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Detection of periodontopathogenic bacteria in pregnant women by traditional anaerobic culture method and by a commercial molecular genetic method

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ABSTRACT

To culture facultative and strict anaerobic bacteria is a well-established method for analyzing subgingival plaque samples. Micro-IDent® and micro-IDent® *Plus* (HAIN Lifescience GmbH, Nehren, Germany) tests are two commercially available rapid PCR-based methods for the identification and quantification of putative periodontopathogen bacteria. In this study, we compared these commercial PCR-based hybridization methods with conventional anaerobic culture technique. A total of 36 subgingival plaque samples were collected from periodontal pockets of pregnant women with chronic localized periodontitis. Aliquots of these samples were evaluated with species-specific probes provided by micro-IDent® and micro-IDent® *Plus* tests simultaneously, and from the same samples anaerobic and capnophylic bacteria were cultured on selective media. The overall agreement between both methods was excellent for *Eubacterium nodatum, Tannerella forsythia* and *Porphyromonas gingivalis* (97–92%), fair for *Capnocytophaga* sp, *Eikenella corrodens, Actinobacillus actinomycetemcomitans*, and *Prevotella intermedia* (91–89%) and poor for *Fusobacterium nucleatum, Parvimonas micra (Micromonas micros)*, and *Campylobacter rectus* (86–78%). Discrepancies in the results may be explained by inability of culture method to distinguish between closely related taxa (e.i *P. intermedia/ Prevotella. nigrescens*), and problems of keeping periodontopathogen bacteria viable, which is required for successful detection by standard culture method. Nucleic acid-based methods may replace cultivation method as frequently used methods in microbiological diagnosis of progressive periodontitis, thus micro-IDent® andmicro-IDent® *Plus* tests can be recommended where culture of periodontopathogenic bacteria is not performed in routine microbiology laboratories to analyze subgingival plaque samples.

1. Introduction

More than 300 bacterial species may participate in the colonization of the oral cavity. Some of these bacteria may play significant role in the development of periodontitis, such as *Aggregatibacter (Actinobacillus) actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia* (*Bacteroides forsythus*), as the most important species [1], but many other species are considered to be closely associated with this clinical entity, such as *Prevotella intermedia*, *Fusobacterium nucleatum*, *Capnocytophaga* sp., *Parvimonas micra (Micromonas micros)*, *Campylobacter rectus*, *Treponema denticola*, etc. [2,3]. The presence of these microorganisms in the periodontal pocket can be considered as a marker in the development of periodontitis or an indicator in the progression of inflammation [4]. Periodontitis is a chronic infectious disease, the primary etiological agents being Gram-negative anaerobic bacteria that occupy the tooth-associated biofilm in the subgingival plaque. These bacteria can maintain chronic inflammation in the human body, and may contribute to the development of infective endocarditis, aspiration pneumonia, and in pregnant women chronic periodontal disease may be important risk factor for preterm delivery [5–7]. In acute or chronic periodontitis beside the clinical symptoms monitoring for the presence and amount of periodontopathogenic bacteria may help to establish the diagnosis, to follow the therapeutic efficacy or the progression of the disease. Culture method is recently known as gold standard to identify the major putative periodontopathogenic bacteria, to study the mechanism and nature of oral colonization, or to predict treatment outcome. However it is expensive, time-consuming and labour-intensive; in addition to these, only an experienced microbiologist can recognize the afore mentioned bacteria in the rich, human subgingival community. Culture and identification of anaerobic bacteria are not uniformly used method in the routine clinical microbiology laboratories, accordingly the diagnosis of periodontitis is often based only upon clinical findings.

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Because of these drawbacks, many laboratories try to find new methods, such as single or multiplex PCRs followed by DNA–DNA hybridization for the detection of putative periodontopathogen bacteria. In many cases, these tests detect only the most frequently studied bacteria, such as *Actinobacillus actinomycetemcomitans*, *T. forsythia* and *P. intermedia* [8]. However, there is no single bacterium or pathogen group, which can be justified as the main causative agent in periodontitis. Some bacteria in this group are associated more frequently with periodontitis, these are the so called "key" pathogens, while others can be found in low numbers, and less frequently in the periodontal pockets, therefore it may be more relevant if a series of periodontopathogen bacteria could be easily detected by routine microbiological laboratories by a quantitative way.

