

Research Article

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Species and number of bacterium may alternate IL-1 β levels in the odontogenic cyst fluid

Odontojenik kist sıvılarındaki bakteri tür ve sayıları IL-1 β düzeyini değiştirebilir

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Abstract

Objectives: The role of oral bacteria in the etiopathogenesis of odontogenic cysts (OC) is controversial. Immune response is regulated by the cytokines secreted during infection. This study aims to describe the association in between bacteria and levels of cytokines in OC.

Methods: Infected OC fluid samples were obtained from 25 odontogenic keratocysts and 14 radicular cysts (RC). Bacteria detection was performed by polymerase chain reaction on bacterial 16S rRNA genes. Cytokine levels in OC fluids were determined using “luminex” method.

Results: *Porphyromonas gingivalis* was the most common bacteria in all samples (41.03%). Bacteria species number was higher in RCs. The significant difference was detected in terms of interleukine (IL)-1 β levels to the number of bacteria contained in cyst fluids ($p < 0.05$). IL-1 β level of cyst fluid group containing three or more species of bacteria increased compared with cyst fluid group containing two types of bacteria ($p < 0.05$). IL-1 β level was high in cyst fluids with *Campylobacter rectus* and *Treponema denticola* or with three or more bacteria species. IL-1 β level was

higher in the cyst fluids with *Enterococcus faecalis* negative than *E. faecalis* positives.

Conclusions: Our results suggest that species and the number of bacterium may differ IL-1 β levels in the OC fluid.

Keywords: Bacteria; IL-1 β ; Odontogenic cyst fluid; Polymerase chain reaction (PCR).

Özet

Amaç: Odontojenik kistlerin (OK) etyopatogenezinde oral bakterilerin rolü tartışmalıdır. Bağışıklık yanıtı, enfeksiyon sırasında salgılanan sitokinler tarafından düzenlenir. Bu çalışma, enfeksiyon ajanları olarak bakteriler ile OK sitokin seviyeleri arasındaki ilişkiyi tanımlamaktadır.

Metod: Enfekte OK sıvı örnekleri 25 keratokist ve 14 radiküler kist (RK)’dan elde edildi. Bakteri tespiti bakteri 16S rRNA genlerine dayanan bir polimeraz zincir reaksiyonu (PZR) ile gerçekleştirildi. OK sıvılarındaki sitokin seviyeleri “luminex” yöntemi kullanılarak belirlendi.

Bulgular: *Porphyromonas gingivalis*, tüm örneklerde en sık görülen bakteriydi (% 41.03). Bakterilerin tür sayısı RK’lerde daha yüksekti. Kist sıvılarında bulunan bakteri sayısına göre interleukin (IL)-1 β düzeyleri bakımından anlamlı fark tespit edildi ($p > 0.05$). Üç veya daha fazla bakteri içeren kist sıvı grubunun IL-1 β seviyesi, iki tür bakteri içeren kist sıvı grubuna kıyasla artmıştır ($p < 0.005$). Özellikle üç veya daha fazla bakteri bulunan veya *Campylobacter rectus* veya *Treponema denticola*’lı kist sıvılarında IL-1 β seviyesi yüksekti. İlginç şekilde, IL-1 β düzeyi *Enterococcus faecalis* negatif kist sıvılarında *E. faecalis* pozitiflerinkinden daha yüksekti.

Sonuç: Sonuçlarımız, OK sıvısındaki bakteri türlerinin ve sayısının IL-1 β düzeylerinde farklılığa neden olabileceğini düşündürmektedir.

Anahtar Kelimeler: Bakteri; IL-1 β ; Odontojen kist sıvısı; Polimeraz zincir reaksiyonu (PZR).

