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ORIGINAL RESEARCH

INTERFERON- GAMMA LEVELS IN THE ODONTOGENIC CYST FLUIDS REGARDING BACTERIUM CONTENT

Odontojenik Kist Sıvılarının Bakteri İçeriğine Göre İnterferon-gamma Düzeyleri

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ABSTRACT

Purpose: Odontogenic cysts (OCs) are pathological lesions that include liquid or semi-liquid surrounded with epithelium. Radicular cysts' (RCs) and odontogenic keratocysts' (OKCs) are common odontogenic cysts of jaws, and may reach to a substantial size without symptoms for a long time. It is known that cytokines secreted during infection and inflammation regulate the immune response. This study aims to describe the relationship between bacteria as infection agents and the levels of interferon-gamma (IFN-γ) cytokine of innate and adaptive immune response.

Materials and Methods: OC fluid samples with a history of infection were collected from a total of 39 OCs consisting 25 samples of odontogenic keratocysts (OKC) and 14 samples of radicular cysts (RC). Anaerobic bacteria detection was performed by a polymerase chain reaction (PCR) based on bacterial 16S rRNA genes. IFN-γ levels in OC fluids were determined using the luminex method.

Results: No significant differences in IFN-γ levels and T cell type 1 cytokine responses were observed between the cystic fluid samples classified on the basis of age, gender, cyst-type, cyst-size, bacterial species and the number of bacterial species contained. The measured concentrations IFN-γ, which is a helper T cell type 1 cytokine were consistent with published data from experimental animal models, immunohistochemical studies, and molecular studies.

Conclusion: Luminex method can detect the concentration of many different types of protein in a small sample volume and is suitable for determining the protein content of odontogenic cysts.

Keywords: *Odontogenic keratocyst, radicular cysts, interferon-gamma, bacterial species, polymerase chain reaction (PCR)*

ÖZ

Amaç: Odontojenik kistler (OK), etrafı epitel ile çevrili içinde sıvı veya yarı-sıvı bulunan patolojik oluşumlardır. Odontojenik keratokistler (OKC) ve radiküler kistler (RC) uzun zaman belirti vermeyerek belirli bir büyüklüğe ulaşabilen, ağızın yaygın odontojenik kistleridir. Enfeksiyon ve inflamasyon sırasında salgılanan sitokinlerin bağışıklık yanıtını düzenlediği konusunda da bilgiler mevcuttur. Bu çalışma, enfeksiyon ajanları bakteriler ile doğal ve kazanılmış immün yanıt sitokinlerinden interferon-gamma (IFN-γ) düzeyi ilişkisinin tanımlanması amacıyla düzenlenmiştir.

Gereç ve Yöntem: Yirmibeş odontojenik keratokist (OKK) ve 14 radiküler kist (RK) olmak üzere enfeksiyon geçmişine sahip toplam 39 OK sıvı örneği alınmıştır. Anaerobik bakterilerin saptanması amacıyla, bakteriyel 16S RNA gen temelli polimeraz zincir reaksiyonu (PZR) kullanılmıştır. OK sıvısındaki IFN-γ düzeyi luminex yöntemiyle belirlenmiştir.

Bulgular: Yaşa, cinsiyete, kist tipine, büyüklüğüne, içerdiği bakteri türü ve tür sayısına göre gruplandırılan kist sıvılarının IFN-γ düzeylerinde anlamlı fark ve buna bağlı olarak T hücre tip 1 sitokin yanıtı saptanmamıştır. Yardımcı T hücre tip 1 sitokinlerinden olan IFN-γ düzeyleri daha önce yayınlanan hayvan deneyleri, immünohistokimya ve moleküler çalışmalarla uyumlu bulunmuştur.

Sonuç: Az miktardaki örnekte pek çok proteinin düzeyini belirleyen lumineks metodu odontojenik kist sıvılarının protein içeriğini belirlemek için uygundur.

