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BACTERIALAND MOLECULAR CHARACTERIZATION OF BACTERIA ASSOCIATED WITH INFECTED ROOT CANALS

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Abstract

The infections of root canal are assorted and specific infections with a popular of anaerobic bacteria. The study was examine the presence of *P. intermedia* in 1st and 2nd endodontic visits by traditional culture and (PCR) method. A total of 48 patients aged 33 to 56 years old were convoluted in this work. About 26 and 22 of them were in 1st and 2nd visit of endodontic treatment respectively. Then detection of *P. intermedia* in infected root canals of both groups were studied using selective media and methods of polymerase chain reaction. The forty eight patients existing infected root canal were included in this study. The result were shown about 5 of 26 (19.2) and 3 of 22 (13.6) were isolated by culture in 1st fist and 2nd visit of endodontic treatment respectively, while the detection by PCR showed about 11 of 26 (42.3) and 7 of 22 (31.9) were detected in 1st fist and 2nd visit of endodontic treatment respectively. On the other hand, the methods of PCR and growth detected this species, where *P. intermedia* was only 8/ 48 (4.2) % positive culture from total infected root canals. While PCR method were identified the target species in 16/48 (33.4%). The results confirm that PCR method is very sensitive, specific and time consume technique for detecting *P. intermedia* DNA in infected root canals than culture method.

Key words : *P. intermedia*, endodontic treatment, infected root canals.

Introduction

The infections of root canal (RCI) is specific assorted and infection with a popular of anaerobic bacteria. The canal of root bacteria is facultative, gram negative, specifically the pigmented black bacterium has isolated from canals with infecting, which have role in the virulence and pathogenesis of infection of endodontic (Tomazinho and Avila-Campos, 2007). "Mostly *P. intermedia* s is a gram-negative, anaerobic, non-motile, asaccharolytic and black pigmented bacilli that form greenish-black colonies in blood agar plates". Therefore, their growth ability in the endodontic microenvironment was important to define their presence and their role in endodontic infections (Yoshino, 2007). "Black-pigmented anaerobic rods such as *Prevotella* spp. and *Porphyromonas* spp. are involved in the etiology and perpetuation of endodontic infections". Moreover, it is known that these organisms represent important pathogens in destructive periodontal disease and have been recovered from several other infectious processes, such as in acute endodontic infections (Ke *et al*, 1999; Jung *et al*, 2000). The growth culture of bacteria are only able to reveal living bacteria and this is decrease sensitive to identification of low number of microbial cell, whereas the assay of PCR represent the newest method and most delicate and well assay applied to the research of endodontic organism (Fouad *et al*, 2002; Gomes *et al*, 2005; Yang *et al*, 2013). Identification of bacteria has involved culturing on solid culture media with biochemical growth methods, but those procedures are laborious, expensive and time-consuming". In the last decade, genetic approaches have been used to recognize the bacteria in medical models, which have been shown to be prompt, delicate, accurate and allowing the discovery of fastidious organisms that are impossible to culture at this time (Ahmed *et al*, 2012). Eventhough, Foschi *et al*. (2005) using molecular method to identify a variety of microorganisms, including periodontal or endodontic pathogens. So the current study aimed to appraise the

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prevalence of this bacterium in the infected root canals by the PCR techniques and growth culture.

Materials and Methods

Forty eight patient were taken in this research, awarding at the Unit of Operative Department in College of Dentistry, Babylon University. All patients had good wellbeing and had no medical diseases. Those had received antibiotics before the canal of root therapy was excluded from this work. The ages of Patients ranged from 933 to 560 years old.

Sample collection

Root canal samples for bacteriological and genetic Study taken by paper point size 40mm were inserted in infected root canal for 2mint and placed in tube contain 0.5 ml of normal saline (Al-Rawi, 2012).

Bacterial detection by culturing

Root canal sample were transfer to laboratory for isolation and detection of studied bacteria by routine culture method which was done by using selective media and anaerobic condition, all gram negative anaerobic bacilli inoculated on blood agar give black-pigmented colonies. All bacteria gave catalase negative, indol positive results, vancomycin sensitive and inability to ferment any sugars. Then cultivation on enriched selective media that used for the isolation and possible documentation of *P. intermedia* (Al-Khafagee *et al*, 2013; NCCLS, 2004).

Bacterial detection by PCR

Root canal samples for genetic study used for species specific molecular technique after DNA extraction from samples and its concentration and transparency were determined, the primer specific to P. intermedia have sequences of forward and revers primer as shown in table 1 under following amplification conditions at 256 bp: 35 cycles: 94-C 30 s; 59-C 45 s; 73-C 20s, the product of PCR was analyzed with one present of gel agarose of electrophoresis. The ethidium bromide were using to staining the gel of agarose, moreover the image of the gel were capturing by gel document. A sample which yielded the amplicon of predicted of 256 bp was predicted to pigmented bacterium (*P. intermedia*) this molecular study was done according to Fouad *et al*. (2002) and Foschi *et al*. (2005).

Results

Among 48 patients existing infected root canal were included in the study. Patient ages ranged from 33 to 56 years.

