The effect of dental caries and restorative biomaterials on IL-1β and TNF-α levels in the gingival crevicular fluid

Uticaj karijesa i zubnih ispuna na nivo IL-1 β i TNF-α u gingivalnoj tečnosti

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Abstract

Background/Aim. In the spirit of personalized medicine, determining caries biomarkers in the saliva and gingival crevicular fluid (GCF) attracts great attention in the current dental research. The concentration of GCF cytokines is illustrative in depicting the processes in tooth structures. Their relevance must be inspected with aspects of tooth position and caries lesion level. Different impacts of dental restoration materials on GCF IL-1β and TNF-α could be used as a parameter for estimating local inflammation. This paper aimed to estimate the concentrations of the proinflammatory cytokines (IL-1β and TNF-α) in the GCF and to correlate them with caries extension, tooth position, and different restorative biomaterials.

Methods. GCF samples were collected from 90 periodontally healthy patients demonstrating at least one tooth with proximal caries and one intact tooth, at the baseline, 7 and 30-days post-treatment. The biomarkers’ profile was investigated in relation to different levels of caries extension (superficial, pulpal, gencious, root affection), defect size, and restorative biomaterial.

Results. Before therapy, caries level was significantly associated with IL-1β concentration, demonstrating the lowest level in gencious (C4) and superficial caries (C2). Thirty days after therapy, root affection (C5) was characterized by the highest IL-1β concentration. Different dental fillings showed various GCF cytokine changes. CPC induced a significant IL-1β increase in more than 70% of treated patients. Caries lesion size was insignificantly associated with GCF levels of these proinflammatory cytokines, where larger defects were followed by an average cytokine increase. Considering the tooth position before therapy, IL-1β had the highest level in GCF samples from caries-affected canines and second molars, while TNF-α showed the highest levels from canines GCF. Dental restoration induced cytokine increase in canines (IL-1β and TNF-α), 1st and 2nd molars GCF (IL-1β). Conclusion. Inflammation intensity of tooth structures was directly reflected in IL-1β and TNF-α concentrations. Dental restoration significantly affects IL-1β and TNF-α levels, depending on the used dental filling-type material. The profile of these cytokines varied in GCF samples of the tooth with different anatomical positions, where canines and molars demonstrated the highest level. An increase of these proinflammatory cytokines in the absence of any symptomatic manifestation of the inflammatory response can be considered as a possible tooth reparation parameter.

Key words: dental caries; dental restoration, permanent; dental materials; gingival crevicular fluid; interleukin-1; tumor necrosis factor-alpha.

Apstrakt

Uvod/Cilj. U duhu personalizovane medicine, određivanje biomarkera za karijes u pljuvački i gingivalnoj crevikularnoj tečnosti (gingival crevicular fluid – GCF) privlači veliko interesovanje u novijim stomatološkim istraživanjima. Koncentracija citokina u GCF reflektuje procese u zubnim strukturama. U smislu interpretacije njihovih koncentracija, treba uzeti u obzir uticaj položaja zuba i obim karijesne destrukcije. Nirvo IL-1β i TNF-α u GCF mogu poslužiti kao indikator zapaljenog odgovora na biomaterijale koji se koriste za zubne ispune. Cilj rada bio je određivanje proinflamatornih citokina IL-1 β i TNF-α u GCF poreklom od karijesom zahvaćenih i intaktnih zuba i njihovo...
Introduction

Dental caries is the most frequent health problem in population. It is caused by bacterial biofilms whose maturation is associated with an anaerobic shift in microflora, while the subsequent acidification leads to demineralization of the dental enamel representing the pathognomonic sign of the disease. Despite outstanding prophylactic strategies, dental caries and related complications are still highly prevalent in the population. Prophylactic strategies, dental caries and related pathognomonic sign of the disease. Despite outstanding demineralization of the dental enamel representing the reaction is usually sufficient to control the tooth infection and formation of reactionary dentin. Indeed, more intensive functions and play an important part in the local immune response against infective threats. They express mediators, such as cytokines and chemokines (IL-6, IL-8, IL-10, IL-1β, TNF-α, CCL2, CCL20, CXCL10) and defensins. This inflammatory reaction is directed to eliminate or attenuate cariogenic pathogens in odontoblasts’ proximity. In the case of low-intensity inflammation, this reaction is usually sufficient to control the tooth infection and to induce the formation of reactionary dentin. Indeed, more intensive or prolonged inflammation interrupts the regeneration processes and results in intensive mediator response from odontoblasts, dental pulp resident cells, and infiltrating immune cells. Further progression of bacterial invasion through the odontoblast barrier generates an immune response in the dental pulp complex, resulting in pulpitis and progression of the inflammatory process toward periodontium. Moreover, the in vitro stimulation of the tooth crown odontoblasts with TLR2 or TLR4 agonists resulted in a completely different profile of IL-1β, TNF-α, IL-8 CCL20, and β-defensin-2 production, indicating a differential response to aerobic or anaerobic bacteria. The inflamed dental pulp is a significant source of IL-1β and IL-8. On the other hand, locally produced IL-1β and TNF-α exert significant influence on odontoblast functions, inducing further β-defensin production, production of dental matrix protein-1, and inducing proliferation of odontoblast-like cells derived from stem cells. Moreover, the studies that investigated the cytokine profile in the GCF samples following dental restoration reported controversial data.