Anahtar kelimeler: *Odontojenik keratokist, radiküler kist, interferon-gama, bakteri türleri, polimeraz zincir reaksiyonu (PZR)*

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Introduction

Odontogenic cysts (OCs) originating from odontogenic epithelium are pathological lesions often seen in the jaw bones and are divided into two groups as developmental and inflammatory cysts. 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 most commonly encountered ones. RCs originate often from the infection of necrotic teeth and progress as apical lesions whereas OKCs occur with a non inflammatory response to chronic irritation; as such, its development is different from RCs (2). Although studies have shown that oral bacteria play a role in the initiation of periodontal inflammations including periapical lesions, research to define the responsible bacteria is still limited (3). As a result of the stimuli that induce the regulation of the immune response, the proteins secreted by leukocytes and other cells of the organism are named cytokines (4, 5). Some cytokines were detected in the radicular cyst wall and cyst fluid. It has been suggested that these cytokines play an active role in the growth of the cyst by activating osteoclasts to bone resorption, providing keratinocyte proliferation and inducing collagenase production, secretion and activation (4, 6, 7). Viral infections are known to be associated with the secretion of IL-15, interferon alpha (IFN-α) and IFN-gamma (IFN-γ). IFNs have the pleiotropic nature of cytokines. IFN-α, IFN-β and IFN-γ possesses antiviral activity. IFN-γ is also an activator of the pathway that leads to cytotoxic T cells. However, IFN-γ is considered a proinflammatory cytokine because it augments TNF activity and induces nitric oxide (NO) (8).

IL-12 (natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2, p40) results in the local release of IFN-γ by natural killer cells (NK) and T lymphocytes. IFN-γ stimulates macrophages to phagocyte and destroy microorganisms (9). Kubota et al.(7) proposed that macrophage cell death caused by bacteria may trigger the initial IFN-γ production at an early stage of bacterial infection.

The purpose of this study is to determine the levels of IFN-γ in OC samples detected to contain bacteria using the Polymerase Chain Reaction (PCR) method and detect the incidence of infectious agents and cytokine levels.

Materials and Methods

Patients and clinical data

The data used in the present study were collected from the Department of Oral and Maxillofacial Surgery of the Faculty of Dentistry, Istanbul University. The study population consisted of systemically healthy patients who had either RCs or OKCs with jaw expansions, along with a history of infection and purulent cyst fluid (CF). A total of 39 patients with 25 OKCs and 14 RCs were enrolled in the study. Only expansive cysts were chosen because of the usefulness of obtaining CF. Computerized tomography scans were used to evaluate the expansion size of the buccal and/or lingual bony cortices. The differentiation of purulent CF was made by simple inspection, but the certain diagnosis was achieved with a histopathological report. Patients who were under antimicrobial therapy, who were receiving antiviral or immunosuppressive therapies, or had an obvious mucosal breach or portal entry for infection via the oral cavity were excluded from the study. Patients who had OCs with a diameter of less than 1 cm (calculated via an orthopantomograph) were also excluded be-

cause of the inability to obtain adequate CF. Ethical Committee permission from Local Ethical Committee of Istanbul University, Istanbul Faculty of Medicine (no: 2008/3205) was obtained prior to the commencement of the study.

Collection of samples

Preoperatively, the operation area was treated with a 0.21% chlorhexidine solution. Fluid from OCs was collected using disposable sterilized 19-gauge needles attached to syringes which are then transferred into sterilized Eppendorf tubes. During the entire surgical procedure, the risk of salivary contamination of samples was avoided using meticulous high-volume evacuation. The remaining part of the cyst was then enucleated for a histopathological examination. Regarding the final histopathological diagnosis, samples of the OKCs and RCs were separated for the investigation. All samples were immediately transferred and stored at -20°C before extraction of genomic DNA and determination of cytokine levels.

Identification of bacterial content in cyst fluid by PCR

DNA was extracted from OCs fluid using a MagNA Pure Compact DNA Isolation Kit (Roche Diagnostics GmbH, Germany) according to the manufacturer's instructions.