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Introduction

Odontogenic cysts (OCs) originating from odontogenic epithelium are commonly seen pathological jaw lesions in the oral and maxillofacial surgery practice. They are divided into two groups, one being developmental and the other being inflammatory. Inflammatory cysts are thought to arise from inflammation-induced epithelial proliferation with subsequent central liquefaction [1]. Inflammatory radicular cysts (RCs) and developmental odontogenic keratocysts (OKCs) are the two most commonly encountered types in our practice. Infection of necrotic teeth are responsible for the formation of RCs and progress as apical lesions whereas OKCs react with an inflammatory response to chronic irritation caused by necrotic teeth [2]. Although studies have shown that periodontal inflammations' initiation is often caused by oral bacteria including periapical lesions, there's still limited research to define the responsible bacteria [3]. The proinflammatory cytokines and inflammation-associated growth factors are playing a role in the development of OKCs [4, 5]. Interleukin-1 (IL-1), IL-12, tumor necrosis factor-alpha (TNF- α) and IL-15 are secreted from macrophages during bacterial infections and regulate the immune response [6, 7]. Cytokines (IL-1, IL-12, TNF- α) are also responsible for systemic response to infections. IL-1 α and TNF- α are important mediators contributing to the development of cysts. IL-1 α and TNF- α were shown to stimulate the production of osteoclast-like multinucleated cells and enhance the activity of osteoclasts' bone resorption [5]. IL-12 (natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2, p40) causes local release of interferon-gamma (IFN- γ) from natural killer cells (NK) and T lymphocytes, stimulates the cytolytic activity of NK cells and also alerts the adaptive immune response by forwarding type-1 helper T cells (Th1) [7]. IL-15 is a pleiotropic cytokine that plays an important role in both innate and adaptive immune systems [7–9]. There are limited studies investigating the levels of cytokines in OCs and these have usually examined pro-inflammatory cytokines [5, 10, 11] or growth factors [4]. The aim of this study is to detect the levels of macrophage-originated cytokines (IL-1 β , IL-12, IL-15 and TNF- α) as response to bacterial infection and the bacterial content in OC fluids.

Materials and methods

Study population

The cyst fluid (CF) samples were collected at Istanbul University, Faculty of Dentistry, Department of Oral and

Maxillofacial Surgery. The study population consisted of otherwise healthy subjects who had either RCs or OKCs. Of those, study sample was chosen from the subjects only with clinically visible jaw expansions due to the cyst wall's pressure, and with a history of symptomatic infection with purulent cyst fluid (CF) on aspiration. Exclusion criteria included usage of antimicrobial therapy, receipt of antiviral or immunosuppressive therapies, or having an obvious mucosal breach or portal entry for infection via the oral cavity. Subjects who had OCs with a diameter <1 cm (measured on an orthopantomograph) were also excluded due to the possibility of inability to obtain adequate CF. As such, the final study sample consisted of 39 subjects with 25 OKCs and 14 RCs. Cone beam computerized tomography (CBCT) scans were used for the evaluation of the borders of the lesions, including the buccal expansions. Purulent CF was observed by inspection, and confirmed by histopathological analysis. Ethical Committee permission from Local Ethical Committee of Istanbul University, Istanbul Faculty of Medicine (no: 2008/3205) was obtained and the study protocol was in compliance with the Helsinki Declaration. Written informed consent was obtained from all subjects of the study.

Collection of samples

Disposable 19 gauge needles were used to collect OC fluids, attached to syringes, and then these were transferred into eppendorf tubes. During the entire procedure, salivary contamination was avoided using meticulous high-volume suction. The remaining cyst epithelium was then enucleated and sent for histopathological examination. Regarding the histopathological diagnosis OKCs and RCs samples were isolated for further analysis. Before extraction of genomic deoxyribonucleic acid (DNA) and determination of cytokine levels, all samples were immediately transferred and stored at -20°C .

DNA extraction

DNA was extracted from OCs fluid using a MagNA Pure Compact DNA Isolation Kit (Roche Diagnostics GmbH, Germany). The polymerase chain reaction (PCR) method was used to detect bacteria.