The present study hypothesized that IL-1β and TNF-α profile in the GCF from caries-affected and intact teeth are different, while the caries extension, tooth position, and different restorative biomaterials alter the biomarker profile as well.

The aim of the study was to investigate GCF IL-1β and TNF-α profile between caries-affected and healthy teeth, and estimate the effect of caries destruction, restorative material and tooth position on their respective concentrations.

Methods

Study design

The study was designed as a short-controlled prospective study, longitudinally assessing the effect of caries and its respective treatment on the local levels of the IL-1β and TNF-α in the split mouth-design.
Study population and inclusion criteria

The study population was comprised of 90 outpatients attending the Clinic for Stomatology at the Military Medical Academy, Belgrade, Serbia, in the period between January 2015 until June 2018. The population consisted of younger participants (mean age of 31 ± 6.15 years) with similar distribution in gender. The study was conducted in accordance with the International Ethical Guidelines and Declaration of Helsinki (1964/1975) and was approved by the Institutional Ethics Committee (reference number VMA/10-12/A.1). The participants were informed about the study characteristics and the scheduled procedures and accepted to participate by signing informed consent.

The enrolled participants had to be systemically healthy non-smokers, presenting at least one caries-affected and one intact tooth from the same morphological group of teeth, with intact periodontal tissues. The exclusion criteria were as follows: active periodontal disease; subgingival periodontal treatment in less than 6 months; antibiotic and anti-inflammatory intake in the last 3 months; health conditions and chronic diseases affecting the inflammatory status and/or bone metabolism; unsatisfying oral hygiene.

Caries lesions were diagnosed using a visual-tactile technique combined with the radiological exam and according to the Black’s Classification 31, while the periodontal condition was assessed using a combination of clinical parameters and panoramic radiographs according to the recent Classification of periodontal and peri-implant diseases and conditions 32, 33. Based on the progression levels, caries lesions were classified as superficial (C2), pulp involvement (C3), gangrene (C4), and root involvement (C5).

Restorative biomaterials

Six different restorative materials were used for dental filling – two temporary materials: zinc-phosphate cement (ZPhC-Cegal NV, Galenika, Serbia) and carboxylate cement (ZPoC-Harvard, USA); two permanent restorations: amalgam (Amg-Extracap D caps, Galenika, Serbia); nanohybrid composites: BF (the mixture of bisphenol-A-glycidyl-dimethacrylate (BisGMA) 15–25%, triethylene glycol dimethacrylate (TEGDMA) 12–14%, aluminofluoroborosilicate glass 50–60% [aluminium trioxide (Al2O3) 1–2%, and DL-camphorquinone, Shofu, Japan] and TEC (Tetric EvoCeram), the mixture of 2.5–10% of BisGMA and 2.5–10% of urethane-dimethacrylate (UEDMA) and nonhazardous additions (Ivoclar Vivadent, USA); GIC (glass ionomer cement, GIC Fuji PLUS®, Green Circle, USA) was used for both settings, standalone restorations and the base for nanohybrid composites (BF and TEC). Dental fillings (temporary and permanent) were sealed in one session while the placed mass counted between 0.07–2.03 g (Table 1).

| Parameters | IL-1β | TNF-α |
|------------|-------|-------|
|            | 7 days | 30 days | 7 days | 30 days |
| All        | n/total | %       | n/total | %       | n/total | %       | n/total | %       |
| Dental filling type | 39/86 | 45 | 33/74 | 45 | 35/84 | 42 | 25/74 | 34 |
| TEC        | 9/17 | 53 | 8/17 | 47 | 6/17 | 35 | 9/17 | 53 |
| AMA        | 2/14 | 14 | 4/11 | 36 | 4/13 | 31 | 2/11 | 18 |
| BEA        | 8/15 | 53 | 3/15 | 20 | 6/15 | 40 | 2/15 | 13 |
| CFC        | 6/14 | 43 | 4/9 | 44 | 6/13 | 46 | 3/9 | 33 |
| GIC        | 7/13 | 54 | 6/11 | 55 | 6/13 | 46 | 6/11 | 36 |
| CPC        | 7/13 | 54 | 8/11 | 73 | 7/13 | 54 | 5/11 | 45 |
| Caries level | 31/58 | 53 | 19/51 | 37 | 23/58 | 53 | 51/58 | 37 |
| C2         | 3/5 | 60 | 2/4 | 50 | 2/5 | 40 | 3/4 | 75 |
| C3         | 5/9 | 56 | 4/9 | 44 | 6/9 | 66 | 3/9 | 33 |
| C4         | 5/7 | 71 | 4/7 | 57 | 4/7 | 57 | 4/7 | 57 |
| C5         | 4/11 | 55 | 2/10 | 20 | 3/11 | 27 | 2/10 | 20 |
| < 0.5 g    | 35/69 | 51 | 23/62 | 37 | 30/69 | 44 | 22/62 | 35 |
| < 1.0 g    | 4/9 | 44 | 1/7 | 14 | 4/9 | 44 | 1/7 | 14 |
| > 1.0 g    | 2/8 | 25 | 4/8 | 50 | 1/8 | 13 | 3/8 | 38 |
| Tooth position | 1 | 3/5 | 60 | 2/4 | 50 | 2/5 | 40 | 3/4 | 75 |
| 2         | 5/9 | 56 | 4/9 | 44 | 6/9 | 66 | 3/9 | 33 |
| 3         | 5/7 | 71 | 4/7 | 57 | 4/7 | 57 | 4/7 | 57 |
| 4         | 6/11 | 55 | 2/10 | 20 | 3/11 | 27 | 2/10 | 20 |
| 5         | 6/24 | 25 | 6/18 | 33 | 9/24 | 38 | 6/18 | 33 |
| 6         | 7/12 | 58 | 5/11 | 45 | 6/12 | 50 | 4/11 | 36 |
| 7         | 7/16 | 44 | 6/14 | 32 | 7/16 | 44 | 6/14 | 32 |
Biomarker measurement

