African Journal of Microbiology Research Vol. 5(14), pp. 1999-2002, 18 July, 2011
Available online http://www.academicjournals.org/ajmr
DOI: 10.5897/AJMR11.550
ISSN 1996-0808 ©2011 Academic Journals

Full Length Research Paper

Detection of the anaerobic bacteria in the odontogenic cyst fluids using polymerase chain reaction (PCR) method

Fahriye Keskin[1], Sevgi Ciftci[1], Sirmahan Cakarer[2], Firat Selvi[2], Taylan Can[2], Sevda Ozel[3] and Cengizhan Keskin[2]

[1]Department of Microbiology, Dentistry Faculty, Istanbul University, Istanbul, Turkey.
[2]Department of Oral and Maxillofacial Surgery, Dentistry Faculty, Istanbul University, Istanbul, Turkey.
[3]Department of Biostatistics and Medical Informatics, Medical Faculty, Istanbul University, Istanbul, Turkey.

Accepted 15 July, 2011

Odontogenic cysts are slow growing lesions which are formed by epithelium. They may reach to a substantial size without symptoms for a long time. Radicular cysts' (RCs) and odontogenic keratocysts' (OKCs) are common odontogenic cysts of jaws. The main purpose of this study is to evaluate if anaerobic bacteria play a role in the pathogenesis of the RCs and OKCs fluids by polymerase chain reaction (PCR). Odontogenic cyst fluid samples with a history of infection were collected from a total of 28 odontogenic cysts consisting of 16 samples of OKCs and 12 samples of RCs. Anaerobic bacteria detection were performed by PCR based on bacterial 16S rRNA genes. *Porphyromonas gingivalis* existed more frequently compared to the other bacteria, in all samples (39.2%). Following this, *F. nucleatum* (32.1%), *Provetella intermedia* and *Campylobacter rectus (* 25.5%), *Treponema denticola* (25%), *Provetella nigrescens* and *Tannerella forsythia* (17.8%), *Dialister pneumosintes (*14.2%), *Filifactor alocis* (10.7%), *Porphyromonas endodontalis* and *Provetella pallens* (7.1%) were seen. The 58.3% of the *Fusobacterium nucleatum* positive cyst fluids were in the RCs group. In *D. pneumosintes* positive cysts liquid samples, *C. rectus* was found to be positive (p=0.025). The same correlation was observed between *F. alocis* and *C. rectus* (p= 0.003). On the other hand, in *F. alocis* positive cysts liquid samples, *F. nucleatum* also was found to be positive (p=0.026). Odontogenic cysts fluid contained numerous anaerobic bacteria of various types, thus suggesting that oral bacteria may cause symptoms in odontogenic cyst fluids. Further studies are needed to assess the role of these bacteria in the pathogenesis of odontogenic cysts.

Key words: Polymerase chain reaction (PCR), odontogenic cyst, anaerobic bacteria.

INTRODUCTION

Odontogenic cysts are the most commonly seen cystic lesions that affect the maxillofacial region. In the usual etiology of the radicular cysts (RCs), a tooth is infected which then leads to the necrosis of the pulp (Koseoglu et al., 2004; Shear, 1992). Odontogenic keratocysts (OKCs) are common, clinically aggressive lesions that are thought to arise from the dental lamina or its remnants. These types of cysts grow more rapidly than radicular cysts. The inflammatory stimuli which are found in the RCs, do not exist in the keratocysts (Regezi, 2002). Bacteria may act in synergy to produce pathology and it is well documented that fluids of the odontogenic cysts are associated with these (Sunde et al., 2008).

Various studies investigated the bacterial role in odontogenic cysts. On the other hand, the studies which evaluate the odontogenic cysts fluids are limited (Iatrou et al., 1998; Rudelt, 1985). The present study was to determine the anaerobic bacteria of the cyst fluids and to examine the presence of 11 anaerobe oral bacteria by PCR based on bacterial 16S rRNA genes.