Identification of bacterial content with PCR

Species-specific oligonucleotide primers were used to detect the target microbial species. A pair of bacterial

primers that matched almost all bacterial 16S ribosomal ribonucleic acid (rRNA) genes in the same position, except the 18S rRNA gene from the eukaryotic cells, was used as a positive control for the PCR reaction. This served as an indicator of the presence of bacteria in clinical samples. Specific primers for *Tannerella forsythia* (*T. forsythia*), *Treponema denticola* (*T. denticola*), *Campylobacter rectus* (*C. rectus*), *Porphyromonas gingivalis* (*P. gingivalis*), *Provetella intermedia* (*P. intermedia*), and *Provetella nigrescens* (*P. nigrescens*), were described by Ashimoto et al. [12]. *Porphyromonas endodontalis* (*P. endodontalis*), *Provetella pallens* (*P. pallens*), *Dialister pneumosintes* (*D. pneumosintes*), *Filifactor alocis* (*F. alocis*), *Fusobacterium nucleatum* (*F. nucleatum*) [13, 14] and *Enterococcus faecalis* (*E. faecalis*) were described by Siqueira and Rocas [13]. PCR amplification and methods were performed according to the aforementioned literature [12–14]. PCR products were analyzed by 1.5% agarose gel electrophoresis performed at 4 V/cm in a tris-acetate EDTA buffer. The gel was stained with 0.5 L/mL ethidium bromide and photography was performed under a 300 nm ultraviolet transilluminator. As size markers, either 100 base pair (bp) or 1 kb DNA ladder digest (MBI Fermentas) was used. Band of the expected size's presence was considered “positive” in the results.

Determination of cytokine levels in cyst fluid

The profiles of cytokine levels (IL-1 β , IL-12, IL-15 and TNF- α) in CF were determined by authors SC and GD using a Human Cytokine LINCoplex kit (LINCO Research, Inc. USA) by Luminex technology. CF samples were centrifuged at 1000 g for 5 min, and the analyses of supernatant fractions were performed. The kit is capable of detecting four cytokines in one well up to 10,000 pg/mL. Cytokine concentrations were calculated via mean fluorescent intensity (MFI) found on BioPlex (Bio-Rad Laboratories, Inc.) with Bio-Plex manager Software 4.1.

Statistical analysis

Non-parametric tests as Mann-Whitney U (MW-U), Kruskal-Wallis (KW), and Chi-square test were used instead of Shapiro-Wilks Test due to the fact that the data was not normally distributed. Data were analyzed with KW and post-hoc MW-U tests. The Bonferroni correction was used. Statistical analysis was carried out using SPSS 21 statistical software (SPSS Inc, Chicago, IL, USA). p-Values less than 0.05 were considered statistically significant.

Results

Cysts and patient

The study sample consisted of 28 males (M, 71.8%) and 11 females (F), and ages ranged from 21 to 68 years. The OKCs group included 18 M (72.0%), 7 F and RCs included 10 M (71.4%), 4 F (Table 1). In both groups, male-to-female ratio was 2.5:1. Gender variable was statistically significant in favor of males in all cyst groups (Chi-square test $p=0.006$, $p=0.028$, respectively). No statistically significant difference was observed in the OC type regarding the age of patients and the size of lesions (MW-U test $p>0.05$, $p>0.05$, respectively).

Bacterial content

In 34 (87.18%) of the studied specimens, bacteria presence was positive. The red complex (*T. forsythia*, *P. gingivalis*, *T. denticola*) was negative in all samples. *P. gingivalis* was the most common type, in all samples ($n=16$, 41.03%, Table 2). Additionally, *F. nucleatum* ($n=10$, 25.64%), *P. intermedia* ($n=9$, 23.08%) were identified. During analysis of the cysts to determine the number of bacterial species, seven different species of bacteria were identified in one sample,

Table 1: The median and interquartile range of data in the patients of the OKC and RC groups.

	Age (years)	Male	Female	IL-1 β (pg/mL)	IL-12 (pg/mL)	IL-15 (pg/mL)	TNF- α (pg/mL)
OKC group (n = 25)	43 (21–68)	18	7	5.33 (0–335.21)	0 (0–9.42)	4.06 (0–16.95)	0 (0–61.24)
RC group (n = 14)	47 (24–64)	10	4	11.5 (0–2043)	0 (0–10.38)	3.34 (0–16.31)	2.495 (0–111.01)
Assay sensitivities ^a	–	–	–	0.19	0.23	0.63	0.22
p ^b				NS	NS	NS	NS
Total (n = 39)	45 (21–68)	28	11				

IL-1 β , Interleukine-1 beta; IL-12, interleukine-12; IL-15, interleukine-15; NS, non significant; OKC, odontogenic keratocyst; RC, radicular cysts; TNF α , tumor necrosis factor-alpha. ^aMinimum detectable concentrations, ^bMann-Whitney U test.

