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

Uticaj karijesa i zubnih ispuna na nivoe 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, pulpitis, gangrenous, root affection), defect size, and restorative biomaterial. **Results.** Before therapy, caries level was significantly associated with GCF IL-1β concentration, demonstrating the lowest level in gangrenous (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 creviculr 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. Nivoi IL-1β i TNF-α u GCF mogu poslužiti kao indikator zapaljenskog 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

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korelisanje sa obimom karijesa, položajem zuba i različitim zubnim ispunima. **Metode.** Uzorci GCF sakupljeni su od 90 parodontološki zdravih osoba koji su imali najmanje jedan zub sa proksimalnim karijesom i jedan intaktni zub, na početku terapije, kao i 7 i 30 dana nakon terapije. Profil biomarkera ispitivan je u odnosu na obim karijesnih lezija: površne (C2), pulpitis (C3), gangrena (C4) i karijes korena (C5), veličinu defekta i vrstu biomaterijala. **Rezultati.** Obim karijesnih lezija značajno je bio udružen sa IL-1β, čije su koncentracije bile najniže u grupi gangrenoznih zuba (C4) i površnog karijesa (C2). Trideset dana nakon terapije grupa C5 pokazala je najviše vrednosti IL-1β. Različiti materijali za zubne ispune pokazali su različiti profil citokina u GCF. CPC je uzrokovao porast IL-1β kod više od 70% bolesnika. Obim karijesne lezije nije pokazao značajnu korelaciju sa merenim citokinima, dok su veći defekti bili udruženi sa povećanjem srednjih vrednosti citokina. U pogledu položaja zuba, IL-1β je pokazivao najviše vrednosti kod karijesom zahvaćenih očnjaka i drugih molara, dok je TNF-α imao najveće vrednosti kod očnjaka. Nakon terapije, povećanje koncentracije citokina je utvrđeno kod očnjaka (IL-1β i TNF-α), prvog i drugog molara (IL-1β). **Zaključak.** Intenzitet zapaljenja zubnih struktura se direktno reflektovao na koncentraciju IL-1β i TNF-α. Zubni ispuni su značajno uticali na nivoe IL-1β i TNF-α u odnosu na vrstu korišćenog biomaterijala. Profil merenih citokina je varirao u odnosu na različiti položaj zuba, pri čemu su očnjaci i molari pokazali najviše vrednosti. Uočeni porast merenih pro-inflamatornih citokina u odsustvu klinički manifestne patologije može ukazivati na reparatorne efekte.

Ključne reči:
zub, karijes; zub, trajni ispuni; stomatološki materijali; gingivalna sulkusna tečnost; interleukin-1; faktor nekroze tumora.

Introduction

Dental caries is the most frequent health problem in population [1, 2]. It is caused by bacterial biofilms whose maturation is associated with an anaerobic shift in microflora [3], 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 and provide a negative impact on oral and systemic health [4]. Therefore, many efforts are invested in a better understanding of caries pathogenesis in order to improve respective preventive strategies, diagnostic approaches, and predictive treatment protocols with decreased complication rates. In the spirit of personalized medicine, the search for caries biomarkers in the saliva and gingival crevicular fluid (GCF) attracts great attention in the current dental research.

The cariogenic microorganisms and their byproducts, following the initial invasion of tooth enamel, reach the dental tubules and get in contact with dental odontoblasts' cellular extensions. Odontoblasts are specialized cells that, apart from producing the dentin, express many metabolical functions and play an important part in the local immune response against infective threats [5, 6]. They express numerous pathogen recognition receptors that bind di- and/or tri-acetylated lipoproteins, lipopolysaccharides (LPS), flagellin, viral dsRNA, and unmethylated CpG motif-containing DNA [7–10]. As a response to toll-like receptors (TLR) and nucleotide-binding oligomerization domain (NOD), stimulation odontoblasts secrete numerous mediators, such as cytokines and chemokines (IL-6, IL-8, IL-10, IL-1β, TNF-α, CCL2, CCL20, CXCL10) [6, 11–14], and defensins [6, 15, 16]. 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 regenerative process that finally results in 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 [17, 18]. 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 [19, 20]. 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 [21]. On the other hand, locally produced IL-1β and TNF-α exert significant influence on odontoblast functions, inducing further β-defensin production [22], production of dental matrix protein-1, and inducing proliferation of odontoblast-like cells derived from stem cells [23]. Moreover, the studies that investigated the cytokine profile in the GCF samples following dental restoration reported controversial data [24–30].