These species have frequently been isolated as putative pathogens from various oral diseases such as endodontic and periodontitis infections (Ashimoto et al.,

*Corresponding author. E-mail: k_fahriye@yahoo.com or Fahriye.keskin@gmail.com. Tel: +90 5322059794.

1996; Siqueira et al., 2004; 2005).

MATERIALS AND METHODS

Patients and clinical data

The data, used in the present study, were collected at the Department of Oral and Maxillofacial Surgery of Dentistry Faculty, in Istanbul University. The study population consisted of systemically healthy patients having either RCs or OKCs with jaw expansions, along with a history of infection and purulent cyst fluid. A total of 28 patients, with 16 OKCs and 12 RCs, were enrolled in the study. Only the expansive cysts were chosen because of the easiness in obtaining the cyst fluids. CT scans were used to evaluate the expansion size of the buccal and/or lingual bony corteces. The differentiation of purulent cyst fluid was made by simple inspection but the certain diagnosis was achieved by the histopathological report. Only the cysts which have been reported as ‘‘infected”, were included in the study. The patients, who were under antimicrobial therapy, who were receiving antiviral or immunosuppressive therapies, and the patients with an obvious mucosal breach or portal entry for infection via the oral cavity were excluded from the study. The patients who had odontogenic cysts with a diameter of less than 1 cm in size (calculated via the orthopantomographs) were also excluded from the study because of the inability to obtain adequate cyst fluid. The Ethical Commitee permission was obtained prior to the commencement of the study.

Collection of samples

Preoperatively, the operation area was treated with 0.21% chlorhexidine solution. Odontogenic cyst’s fluid was collected using a disposible sterilized 19-gauged needles attached to a syringes band poured into sterilized eppendorf tubes. During the entire surgical procedure, the risk of salivary contamination of the samples was avoided using meticulous high volume evacuation. The remaining part of the cyst was then enucleated for the histopathological examination. Regarding the final histopathological diagnosis, the samples of the OKCs and RCs were separated for the investigation. All samples were immediately transferred and stored at -20°C before the extraction of genomic DNA.

DNA extraction

DNA was extracted from odontogenic cyst fluids using a MagNA Pure Compact DNA Isolation Kit (Roche Diagnostics GmbH, Germany) according to the manufacturer’s instructions. PCR was used to detect bacteria.

PCR identification

Species-specific oligonucleotide primers were used to detect the target microbial species. A pair of bacterial primers that match almost all bacterial 16S rRNA genes at the same position, except the 18S rRNA gene from the eukaryotic cells, was used as a positive control for the PCR reaction. It 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 (Ashimoto et al., 1996), *Porphyromonas endodontalis (P. endodontalis), Provetella pallens (P. pallens), Dialister pneumosintes (D. Pneumosintes), Filifactor alocis (F. alocis), Fusobacterium nucleatum (F. nucleatum)* were described by (Siqueira et al., 2004; 2005). PCR amplification and methods were performed according to the literatures mentioned above (Ashimoto et al., 1996; Siqueira et al., 2004; 2005). PCR products were analyzed by 1.5% agarose gel electrophoresis performed at 4 V/cm in Tris-acetate EDTA buffer. The gel was stained with 0.5 µl/ml ethidium bromide and photographed under 300-nm ultraviolet transilluminator. As size markers, either 100 bp or 1 kb DNA ledder digest (MBI Fermantas) was used. The result was considered to be positive if a band of the expected size was present.

Statistical evaluation

Statistical analysis was carried out using Chi-squared test, Chi-squared yates, Fisher’s exact test and (SPSS 17.0 statistical software; SPSS Inc, Chicago, IL, USA); *P*-values of less than 0.05 were considered to be statistically significant.