Table 2: Distribution of bacterium species in Ocs.

Name of bacterium species	OKC (F:M)		RC (F:M)		Total/OC (F:M)	
	n=25	%	n=14	%	n=39	%
<i>Campylobacter rectus</i>	3 (2:1)	12.00	3 (1:2)	21.43	6 (3:3)	15.38
<i>Dialister pneumocintes</i>	1 (0:1)	4.00	2 (1:1)	14.29	3 (1:2)	7.69
<i>Enterococcus faecalis</i>	5 (1:4)	20.00	3 (1:2)	21.43	8 (1:6)	20.51
<i>Filifactor alocis</i>	1 (0:1)	4.00	2 (0:2)	14.29	3 (0:3)	7.69
<i>Fusobacterium nucleatum</i>	4 (2:2)	16.00	6 (1:5)	42.86	10 (3:7)	25.64
<i>Porphyromonas endodontalis</i>	0	0	2 (0:2)	14.29	2 (0:2)	5.13
<i>Porphyromonas gingivalis</i>	9 (2:7)	36.00	7 (1:6)	50.00	16 (3:13)	41.03
<i>Prevotella intermedia</i>	6 (2:4)	24.00	3 (2:1)	21.43	9 (4:5)	23.08
<i>Prevotella nigrescens</i>	4 (2:4)	16.00	4 (1:3)	28.57	8 (3:7)	20.51
<i>Prevotella pallens</i>	0	0	1 (0:1)	7.14	1 (0:1)	2.56
<i>Treponema denticola</i>	2 (0:2)	8.00	4 (1:3)	28.57	6 (1:5)	15.38
<i>Tannerella forsythia</i>	5 (0:5)	20.00	1 (1:0)	7.14	6 (1:5)	15.38

OC, Odontogenic cyst; OKC, odontogenic keratocyst; RC, radicular cysts; F, female; M, male.

Table 3: Comparison of cytokine levels between RC and OKC [median (min-max) pg/mL].

Cytokine	Assay sensitivities ^a	OKC	RC	p ^b
IL-1 β	0.19	5.33 (0–335.21)	11.5 (0–2043)	NS
IL-12	0.23	0 (0–9.42)	0 (0–10.38)	NS
IL-15	0.63	4.06 (0–16.95)	3.34 (0–16.31)	NS
TNF α	0.22	0 (0–61.24)	2.495 (0–111.01)	NS

IL-1 β , Interleukine-1 beta; IL-12, interleukine-12; IL-15, interleukine-15; NS, non significant; OKC, odontogenic keratocyst; RC, radicular cysts; TNF- α , tumor necrosis factor-alpha. ^aMinimum detectable concentrations, pg/mL; ^bMann-Whitney U test.

5 in two, 4 in three, 3 in four, 2 in 13 and 1 in 11 samples. No bacteria was detected in five subjects (12.82% in total, 20.00% in OKC). There was no bacterium-free CF in the RC group. More bacteria species were observed in RCs 2 (1–5) [median (min-max)] bacteria than in OKCs 1 (0–7) bacteria (MW-U $p=0.009$).

Cytokines levels: IL-1 β , IL-12, IL-15 and TNF- α

Macrophage-derived cytokines IL-1 β , IL-12, IL-15 and TNF- α levels in response to CFs content bacterial infections were determined by luminex (Table 3). The correlation (R) of curves obtained from the standards of all cytokines was 0.900–0.999. High and low concentrations of control serum of the kit were within the expected range. There was no significant difference compared with respect to gender, age, cyst size and type. After the division of CFs in four groups according to the number of bacteria content (0, 1, 2 and ≥ 3 bacteria, Table 4), a significant difference was detected in terms of IL-1 β levels (KW $p=0.009$). IL-1 β level of CF group containing three or more species of bacteria increased compared with CF groups containing two types of bacteria (Figure 1).