The GCF sampling was performed using the filter paper technique as previously described. Strips contaminated with blood or saliva were discarded. The GCF volume was measured using Periotron 6000 (Interstate Drug Exchange, Amityville, NY, USA), calibrated prior to each set of measurements. Following that, the paper strips were placed into microcentrifuge plastic tubes, and elution was performed with 500 μL phosphate-buffered saline by vortexing for 10 seconds and centrifugation at 3,000 g for 5 min, in order to remove plaque and cellular detritus. The supernatants were stored in plastic tubes at -70°C until further analysis. The biomarker estimation was performed using flow cytometry (Beckman FC500; Beckman, USA) with commercial assays BioLegend’s LEGENDplex™, Human Inflammation Panel (Cat No 740118, USA). Detection limits: TNF-α (1.0 pg/mL), IL1-β (1.0 pg/mL).

Statistical analysis

Inter-group comparisons of the parameters were tested with the ANOVA test, with Bonferroni post hoc test. Thereafter, the p-values lower than 0.05 were considered significant. The correlations between the variables were tested with Spearman’s rank correlation test. The average concentrations of IL-1β and TNF-α were expressed as pg of biomarker/μL of GCF, mean ± standard deviation (SD). The statistical analysis was performed using commercial software (GraphPad Prism, USA).

Results

The average concentration of IL-1β and TNF-α in GCF samples of patients according to different time points

The IL-1β and TNF-α concentrations between caries-affected and healthy teeth are depicted in Table 2. At the baseline, IL-1β showed significantly increased levels in caries-affected teeth when compared to the healthy controls (HC), while 30-days post-treatment, TNFα levels were significantly higher in the treated sites than in HC (Table 3).

Table 2

| Parameters | IL-1β (pg) mean ± SD | TNF-α (pg) mean ± SD |
|------------|---------------------|----------------------|
|            | 0 days  | 7 days | 30 days | 0 days  | 7 days | 30 days |
| Dental filling type | TEC     | 111 ± 211 | 92 ± 122 | 158 ± 188 | 24 ± 24 | 19 ± 17 | 28 ± 23 |
|              | AMA     | 155 ± 197 | 126 ± 177 | 187 ± 210 | 34 ± 37 | 24 ± 44 | 27 ± 25 |
| Caries level | C2      | 76 ± 104 | 94 ± 105 | 115 ± 161 | 20 ± 26 | 24 ± 39 | 29 ± 62 |
|              | C3      | 172 ± 243 | 51 ± 92 | 182 ± 182 | 27 ± 44 | 47 ± 66 | 42 ± 52 |
|              | C4      | 49 ± 59 | 113 ± 144 | 104 ± 119 | 5 ± 6 | 10 ± 9 | 12 ± 12 |
|              | C5      | 101 ± 103 | 156 ± 169 | 286 ± 226 | 36 ± 35 | 30 ± 23 | 35 ± 23 |
| Tooth position | 1      | 59 ± 74 | 43 ± 25 | 141 ± 190 | 2 ± 2 | 5 ± 9 | 14 ± 12 |
|              | 2      | 46 ± 48 | 44 ± 54 | 149 ± 205 | 15 ± 12 | 15 ± 17 | 15 ± 20 |
|              | 3      | 117 ± 152 | 135 ± 93 | 253 ± 227 | 49 ± 62 | 35 ± 30 | 47 ± 62 |
|              | 4      | 44 ± 37 | 65 ± 58 | 77 ± 75 | 17 ± 17 | 20 ± 29 | 18 ± 20 |
|              | 5      | 107 ± 111 | 70 ± 79 | 103 ± 149 | 31 ± 40 | 34 ± 56 | 29 ± 35 |
|              | 6      | 59 ± 41 | 166 ± 164 | 214 ± 198 | 24 ± 27 | 32 ± 45 | 34 ± 44 |
|              | 7      | 133 ± 225 | 175 ± 171 | 223 ± 222 | 20 ± 19 | 26 ± 24 | 23 ± 22 |