RESULTS

Out of 33 enucleated lesions, 28 samples were enrolled in the study. 16 of the samples were OKCs, 12 of them were RC. Four of excluded five samples were dentigerous cysts and remaining one was glandular odontogenic cyst. The study group consisted of males and females whose ages ranged from 21 to 68 years. In keratocysts, the age ranged from 21 to 64 years. The maximum number of cases were in the age group of 30 to 39 years (five cases). In RCs the age ranged from 30 to 38 yars. The maximum number of cases were in the age group of 30 to 39 and 40 to 49 years (four cases) (Table 1). Out of 16 cases of keratocysts, 12 (75%) were male and 4 (25%) were female, with a male to female ratio of 3:1. Out of 12 cases of r9 RCs (75%) were male and 3 (25%) female, with a male to female ratio of 3:1 (Table1).

Lesion size ranged from OKCs 1 to 11 cm (4.0±2.6) and RCs 1,5 to 6,5 cm (3.2±1.4). There was no statistically significant correlation between the gender and the cyst size. (P>0,5). Also no statistically significant correlation was observed in the odontogenic cysts regarding the range of age. (p>0.05). 16 of the 28 cysts, were in the mandible and 12 were in the maxilla. The majority of the OKCs were in the mandible (12/16) the majority of the RCs were in the maxilla (8/12). The majority of the both lesions were seen in males (75%).

In 92.8% 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 more frequently present compared to the other bacteria, in all samples (39.2%). Following this, *F. nucleatum* (32.1%), *P. intermedia* and *C. rectus* (25.5 %), *T. denticola* (25%), *P. nigrescens* and *T. forsythia* (17.8 %), *D. pneumosintes (*14.2%), *F. alocis* (10.7%), *P. endodontalis* and *P. pallens* (7.1%) were present. The 58.3% of the *F. nucleatum* positive cyst fluids were in the RCs group. The remaining material (12.5%) were in OKCs. The difference was found to be statistically

Table 1. Distribition of age and sex in odontogenic cysts.

Age group (Years)	Keratocyst		Radicular cyst	
	Female(n)	Male(n)	Female(n)	Male(n)
20-29	1	1	0	1
30-39	1	4	0	4
40-49	1	0	3	1
50-59	1	3	0	3
60-69	0	4	0	0
Total	4	12	3	9

Table 2. Distribution of anaerobic bacteria in odontogenic cysts.

Bacteria	OKCs	RCs	Total	X^2	P
C. rectus	3	5	8	X^2_y=0.82$^+$	0.365
D. pneumosintes	2	2	4	Fisher^{++}	1.000
T. forysthia	4	1	5	Fisher	0.355
T. denticola	2	3	5	Fisher	0.624
F. nucleatum	3	6	9	X^2_y=1.81	0.179
P. gingivalis	7	4	11	X^2_y=0.03	0.867
P. nigrescens	2	4	6	Fisher	0.354
P. intermedia	5	3	8	X^2_y=0	1.000
P. pallens	1	1	2	Fisher	1.000
P. endotontalis	0	2	2	Fisher	0.175
F. alocis	1	2	3	Fisher	0.560

$^+$: X^2yates, $^{++}$: Fisher.

significant (p=0.031). In the *D. pneumosintes* positive cysts liquids, *C. rectus* was found to be positive. This difference was statistically significant (p=0.003). The same correlation was observed in between *F. alocis* and *C.rectus* (p= 0.003). On the other hand, in the *F. alocis* positive cysts liquids, *F. nucleatum* also was found to be positive (p=0.026) (Table 2).

DISCUSSION

Odontogenic cysts are common lesions of the jaws. Among them, OKCs and RCs draw special attention because RCs have a very high occurrence rate whereas OKCs have special biological features. Essentially, RCs occur as an inflammatory response to a chronic irritation resulting from the necrotic pulp of a tooth. Furthermore, proinflammatory cytokines and inflammation-induced expression of some growth factors are crucial for the development of RCs. On the other hand, OKCs are non-inflammatory lesions, and genetic factors are thought to play a major role in their etiology. However, secondary inflammation on their walls is very frequent which results in some changes of the characteristic histopathological features of OKCs towards some non-specific findings, similar to those of RCs (Andric et al., 2007).