Culture and PCR methods detected the test species, *P.intermedia* was only 8 (4.2%) positive culture from 48 infected root canals. PCR detection identified the target species in 16 (33.4%). And the positive results give 256 pb as shown in figure 1, also results of the present study shown about 5 of 26 (19.2) and 3 of 22 (13.6) were isolated in 1st fist and 2nd visit of endodontic treatment respectively, while the detection by PCR showed about 11 of 26 (42.3) and 7 of 22 (31.9) were detected in 1st fist and 2nd visit of endodontic treatment respectively (table 2). So PCR method was found more subtle than manual method in detection of *P. intermedia*. On the other hand the isolation of *P. intermedia* through culture was very problematic and consuming process.

Discussion

"Black-pigmented rods such as *P. intermedia* are involved in the dissemination of endodontic infections. It was verified because it was reported as major causative agent in the infected root canals. In the existing study the presence of *P. intermedia* in the infected root canals was detected by traditional and PCR approaches. The results were shown about 5 of 26 (19.2) and 3 of 22 (13.6) were isolated in 1st fist and 2nd visit of endodontic treatment respectively, while about 11 of 26 (42.3) and 7 of 22 (31.9) were detected in by PCR in 1st fist and 2nd visit of endodontic treatment respectively (table 2). So the detection of *P. intermedia* during endodontic procedure lead to the success of endodontic treatment which depends on several factors, the most important of which is the reduction or elimination of bacterial infection (Siqueira and Rocas, 2006).

The current study come to an agreement with Tomazinho and Avila- Campos (2007) were evaluate the prevalence of black pigmented species in chronic endodontic infections by taking 100 samples detected by culture and PCR techniques who are concluded that *P. intermedia* and *P. gingivalis* are common organisms isolated from endodontic infection. "Furthermore, PCR technique was more sensitive than culture for detecting anaerobic organisms. Therefore, it is important for dentist to define this pathogen and its growth in the endodontic environment So, these previous methods that used to detect bacterial species in root canal infections (Nakajo *et al.*, 2004; Jung *et al*., 2000).

Bacterial culture identifies the predominant species and has played a key role in the association of specific bacteria of endodontic infections (Hancock *et al.*, 2001). Some researchers, Pinheiro ((2003) and Gomes *et al*. (2005) revealed that molecular techniques, particularly PCR have been used to detect bacteria in endodontic infections. It can detect uncultivable or difficult to culture bacteria, although cultures have been widely used to

Table 1 : PCR primer, with predictable amplicon pb used for detection *P. intermedia*.

Bacteria	Primers	bp	Reference
P. intermedia	F-GTGGACCAAAGATTCATCGGT R- TTACTCCCCAACAAAAGCA	256bp	Fouad *et al*. (2002)

Table 2 : Detection of *P.intermedia* by culture and PCR methods.

Endodontic visit	Types of detections	
	By PCR No. (%)	By culture No. (%)
1st endodontic visit	26 11 (42.3)	26 5 (19.2)
2nd endodontic visit	22 7 (31.9)	22 3 (13.6)

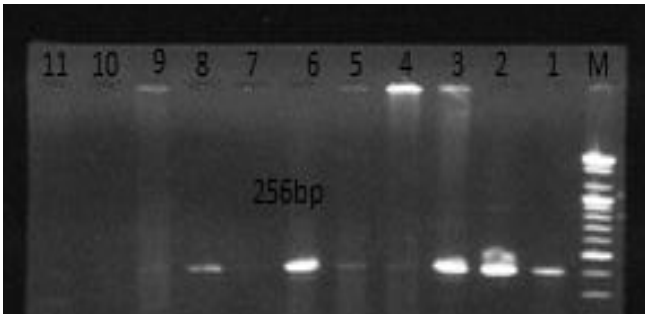

Fig. 1 : Gel electrophoresis of PCR product of *P. intermedia* specific primer. Lane of isolates numbered (1, 2, 3, 5, 6, 8) were positive. M represented 1500bp leader.

detect bacteria in endodontic infections.

Fouad *et al*. (2002) reported a similar occurrence (18% and 8%, respectively) of *P. intermedia* using molecular techniques. It was also found it higher by PCR than culture methods. And Matsuo *et al.* (2003) and Cavrini *et al.* (2005) were found that PCR method more sensitive than culture method. However, the difference was not statistically significant. There may be two explanations for this result. First; most of the previous reports were performed in secondary infection (Yang *et al*., 2013). The failure of the culture method in those studies should be because of the difficulty during sampling procedure (Montebugnoli *et al*., 2004 and Nakajo, 2004). Second; culture methods have some limitations in the detection of especially obligate anaerobic bacteria. Since *P. intermedia* is obligate anaerobic bacteria and difficult to be cultivated, so PCR give higher positive results than culture method. This is important for the treatment plan for the dentist confirm that a PCR method is sensitive and specific to detect *P. intermedia* in general dental infections. It should be emphasized that the PCR technique only detected the target species, but did not enumerate the total number of bacteria present in the samples (Siqueira *et al*, 2001; Hancock *et al*., 2001).

Siqueira and Rocas (2006) were explored culture used to the identification of unexpected species and being considered the gold-plated standard, allows quantification of all the viable bacteria in the samples.

Although the PCR techniques appear to be pretty and offer precise results, but it has certain limitation due to the chance of false positive results due to the detection of any DNA of dead cells in the samples (Saito *et al*., 2006).

Conclusion

The results of the present study confirm that PCR method is very sensitive, specific and time consume technique for detecting *P. intermedia* DNA in infected root canals although culture method being considered the gold standard.

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