* IL-1β, dental filling type, TEC/CPC, 30 days; *; ** IL-1β, dental filling type, BEA/CPC, 30 days; **; † IL-1β, dental filling type, CFC / CPC, 30 days; †; †† IL-1β, caries level, C2 / C5, 30 days; †; †† IL-1β, caries level, C4 / C5, 30 days; †; †† IL-1β, caries level C5, 0d / 30 days; †; †† IL-1β, tooth position 6, 0 / 7 days; †; † IL-1β, tooth position 6, 0 / 30 days; †; SD – standard deviation.
Table 3

| Biomarker | Control (C), mean ± SD | Caries affected teeth, mean ± SD | Baseline vs. C | Day 7 vs. C | Day 30 vs. C |
|-----------|------------------------|----------------------------------|----------------|-------------|-------------|
| IL-1β     | 78.23 ± 90.53          | 245.67 ± 750.10                  | 79.02 ± 84.00 | 148.39 ± 290.12 | p = 0.012 |
| TNF-α     | 24.05 ± 47.67          | 41.45 ± 109.34                   | 84.01 ± 356.50 | 88.14 ± 361.21 | p = 0.010 |

SD – standard deviation

Biomarker levels between sites with different restorative materials

The analysis of average GCF IL-1β level before dental restorative therapy demonstrated a significant variation, with the lowest values in patient samples later treated with BEA and CFC fillings. After restoration, all materials, except BEA, demonstrated GCF IL-1β increase, with the maximal level at a 30-day time interval (Table 2). Temporary dental filling materials (CFC, GIC, CPC) demonstrated a much more intensive local IL-1β increase (from +75 to 210 %) compared to the materials for permanent (TEC, AMA, BEA) dental filling (from -37 to +42 %).

As shown for IL-1β concentration, GCF TNF-α level before dental restoration was the lowest in the patient samples later treated with BEA and CFC fillings. Again, the used dental filling materials induced the increase of GCF TNF-α. The highest average GCF TNF-α was recorded in the samples of GIC and CPC treated patients 30 days after. Temporary dental filling materials (CFC, GIC, CPC) demonstrated again a much more intensive local IL-1β increase (from +12 to 78 %) compared to the materials for permanent (TEC, AMA, BEA) dental filling (from -23 to +17 %).

Association of caries destruction extension with GCF IL-1β and TNF-α concentration

In our study, caries lesion is associated with significant GCF IL-1β concentration even in the initial stage, as a superficial dental change (C2) (Table 2). Before therapy, patients with the gangrenous process (C4) demonstrated the lowest average GCF IL-1β value, while those with pulpitis (C3) had the highest recorded GCF IL-1β concentration. On day 30 after therapy, all patients demonstrated an increase in average GCF IL-1β concentration. This increase was minimal for patients with pulpitis, due to the high initial concentration, but was maximal for patients with the process in the root canal.

Before therapy, GCF TNF-α showed the lowest concentration in the C4 group. However, after dental restoration, the highest average TNF-α concentration was demonstrated in the pulpitis group (C3).

Size of the caries lesion

The size of the caries lesion was determined indirectly, according to the volume of dental filling material needed for restoration. Before therapy, the concentration of GCF IL-1β was the highest in the group with the largest tooth defect caused by caries (> 1.0 g). Interestingly, 30 days after dental restoration, the average concentration increased in the samples of groups with small and very large caries defects, while it decreased in the group with intermediate fillings (0.5–1.0 g) (Table 3). Before therapy, GCF TNF-α demonstrated almost similar values in all groups divided according to caries tooth defect. Contrary to IL-1β findings, dental restoration induced decrement on day 30 in all groups.

Association of tooth position with GCF IL-1β and TNF-α concentration

Tooth position was significantly associated with GCF IL-1β concentration (Table 2). Before therapy, the average concentration was the highest in samples from a canine, second premolar, and second molar. After therapy, GCF IL-1β concentration increased in samples from all teeth except the second molar. The highest average concentration on day 30 was demonstrated in GCF of a canine and second molar.

The concentration of TNF-α before therapy was the highest in samples from canine and second premolar. Dental restoration therapy on day 30 demonstrated an increase of TNF-α in GCF of the first incisor and I and II molars, and contrary to IL-1β showed unchanged or decreased value in GCF of the second incisor, canine, and both molars.

Level of GCF IL-1β and TNF-α after dental restoration varies according to caries extensity, type and volume of dental restoration filling, and tooth position

Seven days after therapy, GCF IL-1β showed an increased value in samples of more than half of the patients treated with both temporary and permanent filling materials, except for those treated with amalgam (AMA) (Table 1). However, after 30 days, GCF IL-1β concentration demonstrated a further decrease in all patients treated with a permanent type of filling (TEC, AMA, BEA), while an increase was demonstrated in all of those treated with a temporary type of filling. This was especially evident for CPC, where almost 75% of treated patients demonstrated a significant GCF IL-1β rise compared to the level before therapy.

On the 7th day, GCF IL-1β was increased in more than half of the patients with superficial caries (C2) or those with the affected root canal (C5). On day 30, a further increase was evident in more than 50% of patients from the more

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profound caries lesion (C3, C4, C5), with a documented decrease only in the C2 group.