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 (Al_2O_3) 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).

Table 1

Percentage of patients with IL-1β and TNF-α gingival crevicular fluid (GCF) increase at time points (at least 20% up increase comparing to 0 time point, before dental filling)

Parameters		IL-1β				TNF-α			
		7 days		30 days		7 days		30 days	
		n/total	%	n/total	%	n/total	%	n/total	%
All		39/86	45	33/74	45	35/84	42	25/74	34
Dental filling type	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
	GJC	7/13	54	6/11	55	6/13	46	4/11	36
	CPC	7/13	54	8/11	73	7/13	54	5/11	45
Caries level	C2	31/58	53	19/51	37	31/58	53	19/51	37
	C3	1/9	11	5/9	55	1/ 9	11	4/7	57
	C4	2/6	33	3/6	50	2/ 6	33	2/5	40
	C5	7/13	54	6/11	55	7/13	54	6/11	55
Dental filling volume	< 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 [34]. 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 comparison of selected groups. The 0 time point before therapy, was the control value for every individual investigated tooth, with the 7th and 30th-day values compared to the initial level. The differences between the two selected groups were evaluated using the Mann-Whitney 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/μLof 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, TNFa levels were significantly higher in the treated sites than in HC (Table 3).

Table 2

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

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	[a] 158 ± 188	24 ± 24	19 ± 17	28 ± 23
	AMA	155 ± 197	126 ± 177	187 ± 210	34 ± 37	24 ± 44	27 ± 25
	BEA	54 ± 84	88 ± 106	[b] 55 ± 107	12 ± 21	25 ± 46	18 ± 43
	CFC	31 ± 26	54 ± 104	[c] 65 ± 100	4 ± 5	7 ± 7	4 ± 5
	GJC	103 ± 104	153 ± 170	192 ± 265	33 ± 33	37 ± 39	37 ± 28
	CPC	243 ± 269	235 ± 245	[a,b,c] 427 ± 331	29 ± 38	44 ± 50	49 ± 57
Caries level	C2	76 ± 104	94 ± 105	[d] 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	[e] 104 ± 119	5 ± 6	10 ± 9	12 ± 12
	C5	[f] 101 ± 103	156 ± 169	[d,e,f] 286 ± 226	36 ± 35	30 ± 23	35 ± 23
Dental filling volume	< 0.5 g	107 ± 183	114 ± 187	167 ± 253	36 ± 93	18 ± 17	33 ± 30
	< 1.0 g	118 ± 174	148 ± 213	56 ± 61	33 ± 63	19 ± 24	25 ± 22
	> 1.0 g	137 ± 204	113 ± 121	236 ± 229	35 ± 55	11 ± 13	30 ± 26
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	[g,h] 59 ± 41	[g] 166 ± 164	[h] 214 ± 198	24 ± 27	32 ± 45	34 ± 44
	7	133 ± 225	175 ± 171	223 ± 222	20 ± 19	26 ± 24	23 ± 22

[a] IL-1β, dental filling type, TEC/CPC, 30 days, *; [b] IL-1β, dental filling type, BEA/CPC, 30 days, **; [c] IL-1β, dental filling type, CFC / CPC, 30days, *; [d] IL-1β, caries level, C2 / C5, 30 days, *; [e] IL-1β, caries level, C4 / C5, 30 days, *; [f] IL-1β, caries level C5, 0d / 30 days, *; [g] IL-1β, tooth position 6, 0/7days, *; [h] IL-1β, tooth position 6, 0/30 days, *; SD – standard deviation.**