The PCR method was used to detect bacteria. Species-specific oligonucleotide primers were used to detect the target microbial species. A pair of bacterial primers that matched almost all bacterial 16S 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. Specific primers for *Tannerella forsythia*, *Treponema denticola, Campylobacter rectus, Porphyromonas gingivalis, Prevotella intermedia,* and *Prevotella nigrescens* were described by Ashimoto et al. (10), *Porphyromonas endodontalis, Prevotella pallens, Dialister pneumosintes, Filifactor alocis, Fusobacterium nucleatum* (11, 12) and *Enterococcus faecalis* were described by Siqueira and Rocas (11). PCR amplification and methods were performed according to the literatures mentioned above (10-12). 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 photographed under a 300 nm ultraviolet transilluminator. As size markers, either 100 bp or 1 kb DNA ladder digest (MBI Fermantas) was used. The result was considered to be positive if a band of the expected size was present.

Determination of IFN-γ levels in cyst fluid

The profiles of IFN-γ levels in CF were determined using a Human Cytokine LINCOplex kit (LINCO Research, Inc. USA) by Luminex technology, according to the manufacturer's recommendations. CF samples were centrifuged at 1,000g for five minutes, and the supernatant fractions were then analyzed. The IFN-γ levels in CF were calculated on BioPlex (Bio-Rad Laboratories, Inc.) with Bio-Plex manager Software 4.1.

Statistical evaluation

The suitability of normal distribution has been examined through Shapiro-Wilks test. Since data did not demonstrate a normal distribution, non-parametric tests; the Mann-Whitney U (MW-U) test and the Kruskal-Wallis (KW) test were used when appropriate. Data were analyzed with Kruskal-Wallis and post-hoc Mann-Whitney U tests. Statistical analysis was carried out using SPSS 21 statistical software (SPSS Inc, Chicago, IL, USA). P-values of less than 0.05 were considered to be statistically significant.

Results

Cysts

39 OC (25 OKCs, 14 RC) samples were enrolled in the study. The study group consisted of males (M, n = 28) and females (F, n = 11) whose ages ranged from 21 to 68 years (43.41 ± 12.06; M, 43.86 ± 13.35; F, 42.27 ± 8.34) (Table 1).

Table 1. Distribution of age and sex in OC (odontogenic cyst) groups (OKC, odontogenic keratocyst: RC, radicular cysts: F, female: M, male).

Age group (years)	OKC	RC	Total (OC)	
	n (F:M)	n (F:M)	n (F:M)	%
20-39	12 (3:9)	4 (0:4)	16 (3:13)	41.03
40-59	10 (4:6)	9 (4:5)	19 (8:11)	48.72
>60	3 (0:3)	1 (0:1)	4 (0:4)	10.26
Total	25 (7:18)	14 (4:10)	39 (11:28)	

Lesion sizes of the cysts ranged from 1.2 cm to 11 cm (3.28 ± 1.92). In the OKCs group (18 M, 72.0%; 7 F, 28.0%), the age ranged from 21 to 68 years (42.72 ± 12.77; M, 43.72 ± 13.85; F, 40.14 ± 9.92). OKC sizes ranged from 1.20 cm to 11.00 cm (3.46 ± 2.24). In RCs (10 M, 71.4%; 4 F, 28.6%), the age ranged from 24 to 64 years (44.64 ± 11.03; M 44.10 ±13.14; 4 F, 46.00 ± 2.45), with a male-to-female ratio of 2.5:1 in both groups RC sizes were from 1.4 cm to 5 cm (2.95 ± 1.15). No statistically significant correlation was observed in the OC type regarding the age of patients and the size of lesions (respectively p = 0.675, p = 0.828 MW-U test). From the gender aspect, in all cysts group (OC) and OKC group, the difference was statistically significant (Chi-square test p = 0.006, p = 0.028, resptivelyand male cases were determined to be dominant.

Bacterial content

After DNA isolation and purification from samples, the presence of bacteria in the CF was determined by species-specific PCR method using specific primers. In 87.18% (34/39) of the studied specimens, the presence of the bacteria was positive. The red complex *(T. forsythia, P. gingivalis, T. denticola)* was negative in all samples. *P. gingivalis* was the most frequently present bacterium compared with the other ones in all samples (n = 16, 41.03%) (Table 2).