Interestingly, the filling volume of less than 1 g was associated with an increase in 44–50%, while a larger filling volume was associated with a decrease of GCF IL-1β in 75% of treated patients. Conversely, on the 30th day, a smaller filling volume was associated with a local IL-1β increase in minor frequency (14–37%).

According to the tooth position, on the 7th day, GCF IL-1β was increased in more than 50% of patients in both incisors, canines, first premolar, and first molar. The 30th day was associated with an IL-1β decrement in GCF of all treated teeth, except the second premolar.

Seven days after dental restoration, the GCF TNF-α value increased in less than half of the patients, both treated with temporary and permanent filling materials. After 30 days, a further decrease of patients percent with documented TNF-α increase was documented in all groups except in those treated with TEC.

As for IL-1β, on the 7th day, GCF TNF-α was increased in more than 50% of C2 and C5 groups. Identically, on day 30, a further increase was evident in more than 50% of patients from the more profound caries lesion (C3, C4, C5), with a documented decrease only in the C2 group.

Again, identically as IL-1β, although in smaller frequency, on the 7th day, GCF TNF-α demonstrated an increase in samples where the filling volume was less than 1 g and a decrease in more than 85% of those treated with a larger filling volume. Conversely, on the 30th day, a smaller filling volume was associated with a local TNF-α increase in minor frequency (14–35%).

Seven days after therapy, GCF TNF-α demonstrated an increase in 57–66% of samples from canines and second incisors. On day 30, there was a TNF-α decrement in GCF of all investigated teeth except the first incisor.

Dental restoration is associated and correlated with IL-1β and TNF-α values in GCF of teeth with superficial caries, small caries extensity, and specific tooth position.

After therapy, coordinated local secretion/liberation of GCF IL-1β and TNF-α was demonstrated in the teeth treated with amalgam (7th day), BEA, and CFC (30th day) (Table 4).

According to the caries level before therapy, only patients with the gangrenous process (C4) did not show a significant correlation of GCF IL-1β and TNF-α. After dental restoration, a significant correlation of GCF IL-1β and TNF-α was demonstrated only in the group with superficial caries lesion, both on the 7th and 30th day.

Caries lesions that needed fillings of less than 1 g were characterized by a significant correlation of GCF IL-1β and TNF-α, both before and after dental restoration.

The specific position of a caries tooth is associated with the correlated production of GCF IL-1β and TNF-α both before and after dental restoration. A significant correlation between IL-1β and TNF-α was demonstrated before and after restoration in GCF of second incisors (7th day), second premolar (7th day), and second molar (7th and 30th day).

### Table 4

| Parameters                        | IL-1β + TNF-α |
|-----------------------------------|---------------|
|                                   | 7 days | 30 days |
| Caries destruction level           |         |         |
| C2                                | 0.0004 | 0.0030  |
| C3                                | ns     | ns      |
| C4                                | ns     | ns      |
| C5                                | ns     | ns      |
| Restorative biomaterial            |         |         |
| TEC                               | ns     | ns      |
| AMA                               | 0.0030 | ns      |
| BEA                               | ns     | 0.0002  |
| CFC                               | ns     | 0.0170  |
| GJC                               | ns     | ns      |
| CPC                               | ns     | ns      |
| Biomaterial amount (g)            |         |         |
| < 0.5                             | 0.0007 | 0.0003  |
| < 1.0                             | 0.0140 | ns      |
| > 1.0                             | ns     | ns      |

*ns – not significant.*

### Discussion

Inflammation in the tooth structures is unequivocally associated with the presence of inflammatory mediators, especially inflammatory cytokines IL-1β and TNF-α. The concentration of GCF IL-1β and TNF-α were extensively studied in local inflammatory conditions as periodontitis 35–38 and periimplantitis 39–44 or even as a systemic inflammatory condition like diabetes 45, 46 or connective tissue disease 47–50. Compared to these inflammatory conditions, cytokines were infrequently investigated in dental caries, especially in GCF of caries teeth 51–53.

Caries is associated with increased local IL-1β and TNF-α levels. Couglo et al. 52 demonstrated that children with high Streptococcus mutans numbers had high salivary IL-1β concentration and low IL1RA. They found that IL-1β was slightly elevated in the saliva and serum of children with caries but was not significantly associated with the caries lesion severity 52. They also showed that IL-1β, IL1RA, and IL-10 gene polymorphism were not significantly associated with dental caries. Eslami et al. 53 demonstrated higher average IL-6 and IL-1β concentrations locally in the inflamed pulpal tissues of subjects with dental caries compared with intact pulp tissue samples. This increase was significantly associated with S. mutans infection. McLachlan et al. 54 documented a significant expression of genes for S100A8, S100A9, S100A10, S100A12, S100A13, TNF-α, IL-1β, IL-8, IL-6, and ENA-78 in the pulp of caries teeth, close to the lesion. Pulp inflammation resulting from carious lesions is characterized by a strong increase in the production of proinflammatory cytokines, including TNF-α, IFN-γ, IL-1β, IL-6, CXCL8, and IL-18 55–57. Therefore, pulpitis intensity is significantly associated with intensive

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local inflammatory mediators production. Additionally, our patients with pulpitis (C3 group) and the largest caries defect demonstrated the highest average IL-1β and TNF-α levels before therapy.

