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Full Length Research Paper

Detection of *Actinobacillus actinomycetemcomitans* from periodontitis and healthy individuals by culture and PCR methods

Farshid Kafilzadeh[1]*, Maryam Ramez[1], Reza Mirnejad[2,3], Faramarz Masjedian[3] and Yaghoob Tahery[1]

[1]Department of biology, Islamic Azad University, Jahrom branch, Iran.
[2]Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
[3]Department of Microbiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.

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The aim of this study was to determine the detection of *Actinobacillus actinomycetemcomitans* from periodontitis and healthy individuals by culture and PCR methods. Clinical isolates were obtained of periodontal pockets by paper point from 50 health subjects with periodontal pockets depth < 5 mm and 50 patients with periodontitis with periodontal pockets depth equal to or exceeding 5 mm that were referred to Dental clinic of Imam Khomeini. These samples were cultured in the culture media TSA and incubated in an anaerobic system. *A. actinomycetemcomitans* were isolated and identified using star like colony structure, gram staining and catalase test. For PCR, samples were analyzed with genus specific primers. The primers were used to multiply the region about 500 bp of *A. actinomycetemcomitans*. Of the 100 samples, *A. actinomycetemcomitans* was isolated from 31 patients (31%), (7 isolate of healthy subjects (14%); 24 isolate of patients (48%)) by using a selective actinobacillus isolation media. Using PCR, a total of 49 (49%) samples were found to be positive for actinobacillus (14 isolate of healthy subjects (28%); 35 isolate of patients (70%)). The presence of *A. actinomycetemcomitans* in dental plagues from patients with periodontitis could indicate a role for periodontal pathogenic bacteria in the periodontal diseases. It is well know that improvements in diagnostic methods are useful in the prevention and treatment of periodontal diseases.

Key word: Periodontitis, *Actinobacillus actinomycetemcomitans*, Culture, PCR.

INTRODUCTION

It is necessary to identify different microorganisms causing different mouth infections in microbiology. In other words, this kind of information will be suitable for preventing mouth and dental infections to occur and progress, especially periodontitus (Avila-Campos et al., 2002). There are different types of periodontitus varying in terms of the age of patient and the progress of the clinical symptoms. It is in several decades ago that the different types of periodontitus, especially in the youth are considered as infectious diseases (Moore et al., 1994, Chen et al., 1999). Today, it is proved that periodontal pockets can be the place of more than 300 different bacterial species, some of which play a special role in the pathogenesis of different types of periodontitus (Slots et al., 2002). *Actinobacillus actinomycetemcomitans* is one of these bacteria causing the local periodontitus to occur especially in the youth and the progressive periodontitus in adults. It is a microorganism with high pathogencite and it is considered as the most important periodontal pathogens in the youth and adults (Slots et al., 1999, Mandel et al., 1987). Also, in cases of not observing the health and doing aggressive dental operations, it plays an important role in occurring and appearing as the extra mouth infections, including endocarditic, pericarditic, pneumonia, septicemia and abscess (Mandel et al., 1981). Therefore, the knowledge of epidemiology in microorganism, which is a pathogen in periodontal

*Corresponding author. E-mail: Dr.Kafilzadeh@yahoo.com. Tel: +98-0917-114-0799

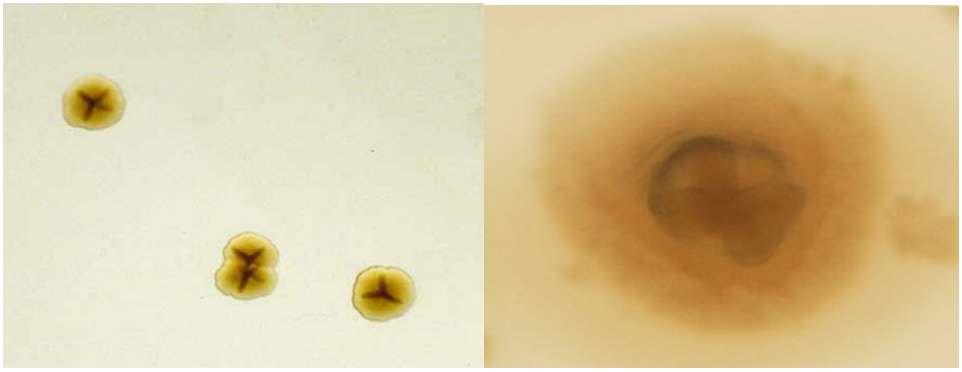

Figure 1. Star-shaped schema of *A. actinomycetemcomitans* bacterium below a phase-contrast microscope.