The purpose of this study was to evaluate the micro-IDent® and micro-IDent® *Plus* tests based on multiplex PCR of 16S rDNA followed by a simultaneous reverse hybridization, detecting altogether 11 putative periodontopathogen bacteria semi-quantitatively, in comparison with conventional culture procedures to isolate anaerobic and capnophylic bacteria from the subgingival plaque of periodontitis patients.

2. Materials and methods

2.1. Patients

A total of 36 pregnant women (age range: 16.7–41.1 years, mean age: 27.6) with clinical signs of chronic localized periodontitis were enrolled in this study. Patients participating in this study were volunteers who had delivery in the University of Szeged, Department of Obstetrics and Gynecology. They were informed about the aim of the study and a detailed Ethics Committee approved Consent Form for dental and microbiological investigations was signed by all of them. Only otherwise healthy women without any sign of systemic or local inflammation except in the oral cavity were assigned to the study. Patients receiving antibiotics during the past three months before a dental examination were also excluded, because antibiotic therapy may change the density and composition of the normal flora and it takes weeks for the microbiota to return to normal. Dental examination and microbial sampling were performed by the dentist member of the team who determined the level of the oral inflammation at the time of the investigation. The dental examinations were performed in accordance with the WHO guidelines [9]. The complete periodontal status was defined, which included determination of the plaque index via the criteria established by Silness and Löe [10]. The plaque index was recorded on a scale 0–3 on Ramfjord teeth, at four surfaces per tooth; the presence or absence of calculus (dichotomously), the recession of buccal marginal gingivae (recorded in mm), tooth mobility; probing depth, and bleeding on probing (BOP) were also recorded [11]. Periodontitis was diagnosed if the patient had $\geq$4 mm probing depth, a "critical probing depth" at least one site, and bleeding on probing at $\geq$50% of the teeth.

2.2. Microbiological sampling

The area to be sampled was isolated with cotton rolls; the tooth surface was cleaned with 70% ethanol and dried with sterile cotton swabs. Samples were obtained from the deepest pockets ($\geq$4 mm) of the most diseased sites with four sterile paper points, which were placed in the gingival crevice for 15 s and moved around the abutment, and then sent to the laboratory: two of them in Portagerm multitransport medium (bioMérieux, S.A., Marcy l'Etoile, France) for culturing, the other two in sterile Eppendorf tube for the molecular genetic investigation. All cultures were commenced within 1 h of sampling [12]. Samples for molecular genetic detection were stored at −20 °C until analyzed.

2.3. Cultivation

Samples (two paper points/patient) for culturing were placed into 1.0 ml pre reduced BHI (Brain Heart Infusion pH 7.2 Oxoid, Basingstoke, UK) broth and mixed on a Vortex shaker for 30 s. The suspensions were diluted (10^{-1}–10^{-5}) in pre reduced BHI broth, and 100 μl aliquot of each dilution and 100 μl sample of the corresponding undiluted suspension were plated on selective and non-selective media. Columbia agar base (Oxoid, Basingstoke, UK) supplemented with 5% (v/v) cattle blood, haemin (5 μg/ml) and vitamin K_1 (1 μg/ml) was used to quantify cultivable facultative and anaerobic bacterial flora. For selective growing of black-pigmented *Prevotella* and *Porphyromonas* species Kanamycin Vancomycin Laked blood agar (KVLB; Oxoid, Basingstoke, UK) was used. For the isolation of anaerobic organisms, cultures were incubated in an atmosphere of 90% N_2, 5% H_2 and 5% CO_2 in an anaerobic cabinet (Bactron Sheldon Man, Cornelius, Oregon, USA) for 6 days at 37 °C. After incubation, a semiquantitative determination was performed for the same species, which was looked for by molecular genetic methods. Each different colony type from positive cultures was subcultured for purity and identification. Results from Gram-staining and atmospheric growth requirements of each colony type were used to determine the additional biochemical tests required to identify the isolates. API 20A and/or ATB ID 32 ANA (bioMérieux, S.A., Marcy l'Etoile, France) tests together with further additional tests according to the Wadsworth Manual [9] were used to identify periodontopathogen bacteria. Spirochetes were looked for by phase contrast microscope at 1000× magnification according to the following score system: 0: no detection, +: not visible in every microscopic field, ++: visible, but in low numbers (1–2) in every microscopic field, +++: visible in high numbers of every microscopic field.