Table 3

The IL-1β and TNF-α concentration between caries affected and healthy teeth

Biomarker	Control (C), mean ± SD	Caries affected teeth, mean ± SD					
		Baseline	Day 7	Day 30	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 restoration 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-a was recorded in the samples of GIC and CPC treated patients 30 days after. Temporary dental filling materials (CFC, GJC, CPC) demonstrated again a much more intensive local TNF-α 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

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 extensivity, 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

Correlation of IL-1β and TNF-α concentration in gingival crevicular fluid (GCF) samples in the different time points

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 [54]. 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 pulpal 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

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 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-1β and TNF-α concentrations vary dramatically according to the tooth position, caries lesion extensivity. It has also been demonstrated that dental restoration material significantly alters its level further. Different groups of teeth are exposed to a different intensity of occlusal forces depending on their anatomical position and primary function, subsequently followed by a different profile of biochemical markers around different teeth. Briefly, the stimulation of periodontal mechanoreceptors is followed by the local release of neuropeptides, growth factors, and cytokines that accordingly regulate the remodeling of periodontal tissues [58–60].

He et al. [57] investigated pulpitis in the experimental model of pulp exposure to oral cavity microorganisms. They succeeded in documenting all the stages of pulpitis, from initial inflammatory cells recrutation to the exposed pulp and initial secretion of IL-1β and TNF-α, to chronic-like inflammation, the disappearance of dental odontoblasts and pulpal necrosis. This elegant study was performed with the micro computed tomography (CT) analysis, histopathological description of the local cell population, as well as RT PCR verification of IL-1β and TNF-α local presence in the time interval from 0h to 72h after pulp exposure. Although in their experimental model caries progressed from the initial lesion to pulpal necrosis in less than 3 days, some parallels could be drawn between caries lesions of the different levels seen in patients. Before therapy, IL-1β GCF increased from the initial C2 caries (enamel + dentin lesion) to pulpitis (C3) and root inflammation (C5), with a modest increase in gangrenous pulp (C4). Similarly, He et al. [57] demonstrated a local pulp IL-1β increase from the initial inflammation to the maximal presence in irreversible pulpitis until the beginning of the necrosis process, after which the value decreased. In our study, only the C2 group had noticeably the smallest increase rate compared to the level before therapy.

Surprisingly, at both control points, on the 7th and 30th day, the average concentration of GCF IL-1β and TNF-α were increased compared to the level before dental restoration practically in all investigated samples. Conclusively, Ilday et al. [27] demonstrated that silorane composite dental restoration after dental caries is associated with a significant increase of average TNF-α, IL-6, and IL-8, while Geraldeli et al. [58] found that amalgam dental restoration induced an increase of local TNF-α but a slight decrease of IL-1β in coronal occlusal dentine of trimmed molars. Since the restored teeth were without any clinical and/or radiological signs, this increase could not be attributed to further progression of caries lesion or any other inflammatory process.

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 [61, 62]. The balanced immune response appears to be essential for a successful bone healing process [63, 64]. The absence of TNF-α delays fracture healing, while prolonged exposure to TNF-α destroys the bone [65, 66]. 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. [67] demonstrated that IL-1β and TNF-α have synergistic 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 [69–72]. 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 [74]. Another inflammatory wave could regulate transdifferentiation of fibroblast-like pulp cells to stem cells [75], 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 reparation 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 [20].

Restorative dental materials significantly influence GCF mediators concentration [29]. Celik et al. [25] and Ilday et al. [26, 27] 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. Sakallioğlu et al. [77] 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. [78] 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 [29, 25, 26], inflammatory mediator increase could be attributed to a healing or reparation process. Calcium hydroxide and mineral trioxide aggregate (MTA) are known to stimulate dentinogenesis and cementogenesis, together with the early inflammation [79], while MTA, at least *in vitro*, demonstrated significant IL-1β stimulating capacity [80]. Hydroxyl ions derived from these restorative materials change the oxido-reductive balance at lesion site [28], 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 [81, 82].

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.

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