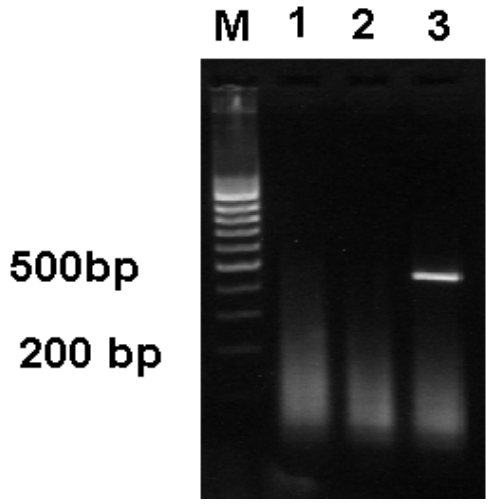

Figure 2. Agarose gel electrophoresis of PCR amplified products generated from patient DNA samples. Lane M is DNA size marker (100 bp DNA ladder, SM#333). Lane 1 is negative control showing no *A. actinomycetemcomitans* infection. Lane 2 is negative sample showing no *A. actinomycetemcomitans* infection. Lane 3 shows 500 bp *A. actinomycetemcomitans*.

lesions would be useful and helpful in selection of treatments and would also prevent the symptoms of infections (Furcht et al., 1996, Boutaga et al., 2007).

A. actinomycetemcomitans is often anaerobic and microaerophilic, so its cultivation and extraction may be very difficult and time consuming, however the development of molecular methods has played a special role in preventing these lesions to occur and causing the early treatment (Ashimoto et al., 1996; Ménard et al., 1992). Several studies have been conducted in order to identify this species on dental plaques of clients affected by periodontitus across the world. But in this study, by using PCR and culture methods, the presence of *A. actinomycetemcomitans* has been studied in the clinical samples of subgingival of healthy individuals and periodontal patients.

MATERIALS AND METHODS

In this study, 100 healthy individuals and patients with the clinical symptoms of periodontitus who were referred to Imam Khomeini specialized dental clinic in Tehran during May 2008 and February 2009, were studied. After registration and receiving oral consents, the individuals were examined in terms of the clinical symptoms of periodontitus and presence of periodontal plaques on their subgingival sections. The sampling of the facial and lateral sections of teeth was carried out through 2 paper point needles being completely sterile. These two paper point needles were placed inside the periodontal pockets for 60 s. Then one of them was placed inside the place of sampling in order to carry out PCR in PBS and the other one was used for culture.

Culture

One paper point needle in the sterile conditions was immediately placed inside the place of sampling over the plates containing triptecas soy agar (including: horse serum, baciteracin and yeast extract) and then transferred to the laboratory in a short time. After incubation period, the plates were studied in term of having the star-shaped colony *A. actinomycetemcomitans* through phase-contrasting microscope (Figure 1).

Finally, after observing the star-shaped colony, the confirmation tests, including gram staining and catalase test were carried out and all samples were confirmed completely.

Samples preparation and PCR

DNA extraction was performed using common method, phenol-chloroform. Oligonucleotide primers were chosen from the published nucleotide sequences of the protected region 16S rRNA of *A. actinomycetemcomitans* according to reference No.1 (Avila-Campos et al., 2002). The primer being used here is as follows:
F-GCT-AAT-ACC-GCG-TAG-AGT-CGG;
R-ATT-TCA-CAC-CTC-ACT-TAA-AGGT. The primers were used to multiply the region about 500 bp of *A. actinomycetemcomitans* (Figure 2). PCR method for final reaction volume of 50 µL included 3 µL buffer, 1.8 µL $MgCl_2$ 1.5 mm, 0.6 µL dNTP, 1.25U of Taq DNA polymerase, 10 µL template DNA, 20 pmol of each forward and reverse primers and sterile distilled water. The PCR process was carried out by using Termocycler Ependorf machine (made in

Table 1. The comparison of PCR results with culture results for the samples of patients group and healthy group.

Microorganism type	Positive culture		Positive PCR	
	Patient group	Healthy group	Patient group	Healthy group
A. actinomycetemcomitans	24(48%)	7(14%)	35(70%)	14(28%)

Table 2. Relative abundance distribution of the *A. actinomycetmcomitans* by the depth of plaque in patients with periodontal through culture method.

Dental plaque depth (mm)	Positive	Negative	Total
<5	0 (0%)	9 (18%)	9 (18%)
5 – 10	11 (22%)	16 (32%)	27 (54%)
>10	9 (18%)	5 (10%)	14 (28%)
Total	24 (48%)	26 (52%)	50 (100%)

Table 3. Relative abundance distribution of the *A. actinomycetemcomitans* by the depth of plaque in patients with periodontal through PCR.

Dental plaque depth (mm)	Positive	Negative	Total
<5 mm	1 (2%)	8 (16%)	9 (18%)
5 - 10 mm	12 (24%)	15 (30%)	27 (54%)
>10 mm	12 (24%)	2 (4%)	14 (28%)
Total	35 (70%)	15 (30%)	50 (100%)

German) according to the following program: predenaturation for 10 min at 95℃ followed by 30 cycles each containing denaturation at 94℃ for 30 s, annealing at 56℃ for 40 s and Extension at 72℃ for 40 s, followed by final extension at 72℃ for 5 min.