2.4. PCR and hybridization

The second two paper points of each patient were used for performing both the micro-IDent® and the micro-IDent® *Plus* tests. The micro-IDent® is able to identify five "key" periodontopathogenic bacteria: *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia, T. forsythia* and *T. denticola,* while the micro-IDent® *Plus* test can detect some other putative pathogens in this disease: *P. micra*, *F. nucleatum*, *C. rectus*, *Eubacterium nodatum, Eikenella corrodens* and *Capnocytophaga* sp., respectively. These two different commercially available tests are based on the same method, but detect different taxa and consist of 2 distinct techniques i.e. PCR and hybridization with species/genus specific probes. The frozen samples were warmed up to room temperature and the DNA was extracted by using the QIAamp DNA Mini preparation kit (Qiagen) according to the manufacturer's recommendations.

PCR amplification was carried out in a reaction volume of 50 μl consisting of 5 μl of template DNA and 45 μl reaction mixture containing 35 μl of primer-nucleotide mix (micro-IDent® and micro-IDent® *Plus*, respectively), 5 μl of 10X PCR buffer (Qiagen), 5 μl of 25 mM $MgCl_2$ and 1U Taq polymerase (Hot Star Taq, Qiagen). PCR cycling was carried out in a TRIO-thermoblock thermal cycler (Biometra, Gottingen, Germany). The cycling conditions comprised an initial denaturation step at 95 °C for 5 min, 10 cycles at 95 °C for 30 s and at 58 °C for 2 min, 20 cycles at 95 °C for 25 s, at 53 °C for 40 s and 70 °C for 40 s and a final extension step at 70 °C for 8 min 5 μl of each reaction product was loaded on a 2% agarose gel to control the length of PCR-amplicons. The subsequent hybridization

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was performed according to the manufacturer's recommendations: the biotin-labelled amplicons were denatured and incubated at 45 °C for 30 min with hybridization buffer. Each strip coated with two control lines and five or six species-specific probes, respectively. The first control is a conjugate control, which can demonstrate the efficiency of conjugate binding and substrate reaction. The second control, namely amplification control can detect the successful amplification. After PCR products had bound to their respective complementary probe, a highly specific washing step removed any non-specifically bound DNA. Streptavidin conjugated alkaline phosphatase was added, strips were washed and hybridization products were visualized by adding substrate concentrate containing dimethyl-sulfoxide. Results could be obtained after approx. 5h. According to the manufacturer, the cut-off of the test is set to 10^3–10^4 genome equivalents. Developed bands were categorized as follows: 0: no band; +: a weak band, ++: a clearly visible band. The DNA-based analysis and the microbial culturing and identification procedures were performed by two separate, blinded examiners. At the end the comparison of the data obtained by the two methods were carried out quantitatively and the overall agreement between the two methods was evaluated qualitatively.

3. Results

3.1. Conventional culture method

Using the conventional anaerobic culture method and direct microscopy, all of the examined 11 periodontopathogenic bacteria could be detected in the samples in different numbers and in different proportion. The number of the isolated species ranged from 1 to 9/sample, with an average number of species/sample: 4.7. *F. nucleatum*, *T. forsythia* and *A. actinomycetemcomitans* were found in the highest number of specimens with a $\geq 10^5$ CFU/ml by the conventional culture method (Fig. 1.A/B). Black-pigmented Gram-negative anaerobic bacilli: *P. intermedia* and *P. gingivalis* were cultured from 58% to 61% of the samples with various CFUs/ml. The putative periodontopathogen *Parvomonas micra* was cultured and identified only in 38% of the samples and only in 14% of the specimens was found in high number ($\geq 10^5$ CFU/ml). Fewer than 20% of the samples contained in detectable numbers of *E. corrodens*, *C. rectus* and *E. nodatum*. Almost in 40% of the patients' samples, spirochetes were detected by phase contrast microscopy.