Table 2. Distribution of bacterium species in odontogenic cyst (OCs), in odontogenic keratocyst (OKC) and in radicular cysts (RC; F, female; M, male).

Name of Bacterium Species	OKC (F:M)		RC (F:M)		Total/OC (F:M)	
	n = 25	%	n = 14	%	n = 39	%
Campylobacter rectus (Cr)	3 (2:1)	12.00	3 (1:2)	21.43	6 (3:3)	15.38
Dialister pneumocintes (Dp)	1 (0:1)	4.00	2 (1:1)	14.29	3 (1:2)	7.69
Enterococcus faecalis (Ef)	5 (1:4)	20.00	3 (1:2)	21.43	8 (1:6)	20.51
Filifactor alocis (Fa)	1 (0:1)	4.00	2 (0:2)	14.29	3 (0:3)	7.69
Fusobacterium nucleatum (Fn)	4 (2:2)	16.00	6 (1:5)	42.86	10 (3:7)	25.64
Porphyromonas endodontalis (Pe)	0	0	2 (0:2)	14.29	2 (0:2)	5.13
Porphyromonas gingivalis (Pg)	9 (2:7)	36.00	7 (1:6)	50.00	16 (3:13)	41.03
Prevotella intermedia (Pi)	6 (2:4)	24.00	3 (2:1)	21.43	9 (4:5)	23.08
Prevotella nigrescens (Pn)	4 (2:4)	16.00	4 (1:3)	28.57	8 (3:7)	20.51
Prevotella pallens (Pp)	0	0	1 (0:1)	7.14	1 (0:1)	2.56
Treponema denticola (Td)	2 (0:2)	8.00	4 (1:3)	28.57	6 (1:5)	15.38
Tannerella forsythia (Tf)	5 (0:5)	20.00	1 (1:0)	7.14	6 (1:5)	15.38

F. nucleatum (n = 10, 25.64%), *P. intermedia* (n = 9, 23.08%), *P. nigrescens* and *E. faecalis* (n = 8, 20.51%), *C. rectus, T. denticola* and *T. forsythia* (n = 6, 15.38%), *D. pneumosintes* and *F. alocis* (n = 3, 7.69%), *P. endodontalis* (n = 2, 5.13%) and *P. pallens* (n = 1, 2.56%) were present. *P. gingivalis* is the most frequent type of bacteria detected in fluids OKC and RC (9/25 and 7/14, respectively). The most frequent bacterial pair is *E. faecalis* / *P. gingivalis* (Ef^{+}/Pg^{+}, 7/39, 17.85%).

When cysts were analyzed to determine the number of bacterial species, 7 different species of bacteria were identified in one case, 5 in 2 cases, 4 in 3 cases, 3 in 4 cases, 2 in 13 cases (33.3%), and one of the bacteria in 11 cases. No presence of bacterium was detected in five cases (12.82% in total, 20.0% in OKC) of the OKC group (Figure 1).

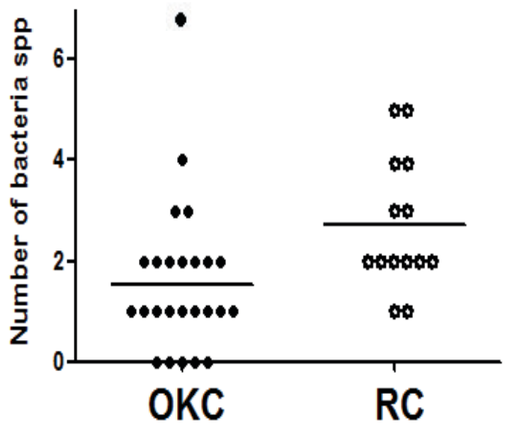

Figure 1. The distribution of the number of species of bacteria according to the cyst groups found positive: the number of species of bacteria in OKCs is 1.60 ± 1.53, 1 (0-7) [mean ± standard deviation, median (min-max)] pg/ml and RCs is 2.71 ± 1.32, 2 (1-5) pg/ml (MW-U p = 0.009).