IL-1 seems to be of extreme importance in the pathophysiology of caries lesion. Horst et al. 56 investigated gene expression of inflammatory mediators in the odontoblast layer of extirpated caries teeth. Both the pulp and the odontoblast layers demonstrated a significant mRNA increase of CCR2, CCR4, CCR5, CCR9, CCL3, CCL23, IL-1β, and TNF-α. More importantly, they showed that TNF-α and especially IL-1β induced an in vitro increase of a human b-defensin 2 (HBD2) mRNA expression in odontoblasts, up to 100 times more intensive than LPS/TLR4 agonist. The only limitation of their study is the selection of teeth because not all 32 samples were third molars, with caries lesion reaching 50 to 75% of dentin thickness. Additionally, the authors did not provide data on whether these teeth were previously treated or not. We have demonstrated that GCF IL-1 seems to be of extreme importance in the pathophysiology of caries lesion. Horst et al. 56 investigated gene expression of inflammatory mediators in the odontoblast layer of extirpated caries teeth. Both the pulp and the odontoblast layers demonstrated a significant mRNA increase of CCR2, CCR4, CCR5, CCR9, CCL3, CCL23, IL-1β, and TNF-α.

According to one group of studies, proinflammatory cytokines are just indispensable in dental regeneration processes. Bone regeneration itself is critically connected to proinflammatory cytokines. The regeneration of bone fracture is associated with biphasic TNF-α and IL-1β increase, with a peak during the initiation of fracture repair, followed by a second peak at the transition from chondrogenesis to osteogenesis during endochondral maturation. The balanced immune response appears to be essential for a successful bone healing process. The absence of TNF-α delays fracture healing, while prolonged exposure to TNF-α destroys the bone. Our study in children with long bone fractures (unpublished results), showed significantly lower IL-1β and MCP-1 serum concentrations in children with insufficient callus formation and minor fragment dislocation (angulation and dislocation less than 1 cm). Therefore, newer studies demonstrated that IL-1β and TNF-α influence the biological behavior of dental stem cells. In a way, they are needed for tooth tissue regeneration. The study from Yang et al. 57 demonstrated that IL-1β and TNF-α have synergic effects on odontogenic differentiation of isolated dental pulp stem cell population. The in vitro treatment with both IL-1β and TNF-α compared to a single treatment with either cytokine demonstrated a significantly faster stem cell proliferation, increased alkaline phosphatase (ALP) activity, increased osteocalcin and bone sialoprotein expression, augmented mRNA expression of ALP, osteocalcin, bone sialoprotein, dentin sialophosphoprotein, and dentin matrix protein-1. Both cytokines synergistically induced significant morphologic dental stem cell changes on the 3rd day at the surfaces of the HA/TCP ceramic scaffolds. The in vivo experiments with dental stem cell implants, pretreated with IL-1 and TNF-2, showed a significant level of hard bone formation, with even bone marrow like hematopoietic tissue.

Goldberg et al. 68 stated that inflammatory processes are very important not only for defense but also for pulp regeneration. Therefore, it seems that local inflammation is overseen only as an unwanted and harmful process, leading only to necrosis in the undesirable outcome. Migration and odontoblastic differentiation of dental stem cells is a crucial step in dental regeneration after caries lesion. Leprince et al. 73 concluded that dental pulp stem cells and mesenchymal stem cells have identical characteristics, and are needed for dental pulp regeneration. According to this aspect, after initial response to local microbiota agents mediated by inflammatory cytokines, after their elimination...
and dental restoration, local stem cells are activated and induced to differentiate into cells that produce reactionary and reparative dentin. Another inflammatory wave could regulate transdifferentiation of fibroblast-like pulp cells to stem cells, or inflammatory monocytes itself could be converged to odonto-progenitor cells.

The balance between the inflammatory process as a defense mechanism and an inflammatory initiated repairation process seems to be influenced by the severity and presence of infection. Controlled, acute production of inflammatory mediators and clearing of microorganisms is associated with tissue repair, while chronic, uncontrolled inflammation is destructive.

Restorative dental materials significantly influence GCF mediators concentration. Celik et al. and Ilday et al. reported that different dental restorative materials induce the various local response, inducing a significant variation of GCF IL-6, IL-8, and TNF-α profile after dental therapy. Sakallioglu et al. investigated the concentration of substance-P, calcitonin gene-related peptide, neurokinin-A, IL-1α, IL-1β, and PGE2 in GCF samples of teeth restored with ceramic, metal, composite, opposite-composite, amalgam, opposite-amalgam, or enamel. Although the study was performed only on 14 patients without any data before therapy or tooth position, they noted significant inter-group variations 4 weeks after restoration. They found the highest level of substance-P in amalgam restored teeth, PGE2 in composite restored, while IL-1α and IL-1β were highly present after metal-based restoration. Similarly, Björkman et al. reported that the removal of amalgam restoration resulted in the normalization of GCF Th1 cytokine levels. We also demonstrated that dental restorative material (both permanent and temporary) induce a significant change in GCF IL-1α and IL-1β levels.