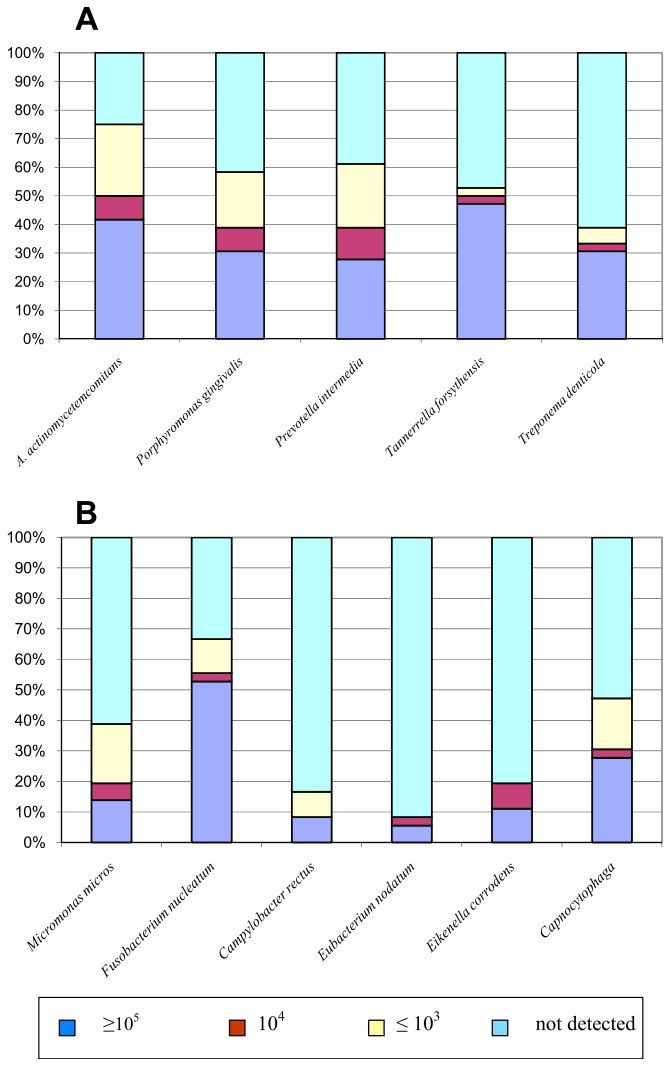

Fig. 1. A, B. Prevalence of the different periodontopathogens in the samples of 36 pregnant women with periodontitis by culture based identification method according to the CFUs/ml.

3.2. Comparison of anaerobe cultivation and nucleic acid-based methods

The best qualitative agreement among the "key pathogen" bacteria was found in the case of *P. gingivalis*. The micro-IDent® kit detected the same number of *P. gingivalis* positive samples than did cultivation procedures (Table 1). Twenty one of the 36 samples were positive for *P. gingivalis* (score + and ++) by molecular genetic method and significant CFUs (10^3–10^5/ml) were found by cultivation, respectively. There were only two samples, which gave discrepant results, one of them was positive by cultivation with a CFU 10^3/ml, but it was negative by micro-IDent® kit, and another one, which had a score + with molecular genetic method, but it was culture negative. Comparison of the results of *P. intermedia* showed an overall agreement of 89% (32/36) of the samples. Three samples gave positive result by culture method and these were negative using molecular genetic method, however there was only one sample, which was negative by the culture method, but low level positivity was detected by the molecular genetic method. The micro-IDent® kit compared with culture method gave a 89% agreement in the case of *A. actinomycetemcomitans*. Cultivation and correct species-level identification of *T. forsythia* by conventional methods are sometimes complicated, therefore this species was more often detected by PCR-based method compared with cultivation (22 versus 19). There were only three samples, which were scored as positive for *T. denticola* by micro-IDent® test, but in this series of samples spirochetes were not seen by microscopy. Spirochetes were not detectable by microscopy too in those cases, where the PCR-based method gave negative results. The best agreement between the micro-IDent® test and the culture method was 94% for *P. gingivalis* and the worst agreements were 89% for *P. intermedia* and *A. actinomycetemcomitans*.