IL-1 β levels of the groups, formed depending on whether the identified bacterial species in CF are positive or negative, were compared (Figure 2). A significant

Table 4: Cyst fluid cytokine (IL-1 β , IL-12, IL-15 and TNF- α) levels [median (min-max) pg/mL] grouped according to the number of bacteria species.

Number of bacteria species	n	IL-1 β	IL-12	IL-15	TNF- α
Bacterium-free	5	10.58 (0–123.26)	0 (0–9.42)	0.19 (0–8.19)	0 (0–0.84)
1 bacterium	11	6.44 (0–335.21)	0 (0–10.38)	4.06 (0–16.95)	0.84 (0–61.24)
2 bacteria	13	0 (0–14.44)	0 (0–0.52)	4.06 (0–16.31)	1.17 (0–16.34)
≥ 3 bacteria	10	23.33 (0–2043.11)	0 (0–3.24)	2.12 (0–15)	15.42 (0–111.01)
p ^a		0.009	NS	NS	NS

IL-1 β , Interleukine-1 beta; IL-12, interleukine-12; IL-15, interleukine-15; NS, non significant; OKC, odontogenic keratocyst; RC, radicular cysts; TNF- α , tumor necrosis factor-alpha. ^aKruskal-Wallis test.

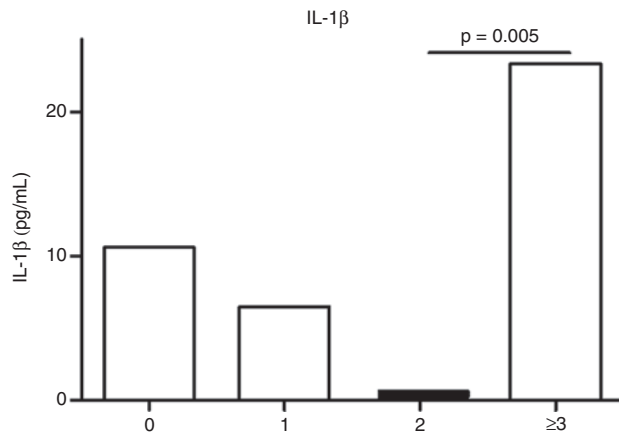


Figure 1: After being grouped according to the number of detected bacteria species [0 (n=5): bacterium-free, 1 (n=11) and 2 (n=13) are positive for 1 or 2 bacteria species, ≥ 3 (n=10): equal or more than three bacteria species] IL-1 β levels of cyst fluids were significantly different (Kruskal Wallis $p=0.009$, Mann-Whitney U test $p=0.005$, $\alpha=0.008$).

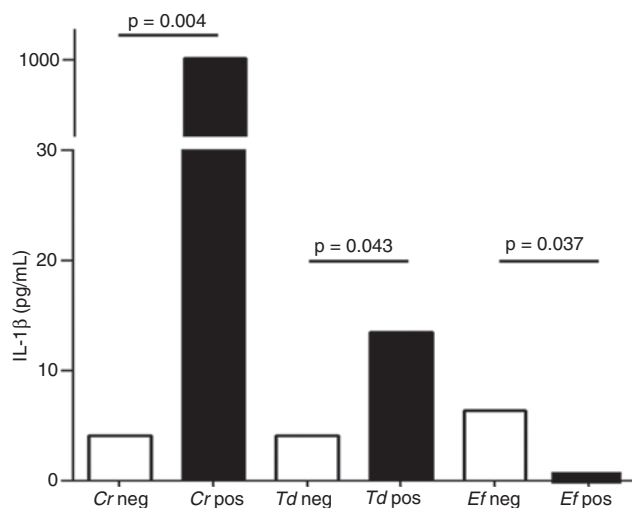


Figure 2: Levels of IL-1 β are higher in *Campylobacter rectus* positive (Cr pos, n=6), *Treponema denticola* positive (Td pos, n=6) and *Enterococcus faecalis* negative (Ef neg, n=31) cyst fluids when compared with *Campylobacter rectus* negative (Cr neg, n=33), *Treponema denticola* negative (Td neg, n=33) and *Enterococcus faecalis* positive (Ef pos, n=8, Mann-Whitney U test).

difference was found in *C. rectus* positive n=6; 1006.27 (6.44–2043.11) pg/mL and *T. denticola* positive n=6; 13.49 (6.44–1706.19) pg/mL, with higher levels of IL-1 β observed than relative bacterium negative cases. Different from these, the presence of higher levels of IL-1 β in *E. faecalis* negative CFs n=31; 6.44 (0–2043) pg/mL than *E. faecalis* positive fluids was observed.