There are several explanations for the increases of GCF IL-1α and IL-1β levels after restoration. Local inflammatory mediators could be induced from dental cells with chemical content liberated from the restorative material, and/or by mediators generated from de novo plaque accumulation. Since there were no clinical signs of any inflammatory process or plaque accumulation after restoration either in our or previous studies, inflammatory mediator increase could be attributed to a healing or repairation process. Calcium hydroxide and mineral trioxide aggregate (MTA) are known to stimulate dentinogenesis and cementogenesis, together with the early inflammation, while MTA, at least in vitro, demonstrated significant IL-1β stimulating capacity. Hydroxyl ions derived from these restorative materials change the oxidative-reductive balance at lesion site, ultimately inducing chemical tissue irritation and cellular necrosis. Necrotic cells release low levels of cytokines and other damage signals to facilitate the removal of the dead or dying cells, leading to the inflammation without microorganisms in the lesion itself.

Conclusion

The significant presence of inflammatory mediators in GCF of the restored teeth without signs of the inflammatory process could be associated with the reparative process. Different influences of various types of dental fillings on GCF IL-1α and IL-1β levels could represent the ground for selecting the optimal restorative material.

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Conflict of interest

None.

REFERENCES

1. Jin L, Lamster I, Greenspan J, Pitts N Scally C, Warnakulasuriya S. Global burden of oral diseases: emerging concepts, management and interplay with systemic health. Oral Dis 2016; 22(7): 609–19.
2. Peter DN, Bourgeois D, Oyawa H, Estupinan-Day S, Ndiaye C. The global burden of oral diseases and risks to oral health. Bull World Health Organ 2005; 83(9): 661–9.
3. Jang JH, Shin HW, Lee JM, Lee HW, Kim EC, Park SH. An Overview of Pathogen Recognition Receptors for Innate Immunity in Dental Pulp. Mediators Inflamm 2015; 2015: 794143.
4. Seng M, Brighton D, Curtis MA, Curn JF, Dry I, Donnici H, et al. Role of microbial biofilms in the maintenance of oral health and in the development of dental caries and periodontal diseases. Consensus report of group 1 of the Joint EFP/ORCA workshop on the boundaries between caries and periodontal disease. J Clin Periodontol 2017; 44 Suppl 18: S5–S11.
5. Cooper PR, Takahashi Y, Graham LW, Simon S, Inuzuta S, Smith AJ. Inflammation-regeneration interplay in the dentine-pulp complex. J Dent 2010; 38(9): 687–97.
Keller JF, Carrouel F, Colomb E, Durand SH, Bandoun C, Misika P, et al. Toll-like receptor 2 activation by lipoteichoic acid induces differential production of pro-inflammatory cytokines in human odontoblasts, dental pulp fibroblasts and immature dentin cells. Immunobiology 2010; 215(1): 53–9.

Ferger JC, Carrouel F, Keller JF, Bandoun C, Misika P, Böcker F, et al. 2010 Cytokine production by human odontoblast-like cells upon Toll-like receptor-2 engagement. Immunobiology 2011; 216(4): 513–7.

He W, Zhang Y, Zhang J, Yu Q, Wang P, Wang Z, et al. Cytidine-phosphate-guanosine oligonucleotides induce interleukin-8 production through activation of TLR9, MsD88, NF-κB, and ERK pathways in odontoblast cells. J Endod 2012; 38(6): 780–5.

Paris S, Wolgin M, Kiiduska AM, Pries A, Zakryzewicz A. Gene expression of human beta-defensins in healthy and inflamed human dental pulps. J Endod 2009; 35(4): 520–3.

Dommisch H, Winter J, Ajil Y, Dausche A, Tiemann M, Ijspeert S. Human beta-defensin (hBD-1, -2) expression in dental pulp. Oral Microbiol Immunol 2005; 20(3): 163–6.

Ferger JC, Allert-Licht B, Renard E, Ducros M, Gaudin A, Smith AJ, et al. Dental Pulp Defence and Repair Mechanisms in Dental Caries. Mediators Inflamm. 2015; 2015: 230251.

Turner MD, Ndjai B, Hurst T, Pennington DJ. (2014) Cytokines and chemokines: At the cross roads of cell signalling and inflammatory response in the dentin matrix. J Endod 2010; 36(1): 64–7.

Hase N, Ozeki N, Hiyama T, Yamaguchi H, Kawai R, Kondo A, et al. Products of dentin matrix protein-1 degradation by inflammatory cytokines reaction elicited by root endosseous infections. J Periodontol 2015; 86(4): 516–22.

Celik, N, Askın, S, Gül MA, Seven N. Ilday NO, Celik N, Dilsiz A, Alp HH, Aydin T, Seven N, et al. Effects of overhang amalgam restoration on levels of cytokines in gingival crevicular fluid. Arch Oral Biol 2017; 84: 139–47.