Several discrepant results were observed when the cultivation and the micro-IDent® *Plus* test were compared (Table 2). *P. micra* was isolated with different CFUs/ml from 5 samples, where the PCR-based method did not detect this species. However, there was only one sample, where the culture method was negative and the PCR-based method could detect this species. The same tendency was seen for *F. nucleatum*, more positive results using culture method than using PCR-based method were given. In the case of *C. rectus*, the micro-IDent® *Plus* test detected more often this species, than the culture methods.We found a good correlation, in the case of *E. nodatum* and *Capnocytophaga* sp., respectively. The best agreement between the micro-IDent® *Plus* test and the culture

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Table 1
Comparison of detection of 5 "key" periodontopathogen bacteria in 36 samples of pregnant women with periodontitis by micro-IDent® and culture method or microscopy in the case of *T. denticola*, respectively.

	Detection by culture based identification (CFU/ml)				
	0	$\leq 10^3$	10^4	$\geq 10^5$	Total
Detection by micro-IDent®	Score				
A. actinomycemcomitans	0	6	0	1	7
	+	3	7	2	3	15
	++	0	2	1	11	14
	Total	9	9	3	15	36
Agreement: 89% (32/36)						
P. gingivalis	0	14	1	0	0	15
	+	1	4	3	3	11
	++	0	2	0	8	10
	Total	15	7	3	11	36
Agreement: 94% (34/36)						
P. intermedia	0	13	1	1	1	16
	+	1	7	3	7	18
	++	0	0	0	2	2
	Total	14	8	4	10	36
Agreement: 89% (32/36)						
T. forsythia	0	14	0	0	0	14
	+	1	1	1	3	6
	++	2	0	0	14	16
	Total	17	1	1	17	36
Agreement: 92% (33/36)						
	Phase contrast microscopy (score)				
	0	+	++	+++	
T. denticola	0	18	0	0	0	18
	+	3	1	2	0	6
	++	0	2	1	9	12
	Total	21	3	3	9	36
Agreement: 92% (33/36)						

Table 2
Comparison of detection of 6 further periodontopathogen bacteria in 36 samples of pregnant women with periodontitis by micro-IDent® *Plus* and culture method.

	Detection by culture based identification (CFU/ml)				
	0	10^3	10^4	10^5	Total
Detection by micro-IDent®	*Plus* Score				
P. micra	0	21	2	1	2	26
	+	1	5	1	3	10
	++	0	0	0	0	0
	Total	22	7	2	5	36
Agreement: 83% (30/36)						
F. nucleatum	0	11	2	1	1	15
	+	1	1	0	2	4
	++	0	1	0	16	17
	Total	12	4	1	19	36
Agreement: 86% (31/36)						
C. rectus	0	22	0	0	0	22
	+	1	3	0	0	4
	++	7	0	0	3	10
	Total	30	3	0	3	36
Agreement: 78% (28/36)						
E. nodatum	0	33	0	1	0	34
	+	0	0	0	2	2
	++	0	0	0	0	0
	Total	33	0	1	2	36
Agreement: 97% (35/36)						
E. corrodens	0	28	0	1	2	31
	+	1	0	2	1	4
	++	0	0	0	1	1
	Total	29	1	3	4	36
Agreement: 89% (32/36)						
Capnocytophaga sp.	0	18	3	0	0	21
	+	0	3	1	4	8
	++	0	0	0	7	7
	Total	18	6	1	11	36
Agreement: 91% (33/36)						

method was 97%, when *E. nodatum* was investigated, and the worst agreement in this test was 78% for *C. rectus*.