Monocyte derived TNF- α , IL-12 and IL-15 cytokine levels were not significantly different.

Discussion

The OKCs are usually asymptomatic and are more commonly found in the posterior mandible of males [15]. RCs represent an inflammatory response to a chronic irritation, whereas OKCs are mostly non-inflammatory lesions [2, 16]. Studies report that the major part of the bacterial content in the OCs are consisted of anaerobes [3, 17]. Scalas et al. [18] observed the number of bacterial species per sample as 2.06 ± 0.93 , but they identified bacterial species *Propionibacterium acnes* (*P. acnes*) (7/16; 43.75%) and *Prevotella spp.* (2/16; 12.50%) by microbiological culture techniques. According to our results, the number of bacterial species per sample was similar and we have observed more bacteria species in the RC fluids. On the contrary, common bacteria in both types of CF were *P. gingivalis*, *F. nucleatum* and *Prevotella spp.* (46.15%). *P. gingivalis* was associated generally with *E. faecalis*. The discrimination depends on using species-specific PCR primers for identification of bacteria in cysts. We were able to identify bacteria in a more sensitive way however the spectrum was limited to these primers. We can suggest that these anaerobic bacteria may trigger the pathogenesis of cyst formation.

The cytokine IL-1 β is a key mediator of host immune response and plays an important role in the development of inflammatory disease and tissue destruction [19], and its precursor is induced in the cytosol of monocytes and macrophages in response to proinflammatory stimuli including pathogenic bacteria [6, 20]. Similarly to IL-6 and TNF- α , it is thought to play a role in bone remodeling, bone resorption, and new bone deposition.

In our study, we have screened IL-1 β , IL-12, IL-15 and TNF- α . Although no significant difference for any cytokine level was determined between cysts groups, IL-1 β and TNF- α were higher in RC fluids similar to report of Hayashi et al. [21]. Our results, where we identified the protein levels on CF, using Luminex, are in correlation with the results of the quantitative multiplex ELISA kit and Human Cytokine Antibody Array [16, 21]. High IL-1 β and TNF- α levels in RC fluid, in addition to higher number of bacteria species in RC, might suggest that a proinflammatory response created by the macrophages might be induced in response to bacteria. Local IL-1 and IL-6 are known to affect epithelial cell proliferation, and as such, IL-1 β and TNF- α could affect cyst formation too [22].

Hayashi et al. [21] considered that the expansion mechanisms of RC and OKC involve similar biologic mechanisms other than infection. Alhashimi et al. [23] showed that after the application of orthodontic force on the maxillary first molars of rats, messenger RNA (mRNA) expression IL-1 β

and IL-6 were induced but the mRNA of TNF- α was not detected. It can be proposed that the trigger of cytokine production could be also due to the application of orthodontic force. IL-1 β level in cysts with no bacteria isolation were not statistically significant yet higher compared to one type of bacteria containing CF group and two types of bacteria containing CF group; this can be explained by the orthodontic forces applied on these subjects.