Generally, the odontonic cyst fluid are sterile but the presence of microorganisms are shown due to the secondary infection. It has been reported that aerob microorganisms were present commonly as a cause of the infection but anaerob microorganisms also are present. Many studies investigated the odontogenic cysts but few of them evaluated the bacterial spectrum (Iatrou et al., 1988; Rudelt, 1985; Yamamura et al., 2005; Browne, 1976). Iatrou et al. (1988) reported that, 89.2% of the bacterial strains were anaerobes in the odontogenic cysts.

Rudelt (1985) investigated the bacterial flora of the infected fluid content of 40 cysts of the jaws and they reported that 77.4% of the bacterial strains were gram positive streptococci. The remaining (22.6%) was reported as anaerobic bacteria. Browne (1976) evaluated 99 cyst fluids and reported that, gram positive bacteria were present in 22.9% of dental cysts, 33.3% of dentigerous cysts and 54.2% of keratocysts. The difference between the different types of odontogenic cysts has been reported to be statistically significant regarding the presence of bacteria. Yamamura et al. (2005) had studied 10 post operative maxillary cyst (POMC) fluids regarding the bacterial density by real time PCR and low (<50%) bacterial detection frequency has been reported in POMC fluids. *P. acnes* has been

reported as the most commonly detected bacteria.

In the present study, positive bacteria were determined in 92.8% of the studied specimens. This is also consistent with the findings of Iatrou et al. (1988) and Rudelt et al. (1985), who had evaluated infected odontogenic cyst fluids. The fact that the source of our samples, being a rather ‘‘closed’’ cavity with a lack of oxygen inside, could give an explanation for this result, as suggested also by Iatrou et al (Iatrou et al., 1988). In addition, it is well documented that the higher percentages of anaerobic cultures were observed in the microflora of orofacial odontogenic infections (Gill and Scully, 1990). In the present study, RCs fluids showed more positive bacteria content than the OKCs fluids. *P. gingivalis* and *F. nucleatum* were more commonly present compared to the other bacteria, in all samples. The 77.8% of the *F. nucleatum* positive cyst fluids were in the RCs group. The second most frequently bacteria were found to be *T. denticola.* and *C. rectus*. Our findings demonstrate that the bacterial content of the RCs are similar with the bacterial content in the endodontic and periodontal infections (Fujii et al., 2009; Ruviére et al., 2007; Chávez de Paz Villanueva, 2002).

The red complex was investigated by Roças et al, in root canal infections. The red complex was found in 4 of 50 cases of necrotic pulps with periradicular pathosis (Rôças et al., 2001). In the authors’ knowledge, there is no documented data about the presence of red complex in odontogenic cysts investigated by PCR method. In the present study, red complex was found to be negative in all samples.

Another interesting finding of this study is that*, C. rectus* was found to be positive in *D. pneumosintes* positive OKCs fluids. This positive correla-tion was also reported by Siquera et al (Siqueira and Rôças, 2003) in primary endodontic infections. The positive correlation in between *F. alocis* with *C. Rectus* and *F. Alocis* with *F. Nucleatum* was shown also by Schlafer et al. (2010) and Siqueira and Rôças (2009a). These findings of bacterial interactions underscore the complex ecological interrelationships that occur among bacteria involved in endodontic infections. The molecular determinants as well as the ecological and pathogenic implications of these various associations remain to be delineated (Siqueira and Rôças, 2009b).

In conclusion, this study demonstrated the presence the anaerobic bacteria in the infected odontogenic cyst fluids by PCR method. No difference was found regarding the presence of these anaerobic bacteria in between RCs and OKCs. Further studies are required to clarify the role of these bacteria, in the pathogenesis of the odontogenic cysts. The relatively small number of the patients can be considered as a limitation of the present study.