There was no bacterium-free CF in the RC group. The mean number (± SD) of bacterial species per sample was 2.00±1.54. The number of species on the type of cyst bacteria was 1.60 ± 1.53 and 2.71 ± 1.32 in OKCs and RCs, respectively (MW-U p = 0.009). More bacteria species were observed in RCs.

IFN-γ levels

IFN-γ levels in CFs were determined by the luminex method with the Human Cytokine LINCOplex kit. Assay sensitivity (minimum detectable concentrations) was 0.86 pg/ml. The correlation (R) of curves obtained from the standards was 0.900 to 0.999. Control serums of the kit with high and low concentrations were within the expected range.

In terms of IFN-γ levels, no significant difference was detected between CF groups RC 2.57 ± 6.74 (14); 0 (0-22.64) [mean ± standart deviation (number); median (min-max)] pg/ml and OKC 0.19 ± 0.54 (25) 0; (0-2.68) pg/ml (MW-U p =0.209). There was no significant difference with respect to gender (MW-U p =0.258), age (three separate groups 20-39 and 40-59, > 60 years; KWp=0.467) and cyst size (MW-Up=0.794).

After the division of CFs in four groups according to the number of bacteria species contained (0, 1, 2, ≥3 bacteria), no significant difference between these groups in terms of IFN-γ levels was detected (KWp = 0.495) (Table 3).

Table 3. Cyst fluid IFN-γ levels [mean ± standard deviation, median (min-max) pg/ml] grouped according to the number of bacteria species.

Number of bacteria species	n	IFN-γ (pg/ml)
Bacterium-free	5	0.14 ± 0.19, 0 (0-0.35)
1 bacterium	11	0.27 ± 0.80, 0 (0-2.68)
2 bacteria	13	1.11 ± 3.59, 0 (0-13.06)
≥3 bacteria	10	2.26 ± 7.16, 0 (0-22.64)
K-W p		0.495

No significant difference in IFN-γ levels was observed when the individual presence or absence of Pg or Fn, the most commonly identified bacterial species (p= 0.682 and p= 0.112, respectively) were considered. Furtermore, when the presence and/or absence of Pg and Fn in CF considered collectively [Fn-Pg-(n=16), Fn-Pg+ (n=13) and Fn+Pg-or+ (n=10], no difference was also detected (KW p = 0.103).

Pg was determined to form infections mostly in association with Ef (Ef+Pg+, n = 7). The levels of IFN-γ in the groups with Ef+Pg+, Ef-Pg+ (n=9) and Ef-Pg- (n=22) were compared and there was no significant difference (KWp=0.540).

Discussion

RCs represent an inflammatory response to a chronic irritation, whereas OKCs are mostly noninflammatory lesions (2). Generally, the odontogenic cyst fluids contain no microorganisms, but might get infected due to the secondary infection. It is found that aerob microorganisms were present commonly as a cause of the infection. However, anaerob microorganisms may also be seen. Previous microbiological studies on odontogenic cysts evaluated the bacteriological spectrum or cytokines individually (4, 13-17), while in our investigation these factors have been investigated simultaneously. It is reported that major part of the bacterial strains in the odontogenic cysts are anaerobes (3, 13, 17). Regarding RCs' and OKCs' anaerobic bacteria content, no difference was found in a study conducted in the infected odontogenic cyst fluids (3, 17). In a study involving three radicular cyts, growth of peptostreptococcus and fusobacterium spp. was observed in three (100%) and one (33.33%) of the specimens under anaerobic conditions, respectively, along with the growth of aerobic bacteria (18).

Kolokythas et al. (19) investigated 16 cytokines including IFN-γ in cystic aspirate from keratocystic odontogenic tumor, ameloblastoma, and dentigerous cysts by a quantitative multiplex ELISA. Their findings showed that specifically the combination of some cytokines might be useful as a diagnostic aid but not IFN-γ.