4. Discussion

Periodontal disease is a very common infectious disease that affects a majority of the world's population to various degrees. It is caused by heavy colonization with various species and a significant number of anaerobic bacteria in the subgingival plaque. A wide range of anaerobic and facultative bacteria can be isolated and identified using standard anaerobic culture techniques, however, only about 50% of bacteria in the oral cavity are cultivable [13]; according to this, the bacterial diversity in periodontic infections is probably still underestimated. Despite of this fact, various studies confirmed the clinical significance of a limited number of "key pathogen" or copathogen species in acute or chronic form of periodontitis. Besides culture method, two different molecular genetic strategies have been introduced for the detection of periodontal "key" and other putative pathogens in subgingival plaques: one technique is based on the use of genomic or oligonucleotide DNA probes and the other is a PCR-based method. The sensitivity of genomic probes to detect periodontopathogen bacteria is 10^2–10^3 bacteria/sample [14]. In contrast to genomic probes, oligonucleotides are synthetically produced, short, stable molecules and can be introduced in automated systems. Chuba et al. [15] were the first who established oligonucleotide probes directed against species-specific sequences of the 16S rDNA to detect periodontopathogen bacteria and to distinguish closely related species such as *P. intermedia* and *P. nigrescens*. When pure cultures are used, the specificity of oligonucleotide probes can be as high as 100%, but this might be reduced during detection of bacteria in complex samples such as subgingival plaque. To further enhance specificity, PCR combined with other molecular genetic techniques was also introduced for routine diagnosis of periodontal pathogens. An extended sensitivity could be demonstrated for the detection of *P. intermedia* or *T. forsythia* in plaque samples and in oral mucous membranes [16]. After amplification of the 16S rRNA gene, specific DNA probes can be used in a reversed hybridization procedure to detect and to quantify bacteria in the amplicon. This two-step method is used by the HAIN micro-IDent® and micro-IDent® *Plus* tests. Molecular genetic methods have enabled the detection of bacterial species that are difficult or even impossible to culture, such as fastidious periodontopathogen bacteria. DNA-based methods are more rapid and in most cases more sensitive techniques, when compared with culture, which needs special laboratory techniques, such as anaerobic incubation. There are only few publications which compared the overall agreement between these two approaches [17,18].

In this recent study, we analyzed plaque samples of pregnant women whith periodontitis on the basis of clinical criteria by classical culture technique and by two commercially available molecular diagnostic kits. Comparison of our overall microbial results with those of other studies is complicated by the facts that different patient populations were investigated, different methods and cut-offs were applied in each study. The overall agreement between the classical culture method and the DNA-base method (micro-IDent® and micro-IDent® *Plus*) for 11 periodontopathogen bacteria was between 78% and 97%. The lowest agreement was found for *C. rectus* (78%), where the DNA-based method gave a positive signal for 8 samples, but the culture method failed to detect this bacterium. The best agreement was found in the case of *E. nodatum* (97%), where 33 out of 36 samples were negative using both methods. In the case of *T. denticola*, no culture method was used, the results of direct microscopy looking for spirochetes in the smear of the samples was compared with the results of DNA-based method. In the case of three samples, the molecular genetic

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method gave weak positive signals, but spirochetes were not seen during direct microscopy, thus resulted in a 92% overall agreement. A 16S rDNA-based PCR detection method was used by Ashimoto et al. [18] to determine the prevalence of *A. actinomycetemcomitans, T. forsythia, C. rectus*, *E. corrodens, P. gingivalis, P. intermedia*, *P. nigrescens* and *T. denticola* in subgingival specimens of 50 advanced periodontitis, 50 adult gingivitis and 50 pediatric gingivitis subjects. PCR detection limits were in the range of 25–100 cells. The prevalences of various species in advanced periodontitis, adult gingivitis and pediatric gingivitis subjects were the following: 30%, 14% and 14% for *A. actinomycetemcomitans*, 86%, 18% and 8% for *T. forsythia*, 74%, 52% and 78% for *C. rectus*, 80%, 70% and 66% for *E. corrodens*, 70%, 10% and 14% for *P. gingivalis*, 58%, 12% and 18% for *P. intermedia*, 52%, 20% and 22% for *P. nigrescens*, and 54%, 16% and 16% for *T. denticola*, respectively. Matching results between the PCR method and the conventional culture method occurred in 28% (*T. forsythia*) to 71% (*A. actinomycetemcomitans*) of the samples; the major discrepancy occurred in the PCR-positive/culture negative category. In this study, lower agreement could be detected between the molecular genetic method and the culture method compared with our recent results. Matching results between PCR and DNA probe method were found in 84% (*T. forsythia*) and 70% (*P. gingivalis*) of the subjects. This study demonstrated the utility of a 16S rDNA-based PCR detection method for identifying important subgingival microorganisms.