REFERENCES

Andric M, Milasin J, Jovanovic T, Todorovic L (2007). Human cytomegalovirus is present in odontogenic cysts. Oral Microbiol Immunol., Oral. Microbiol. Immunol., 22(5): 347-351.

Ashimoto A, Chen C, Bakker I, Slots J (1996). Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lessions). Oral. Microbiol. Immunol., 11: 266-273.

Browne RM (1976). Some observations on the fluids of odontogenic cysts. J. Oral Pathol., 4: 31-36.

Chávez de Paz Villanueva LE (2002). Fusobacterium nucleatum in endodontic flare-ups. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod., 93: 179-183.

Fujii R, Saito Y, Tokura Y, Nakagawa KI, Okuda K, Ishihara K (2009). Characterization of bacterial flora in persistent apical periodontitis lesions. Oral. Microbiol. Immunol., 24: 502-505.

Gill Y, Scully C (1990). Orofacial odontogenic infections: review of microbiology and current treatment. Oral Surg. Oral Med. Oral Pathol., 70: 155-158.

Iatrou IA, Legakis N, Ioannidou E, Patrikiou A (1988). Anaerobic bacteria in jaw cysts. Br. J. Oral Maxillofac Surg., 26: 62-69.

Koseoglu BG, Atalay B, Erdem MA (2004). Odontogenic cysts: a clinical study of 90 cases J. Oral Sci., 46: 253-257.

Regezi JA (2002). Odontogenic Cysts, Odontogenic Tumors, Fibroosseous, and Giant Cell Lesions of the Jaws. Mod. Pathol., 15: 331–341.

Rôças IN, Siqueira JF, Santos KR, Coelho, AM (2001). "Red complex" (*Bacteroides forsythus, Porphyromonas gingivalis*, and *Treponema denticola*) in endodontic infections: a molecular approach. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod., 91: 468-471.

Rudelt HG (1985). Bacterial spectrum of the infected cyst. Dtsch. Zahnarztl Z., 40(6): 590-591.

Ruviére DB, Leonardo MR, da Silva LA, Ito IY, Nelson-Filho P (2007). Assessment of the microbiota in root canals of human primary teeth by checkerboard DNA-DNA hybridization. J. Dent. Child. (Chic)., 74: 118-123.

Schlafer S, Riep B, Griffen AL, Petrich A, Hübner J, Berning M, Friedmann A, Göbel UB, Moter A (2010). Filifactor alocis--involvement in periodontal biofilms. BMC. Microbiol., 1: 10:66.

Shear M (1992). Cysts of the oral lesions. 3 rd ed. Boston, Wright, pp. 136-170.

Siqueira JF, Rôças IN (2003). Positive and negative bacterial associations involving Dialister pneumosintes in primary endodontic infections. J. Endod., 29: 438-441.

Siqueira JF, Roças IN (2004). Polymerase chain reaction-based analysis of microorganisms associated with failed endodontic treatment. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod., 97: 85-94.

Siqueira JF Jr, Rôças IN (2005). Uncultivated phylotypes and newly named species associated with primary and persistent endodontic infections. J. Clin. Microbiol., 43: 3314-3319.

Siqueira JF Jr, Rôças IN (2009a). Diversity of endodontic microbiota revisited. J. Dent. Res., 88(11): 969-981.

Siqueira JF Jr, Rôças IN (2009b). The microbiota of acute apical abscesses. J. Dent. Res., 88(1): 61-65.

Sunde PT, Olsen I, Enersen M, Beiske K, Grinde B (2008). Human cytomegalovirus and Epstein-Barr virus in apical and marginal periodontitis: a role in pathology? J. Med. Virol., 80: 1007-1011.

Yamaura M, Sato T, Echigo S, Takahashi N (2005). Quantification and detection of bacteria from postoperative maxillary cyst by polymerase chain reaction. Oral. Microbiol. Immunol., 20(6): 333-8. Erratum in: Oral Microbiol. Immunol., 2006: 21: 136.