In another study downregulation of the inflammatory response due to the IL-2 and IFN-γ (T helper type 1 cytokines) and the predominant humoral immune response due to the IL-4 and IL-5 (T helper type 2 cytokines) in periapical periodontitis were presented. The researchers demonstrated using immunochemistry that the cells in radicular cysts and the periapical granulomas that expressed IL-4 or IL-6 were far more numerous than cells that expressed either IL-2 or IFN-γ (20). Flow cytometry examination of cell suspensions obtained from tissue samples of radicular cysts and periapical lesions showed a higher number of IL-4 producing cells among radicular cyst samples (21). These findings support the notion that T helper type 2 cells and cytokines are responsible for the etiopathology of the cysts.

In an in vivo experimental study, the mandibular first molars of mice were subjected to pulpal exposure and infection with a mixture of four anaerobic pathogens (Pi, Fn, Streptococcus intermedius and Peptostreptococcus micros) and no significant differences were detected in the levels of IFN-γ (22). In another in vivo study, T helper type cytokine 1 (IL-1 and IFN-γ) response has been proposed to exacerbate the bone resorption due to Pg. (23). Whereas, in an in vitro study, low IL-12 p70 levels produced by macrophages infected with bacteria (*Listeria monocytogenes* and *Staphylococcus aureus*) seemed to increase macrophage death through IFN-γ produced by innate lymphocytes along with the "danger signal" observed at the early phase of infection, rather than initiating IFN-γ production by NK cells (24).

In the present study the presence of bacteria in odontogenic and radicular cyst was investigated using species-specific PCR primers. The number of bacterial species per sample was similar in the findings of Scalas (2.06±0.93) (17). The most common bacteria in both types of cystic fluid was Pg (16/39) [7/25 (OKC), 9/14 (RC)], and it created infection generally in association with Ef (7 in cysts, 4 in keratocysts). In odontogenic cysts, Fn (10/39) is the most frequent bac-

terium determined following Pg. Frequency of Fn in RC (42.86%) observed herein is also similar to published data (18). We can suggest that these two anaerobic bacteria (Pg and Fn) may have a role in the pathogenesis of cyst formation. Luminex method showed low concentrations of IFN-γ, which did not significantly differ according to the number or type of bacteria detected by PCR. This finding is supported by the data of Hou et al. (22). In contrast to the findings of Stashenko et al., we did not observe any increase in the level of IFN-γ in Pg infected cysts (23). We believe that this may be associated with the gradual diminishing of the T helper cytokine type 1 response or with the transformation of chronic inflammation into cyst formation due to the absence of a response.

Conclusion

This study is important because of the determination of the types of bacteria causing infections in odontogenic keratocysts and radicular cysts. It reveals levels of IFN-γ secretion as local immune response to infection. Luminex method can detect the concentration of many different types of protein in a small sample volume and is suitable for determining the protein content of odontogenic cysts. Further studies are required to clarify the role of IFN-γ in the pathogenesis of the OCs. The relatively small number of the samples and bacteria groups can be considered as a limitation of the present study.