Analysis of PCR product

The PCR products were electrophoresed on 1.5% agarose gel and visualized followed by SYBERgreen staining (Figure 2). PCR product was also sequenced in order to confirm the sequence and then the results from PCR cross-section analysis were compared with the available cross-sections by using Blast software. Thus, it was proved that the extracted sections were related to *A. actinomycetemcomitans*.

Statistical analysis

Data were analyzed by means of statistical software (SPSS 12.1). The results were analysed as positive or negative PCR amplify-cation reaction for *A. actinomycetemcomitans*. Descriptive analyses were performed and results are presented as number (%).

RESULTS

The study results showed that isolation rates of the bacterium were 14 and 28% in healthy individuals through culture and PCR respectively. Also, it was clear that the isolation rates of the bacterium in healthy and patient individuals were 28 and 70% through PCR method, respectively (Table 1).

The other result of the study was that, there was no significant difference between the abundance of the bacterium by sex (gender) of 50 patients having referred to the dental clinic, 44% were female and 50% were male. Where as of 50 healthy individuals, 48% were female and 52% were male. There was no significant difference between males and females in relation to fisher test ($P = 0.1$). Also, the study results showed that there was a significant statistical correlation between pocket depth and the presence of the *A. actinomycetemcomitans* in affected individuals ($P < 0.05$). In other words, there is a direct relationship between the depth of pocket and the presence of the *A. actinomycetemcomitans* (Tables 2 and 3)

DISCUSSION

A large number of different microorganisms are found inside the mouth cavity, some of which can only be colonized especially in the sub gingival region, including anaerobic *A. actinomycetemcomitans* bacterium. Indeed, these bacteria play a defined etiologic role in relation to periodontal diseases (Slots et al., 1996, Cortelli et al., 2005).

The bacterium has been identified on the sub gingival plaques of the patients with periodontitus among the youth of the patients with RPP and adult periodontitus. Also, it has been proved that the bacterium has a special role in relation to the extra mouth cavity infections (Lopez et al., 2000; Tan et al., 2001).

The study is to isolate and identify *A. actinomycetemcomitans* bacterium from the periodontal pockets of patients and healthy individuals through the cultivation method on capnophilic conditions and PCR. In the study, 100 individuals healthy and patient were studied in terms of the presence of the *A. actinomycetemcomitans* bacterium by using PCR and the culture method. The study results are comparable with other studies results being carried out in other countries, especially Campos study (Avila-Campos et al., 2002). Campos et al., 2002 studied 50 samples of patients with periodontitus and isolated 90 and 80% of the *A. actinomycetemcomitans* bacterium through PCR and culture method, respectively (Avila-Campos et al., 2002).

The isolation rates of the bacterium from the affected individuals with periodontitus, especially adult periodon-

titus have been reported be 50 to 95% in other studies (Genco et al., 1994; Zambon et al., 1983; Socransky et al., 1992). The difference reported among the studies can be due to differences in the population under study, the age of patients, race, culture, geographical region and laboratorial methods. Also, other significant point is that there are differences in the organism prevalence in different societies, since the bacterium is anaerobic and the culture conditions are very difficult to isolate these bacteria, the exact diagnosis of anaerobic bacteria is impossible in many countries (Trevilatto et al., 2002; Yano-Higuchi et al., 2000; Wu et al., 2007).

In addition to these studies, other studies have been conducted in relation to isolating the bacterium in healthy individuals and patients by using the culture methods and PCR. All of the studies indicate higher rate of the *A. actinomycetemcomitans* bacterium in individuals affected with periodontitus than of the bacterium in the health individuals.

Also, all of the studies, like the current study, show that there is a significant relationship between colonization of the *A. actinomycetemcomitans* bacterium and the pocket depth, so that the isolation rate of the bacterium is higher in those individuals having the pocket depth >5 mm. These findings are consistent with the anaerobic nature of the bacterium (Yano-Higuchi et al., 2000, Sakurai et al., 2007, Wu et al., 2007).

In current study, there was no significant difference between the abundance of the *A. actinomycetemcomitans* bacterium in each of two groups, males and females. The finding are almost comparable with findings of other studies (Avila-Campos et al., 2002, Sakurai et al., 2007). Since in the study, the numbers of male and female clients were not equal, these two groups were not compared with each other.

Generally, the study results shows that the prevalence rate of the *A. actinomycetemcomitans* bacterium in patient group with dental pockets may be higher than the prevalence rate of the bacterium in healthy group. In other words, the bacterium exists on dental plaques of the healthy individuals but its rate is very low, but since the bacterium is a kind of opportunistic bacterium, in the event that there has been a suitable condition as a result of not observing the related hygienic rules, it can grow and multiply as a dangerous, harmful and pathogenic bacterium.

Therefore, all dentists should consider the pathogenicity of the bacterium in relation to the affected individuals with periodontitus in order for them to be diagnosed and treated through taking a sample and cooperation with the laboratory staff.

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