Ibrahim H, Motawi S, Khraisheh M, et al. Assessment of interleukin-1β, interleukin-6, and tumor necrosis factor-α levels in the peri-implant sulcular fluid among water-pipe (narghile) smokers and never-smokers with peri-implantitis. J Clin Periodontol 2016; 43(7): 597–604.
47. Özyakar O, Altıcık E, Nadlantry A, Karabulut G, Kabasakal Y. Clinical periodontal status and inflammatory cytokines in primary Sjögren syndrome and rheumatoid arthritis. J Periodontal 2018; 89(8): 959–65.

48. Čeříková B, Gasibradová E, Opál J, Jelinek S. Proinflammatory and anti-inflammatory cytokines in gingival crevicular fluid and serum of patients with rheumatoid arthritis and patients with chronic periodontitis. J Periodontol 2013; 84(1): 84–93.

49. Jarad F, Ahmed HB, Mikami T, Alam K, Ramana GE, Al-Hazimi K. Cytokine profile in the gingival crevicular fluid of rheumatoid arthritis patients with chronic periodontitis. J Investig Clin Dent 2014; 5(1): 1–8.

50. Beşkederli B, Budanović N, Aksu K, Nadlantry A, Lappin DF, Erzenović E, et al. Periodontal therapy in chronic periodontitis lowers gingival crevicular fluid interleukin-1beta and DAS28 in rheumatoid arthritis patients. Rheumatol Int 2014; 33(10): 2607–16.

51. Kumar NK, Reddy VK, Padakandla P, Yogar H, Kalagatla S, Chandru SN. Evaluation of chemokines in gingival crevicular fluid in children with dental caries and stainless steel crowns: A clinical-biochemical study. J Indian Soc Pedod Prev Dent 2016; 34(3): 273–9.

52. Cangil D, Onay H, Özlem Y, Aksan G, Örkinç F, Kutshekin N, et al. Associations of interleukin (IL)-1β, IL-1 receptor antagonist, and IL-10 with dental caries. J Oral Sci 2015; 57(3): 31–6.

53. Eslami H, Pourabdollah F, Sepehr R, Zerandis A. Evaluation of Relationship between Streptococcus mutans, Dental Caries and IL-1α and IL-6.] Periodontal Implant Dent 2016; 8(1): 33–6.

54. Ma’shedan JL, Saan AJ, Smith AJ, Landini G, Cooper PR. S100 and cytokine expression in caries. Infect Immun 2004; 72(7): 4102–8.

55. Vargas JC, Almeida-Leitão B, Baudouin C, Miskhe P, Béclère V, Carronel F. Odontoblast control of dental pulp inflammation triggered by cariogenic bacteria. Front Physiol 2013;4: 326.

56. Hurst OV, Hurst JA, Samudra R, Dale BV. Caries induced cytokine network in the odontoblast layer of human teeth. BMC Immunol 2011; 12: 9.

57. He Y, Gu Y, Lu J, Feng Q, Wang H, Guan H, et al. Pulpal Tissue Inflammatory Reactions after Experimental Pulpal Exposure in Mice. J Endod 2017; 43(1): 90–5.

58. Garralda S, Li Y, Hogan MM, Tjaderhane LS, Pasley DH, Morgan T, et al. Inflammatory mediators in fluid extracted from the coronal occlusal dentine of trimmed teeth. Arch Oral Biol 2012; 57(3): 264–70.

59. Iwasaki IR, crunch LD, tutor A, Gordon S, Hakemani M, Marc DB, et al. Tooth movement and cytokines in gingival crevicular fluid and whole blood in growing and adult subjects. Am J Orthod Dentofacial Orthop 2005; 128(4): 483–91.

60. George A, Eisens C-A. Detection of root resorption using dentin and bone markers. Orthod Craniofac Res 2009; 12(2): 229–35.

61. Kan T, Choi T, Atsuyama T, Yamanaka M, Noosh N, Greaves D, et al. Expression of osteoprotegerin, receptor activator of NF-kappaB ligand (osteoprotegerin ligand) and related proinflammatory cytokines during fracture healing. J Bone Miner Res 2001; 16(6): 1004–14.

62. Lehmann W, Edgar CM, Wang K, Choi TJ, Barnes GL, Kakear S, et al. Tumor necrosis factor alpha (TNF-alpha) coordinately regulates the expression of specific matrix metalloproteinases (MMPs) and angiogenic factors during fracture healing. Bone 2005; 36(2): 300–10.

63. Kader P, Schmidt-Bleek K, Schell H, Gader T, Tohen D, Schmitzmaier G, et al. The early fracture hematomas and its potential role in fracture healing. Tissue Eng Part B Rev 2010; 16(4): 427–34.

64. Schmidt-Bleek K, Schell H, Lüman J, Schulz N, Hoff P, Pfaff M, et al. Initial immune reaction and angiogenesis in bone healing. J Tissue Eng Regen Med 2014; 8(2): 120–30.

65. Karmas J, Dayfer S, Watkins C. Multiple Roles of Tumor Necrosis Factor - Alpha in Fracture Healing. Bone 2015; 78: 87–93.