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REFERENCES

1. Slootweg PJ. Lesions of the jaws. Histopathology 2009;54(4):401-18.
2. Andric M, Milasin J, Jovanovic T, Todorovic L. Human cytomegalovirus is present in odontogenic cysts. Oral Microbiol Immunol 2007;22(5):347-51.
3. Keskin F, Ciftci S, Cakarer S, Selvi F, Can T, Ozel S, Keskin C. Detection of the anaerobic bacteria in the odontogenic cyst fluids using polymerase chain reaction (PCR) method. Afr J Microbiol Res 2011;5:1999-2002.
4. Muglali M, Komerik N, Bulut E, Yarim GF, Celebi N, Sumer M. Cytokine and chemokine levels in radicular and residual cyst fluids. J Oral Pathol Med 2008;37(3):185-9.
5. Alhashimi N, Frithiof L, Brudvik P, Bakhiet M. Orthodontic tooth movement and de novo synthesis of proinflammatory cytokines. Am J Orthod Dentofacial Orthop 2001;119(3):307-12.
6. Meghji S, Qureshi W, Henderson B, Harris M. The role of endotoxin and cytokines in the pathogenesis of odontogenic cysts. Arch Oral Biol 1996;41(6):523-31.
7. Kubota Y, Ninomiya T, Oka S, Takenoshita Y, Shirasuna K. Interleukin-1alpha-dependent regulation of matrix metalloproteinase-9(MMP-9) secretion and activation in the epithelial cells of odontogenic jaw cysts. J Dent Res 2000;79(6):1423-30.
8. Dinarello CA. Proinflammatory cytokines. Chest 2000;118(2):503-8.
9. Abbas AK, Lichtman AH, Pillai S. Cellular and molecular immunology. 7th ed., Philadelphia: Saunders, 2012, p.225-42.
10. Ashimoto A, Chen C, Bakker I, Slots J. Polymerase chain reaction detection of 8 putative periodontal pathogens in sub-

gingival plaque of gingivitis and advanced periodontitis lesions. Oral Microbiol Immunol 1996;11(4):266-73.
11. Siqueira JF Jr, Rocas IN. Polymerase chain reaction-based analysis of microorganisms associated with failed endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;97(1):85-94.
12. Siqueira JF Jr, Rocas IN. Uncultivated phylotypes and newly named species associated with primary and persistent endodontic infections. J Clin Microbiol 2005;43(7):3314-9.
13. Iatrou IA, Legakis N, Ioannidou E, Patrikiou A. Anaerobic bacteria in jaw cysts. Br J Oral Maxillofac Surg 1988;26(1):62-9.
14. Rudelt HG. [Bacterial spectrum of the infected cyst]. Dtsch Zahnarztl Z 1985;40(6):590-1.
15. 15. Yamaura M, Sato T, Echigo S, Takahashi N. Quantification and detection of bacteria from postoperative maxillary cyst by polymerase chain reaction. Oral Microbiol Immunol 2005;20(6):333-8.
16. Browne RM. Some observations on the fluids of odontogenic cysts. J Oral Pathol 1976;5(2):74-87.
17. Scalas D, Roana J, Boffano P, Mandras N, Gallesio C, Amasio M, Banche G, Allizond V, Cuffini AM. Bacteriological findings in radicular cyst and keratocystic odontogenic tumour fluids from asymptomatic patients. Arch Oral Biol 2013;58(11):1578-83.
18. Ilıcalı A, İnanç D, Öner B, Balkanlı O. Microorganisms isolated from dental infections [Odontojen enfeksiyonlardan izole edilen mikroorganizmalar]. İstanbul Üniv Diş Hek Fak Derg 1989; 23(4):190-4.
19. Kolokythas A, Karas M, Sarna T, Flick W, Miloro M. Does cytokine profiling of aspirate from jaw cysts and tumors have a role in diagnosis? J Oral Maxillofac Surg 2012;70(5):1070-80.
20. Walker KF, Lappin DF, Takahashi K, Hope J, Macdonald DG, Kinane DF. Cytokine expression in periapical granulation tissue as assessed by immunohistochemistry. Eur J Oral Sci 2000;108(3):195-201.
21. Ihan Hren N, Ihan A. T lymphocyte activation and cytokine expression in periapical granulomas and radicular cysts. Arch Oral Biol 2009;54(2):156-61.
22. Hou L, Sasaki H, Stashenko P. Toll-like receptor 4-deficient mice have reduced bon destruction following mixed anaerobic infection. Infect Immun 2000;68(8):4681-7.
23. Stashenko P, Goncalves RB, Lipkin B, Ficarelli A, Sasaki H, Campos-Neto A. Th1 immune response promotes severe bone resorption caused by Porphyromonas gingivalis. Am J Pathol 2007;170(1):203-13.
24. Kubota K. Innate IFN-gamma production by subsets of natural killer cells, natural killer T cells and gammadelta T cells in response to dying bacterial-infected macrophages. Scand J Immunol 2010;71(3):199-209.

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