Comparison of two methods used in this study showed differences in the case of cultivable periodontopathogen species. The micro-IDent® test more often detected *T. forsythia* compared with cultivation (22 versus 19). This finding may originate from the deficient sensitivity of routinely used culture media. The micro-IDent® kit detected almost the same number of *P. gingivalis* positive samples than did cultivation procedures with only two discrepant results producing 94% overall agreement. This finding is different from the data of Van Steenbergen [19] and Eick [17], they found a higher percentage of *P. gingivalis* positive samples by PCR-based method in comparison with culture, however we do not know how strict anaerobic culture method was used during these studies. The differentiation between *P. intermedia* and *P. nigrescens* using conventional biochemical methods is very difficult, and identification by gene probes also often requires very exact conditions, to avoid false positive results. According to the manufacturer, the micro-IDent® test can detect only *P. intermedia*. The 3 culture positive samples which proved to be negative in the PCR-based hybridization assay were very probably false positive cases due to the inability to differentiate between *P. intermedia* and *P. nigrescens* by routinely used ATB ID 32A kit. Eick et al. [17] published initial difficulties in detecting *A. actinomycetemcomitans*with the first version of the miro-IDent® kit. Because of its high pathogenicity, a lower cut-off for this species was considered to be crucial. New cut-off for *A. actinomycetemcomitans* was set to 10^3 genome equivalents and in addition to this, an internal positive control was incorporated in the test ensuring negative results are not due to failure of the PCR reaction. In our case, this new version of the micro-IDent® kit identified almost all *A. actinomycetemcomitans* containing samples, however one sample with a high CFU for *A. actinomycetemcomitans* ($\geq 10^5$ CFU/ml) was negative. 3 samples, gave a slightly visible band during the hybridization, but were negative using by the culture method resulting in only 89% agreement. The micro-IDent® *Plus* was developed to detect more putative periodontopathogen bacteria. Except for *E. nodatum* and *Capnocytophaga* sp., where the agreements between the two methods were 97% and 91%, respectively, the culture method could detect more positive samples for *P. micra* (5 samples), *F. nucleatum* (4 samples) and *E. corrodens* (3 samples) with high CFUs/ml. Overall agreement for the investigated 10 bacterial species between the culture and the micro-IDent® methods was excellent for *E. nodatum*, *T. forsythia* and *P. gingivalis*, fair for *E. corrodens*, *P. intermedia/nigrescens, A. actinomycetemcomitans* and *Capnocytophaga* sp. and poor for *F. nucleatum, P. micra* and *C. rectus*.

Because of the high cost and time-consuming methods, the quantitative culture of putative periodontopathogenic bacteria is not a routine procedure in most of the clinical microbiological laboratories. In addition, anaerobiosis indispensable for obligate anaerobic periodontopathogen bacteria is sometimes difficult to maintain during sample collection and transportation. Although extensive microbial analyzes have been performed from subgingival plaque samples of periodontitis patients by many studies, systematic PCR-based analyzis of subgingival microbiota has not been carried out in a pregnant population, with chronic localized periodontitis so far. The purpose of this study was to examine the prevalence of major putative periodontopathogens in pregnant patients suffered from characteristic signs and symptoms of periodontitis, by a culture-independent and rapid molecular method. The micro-IDent® and the micro-IDent® *Plus* assays have been shown to give a good overall agreement in “key” periodontal pathogens, and can be recommended for routine laboratory use to establish the rapid microbial diagnosis. However, cultivation may provide detection of multiple bacterial species coincidentally, as well as allow the determination of antimicrobial resistance. Thus cultivation still plays a major role, particularly when examining cases of refractory periodontitis.

Conflict of interest

None.

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