and serum concentrations of IL-10 were not correlated with dental caries or *S. mutans* levels.

Oral fluids mainly consist of whole saliva and include a mixture of proteins, antibodies, hormones, and other molecular analysis, which are frequently measured in standard blood tests. Oral fluid analysis has obvious advantages as compared with blood-based analysis, such as easy access and noninvasive collection (33). In addition, blood collection is considerably more difficult in studies of children. The oral cavity is a distinct environmental niche, and immune biomarkers are influenced by local immune processes. Cytokines are useful in diagnosing and treating diseases of the oral cavity, and saliva can be used as a noninvasive diagnostic fluid for measurement of biomarkers released during disease initiation and progression (34). In the present study both saliva and serum samples were used to measure cytokine concentrations. Salivary and serum concentrations were not correlated. Our study confirms the importance of...
saliva sampling for evaluating immunologic processes involved in dental caries and suggests that elevated IL-1\(\beta\) concentration and diminished IL-1ra concentration contribute to the development of dental caries associated with \emph{S. mutans} colonization.

In the present study IL-1\(\beta\), IL-1ra, and IL-10 gene polymorphisms were not associated with dental caries or \emph{S. mutans} colonization. The only other similar study, by Wang et al., studied correlations between cytokine gene polymorphisms and dental caries and reported a correlation between the IL-1\(\beta\) gene and dental caries (35). To our knowledge, the present study is the first to study the associations of IL-1ra and IL-10 gene polymorphisms with dental caries. Our results confirm those of Wang et al. and offer a new perspective on dental caries. Because of the complex structure of dental caries pathogenesis and progression, it is difficult to identify factors that predict the carious process. Future studies of associations between cytokines and dental caries should investigate additional cytokines and enroll a larger number of participants.

References
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