In vitro studies have been demonstrated IL-1 β and TNF- α secretion induced by live bacteria or by their lipopolysaccharide (LPS) and plays a stimulatory role in maxillofacial abscess formation [6, 24]. Additionally, in adult periodontitis, significant elevation of LPS-stimulated IL-1 β and TNF- α was indicated by the gingival crevicular fluid analysis [25]. IL-1 β level increased when the CF's number of species of bacterial content was three or more. Especially in the RC fluids of our study, more bacteria species and elevated IL-1 β levels were detected. Increasing bacteria species could trigger IL-1 β production. The cytokine response being lower in the few bacteria species containing CFs (one or two bacteria species containing cysts) can be associated with the proteolytic effect of the bacteria. *P. gingivalis*'s proteolytic effect, down regulating the IL-8, is previously shown in oral epithelial cell cultures in vitro [26]. Other bacteria, including the *P. gingivalis* need to be investigated in regards to their skipping the innate immunity mechanisms by down regulating the cytokines. Our results suggest that *E. faecalis* might have a similar effect on IL-1 β . However there was no statistically difference in between *P. gingivalis* positive and *P. gingivalis* negative proteolytic effect, down regulating the IL-8, is previously shown in oral epithelial cell cultures samples' IL-1 β levels, yet we found high IL-1 β level in *C. rectus* positive and *T. denticola* positive CFs; this can stimulate cyst formation. According to our results, *C. rectus*, *T. denticola* bacteria exacerbated the pathogenesis by increasing the production of IL-1 β . However, it was observed that *E. faecalis* suppresses the secretion of IL-1 β . The reason of less IL-1 β in CFs with two species bacteria in comparison with one type could be the antagonistic interaction of bacteria or prevention of host inflammatory response by some bacteria such as *E. faecalis*. In this study, bacterial DNAs were defined as the agent of infection in CF and their relationship was shown with IL-1 β .

Although our study reflects local response to cyst, macrophage derived cytokines (IL-1, IL-12, TNF- α) are also responsible of systemic manifestations of infections. TNF- α , as a pleiotropic cytokine, is considered to play a primary role in modifying inflammatory and immune responses to various inflammatory diseases and tumor. It mostly triggers apoptosis and necrosis in sensitive tissues. It has been linked to osteomyelitis and periodontitis, however it can

also participate in osteoclast formation and thus help in haemostasis, and the bone resorption in the maxillofacial region [27]. Investigations showed that TNF- α is significantly higher in the RC fluids [5, 27]. In our study, although there was no significant difference between the OKC and RC, the TNF- α levels in RC were higher than OKC that were consistent with aforementioned studies. The idea of anti-inflammatory response, being more dominant in the development of RCs, is also supported by the aforementioned finding.

It has been suggested by previous literature that the gradual diminishing of IFN- γ as the T helper cytokine type 1 response or the transformation of chronic inflammation into cyst formation due to absence of the adaptive immune response [28] however getting chronic innate immune response was supported with these findings. This study focuses on determination of the types of bacteria causing infections in OKCs and RCs and it reveals levels of some cytokine secreted by macrophages as local immune response to infection. Previous microbiological studies on OCs evaluated the bacteriological spectrum or cytokines individually, while in our investigation, however these factors have been investigated together in our study [5, 17, 18, 29–31]. One of the advantages of Luminex method was that it is supposed to detect the concentration of many different types of protein in OC fluid, so it can be obtained in a small sample volume. The presence of immune-competent cells in OC fluid samples was not investigated and, the relatively small number of the samples in bacteria groups can be considered as limitations of the present study. There's a need for further in vitro studies are required to clarify the role of each bacterium and other cytokines in the pathogenesis of the OCs.

Conclusion remarks

This study shows the revelation of the relationship between cytokines and bacterial content of OC fluids. It was detected that the increased number of type of bacteria in the CF may cause increased secretion of IL-1 β as a sign of activated macrophages. The most common type of bacteria observed in OC fluids were *P. gingivalis* and *Prevotella spp.* While some bacteria (*C. rectus* and *T. denticola*) cause the secretion of IL-1 β at high levels, others (such as *E. faecalis*) suppress it. IL-1 β is thought to be involved more than IL-12, IL-15 and TNF- α in the cyst formation, but not identified as a specific cyst type. However, the IL-1 β level was slightly elevated in the RC fluid specimens with increased number of type of bacteria. These findings supported the thought that anti-inflammatory response is responsible for the development of this type of cyst. Further studies

are required to clarify the role of these bacteria in the pathogenesis of the OCs. The relatively small number of the samples for bacterial types groups of OC can be considered as a limitation of this study.

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