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CLINICAL ARTICLES

Correlation between Clinical Symptoms and Microorganisms Isolated from Root Canals of Teeth with Periapical Pathosis

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Periapical pathosis cases were classified and the effect of bacteria on clinical symptoms was examined. A positive correlation between bacterial growth and clinical symptoms was found. *Peptococcus magnus* and Bacteroides species were commonly found in clinically acute cases, while oral streptococci and enteric bacteria were frequently isolated from clinically asymptomatic cases.

Periapical pathosis is considered an endogenous infection caused by the oral microflora. Therefore, many investigators have attempted to isolate and identify various microorganisms from root canals or periapical regions. The criteria for clinical case selection are not definitive, except in the cases of necrotic pulps (1, 2) and acute alveolar abscesses (3, 4). Also, the methods for sampling, cultivation, and identification vary among investigators. Improvements in laboratory techniques have permitted the isolation and identification of anaerobic bacteria, and it has become clear during recent years that these bacteria can often be isolated from root canals in cases of periapical lesions with acute clinical symptoms (4, 5). Sundqvist (2) reported that Peptostreptococcus, Campylobacter, Peptococcus, Eubacterium, and especially *Bacteroides melaninogenicus* were found in higher frequency in teeth with acute periapical inflammation than in asymptomatic teeth, but did not examine the relationship between clinical symptoms and the distribution of bacteria.

In this study, all bacteriological procedures were performed using a continuous anaerobic system according to the method of Berg and Nord (6). The purpose of this study was to clarify the relationship between clinical symptoms of periapical lesions and the distribution of bacteria isolated from root canals. The endodontic classification described by Morse et al. (7) and Goodman (8) was first attempted in order to classify the cases of periapical pathosis. However, not all cases were distinctly diagnosed. Therefore, we tried to classify periapical pathosis based on the combination of three representative symptoms, i.e. spontaneous pain, percussion pain, and exudation. Comparative studies of the distribution of bacteria isolated from each group were carried out.

MATERIALS AND METHOD

Selection of Patients

Material from the root canals of 36 permanent teeth of patients seen at the Department of Endodontics, Osaka Dental University, was studied. These patients were diagnosed as having periapical pathosis by clinical and radiographic examinations. They were divided into three groups based on a combination of symptoms: class 1—spontaneous pain, percussion pain, and exudation; class 2—percussion pain but no spontaneous pain; and class 3—no spontaneous pain, percussion pain, and exudation (control).

Sampling Procedures

The involved tooth was isolated with a rubber dam, disinfected with tincture of iodine, and the access cavity was prepared using a sterile round bur. A sterile reamer was inserted into the root canal to the foramen and the root canal contents were obtained. Throughout the procedure, anaerobic gas (80% nitrogen, 10% hydrogen, and 10% carbon dioxide) was blown continuously onto the involved tooth using a syringe while a second syringe was inserted into a test tube containing 4.5 ml of reduced transport fluid (9) so as to maintain an anaerobic condition. The reamer with root canal contents was withdrawn and cut off into the test tube. In

cases with marked exudation or pus, a sterile cotton pellet, paper point, or capillary tube was used for collection. As soon as the material was placed into the transport media, the test tube was sealed tightly with a butyl gum stopper and transferred to the laboratory.

Dispersion, Dilution, and Incubation

The samples were dispersed by Thermomixer for 60 s and diluted in 10-fold steps in an anaerobic chamber (Hirasawa Work Co., Tokyo, Japan). From the tubes of the original and the dilutions, aliquots of 0.1 ml were smeared in duplicate on prereduced anaerobic blood agar plates (BBL). One blood agar plate was incubated anaerobically at 37°C for 7 days and another aerobically for 3 days.

Isolation and Identification of Bacteria

The blood agar plate which had approximately 100 colonies was chosen, colonial numbers were counted, and their morphologies were recorded. All colonies on the plate were inoculated in triplicate onto blood agar plates and incubated under anaerobic, aerobic, or 5% carbon dioxide atmosphere in order to determine whether they were anaerobes, facultatives, or capnophiles. Separate isolates were Gram stained and cellular morphologies were recorded. For the isolates which did not show evident Gram reactions, the simple disc technique described in the Wadsworth Anaerobic Bacteriology Manual (10) was used to confirm the reaction.

Anaerobes were identified according to the method described in the Virginia Polytechnique Institute Anaerobe Laboratory Manual (9), i.e. the genus was determined based on Gram reaction, cellular morphology, spore formation, and end products from glucose. In addition, the existence of spores was confirmed by tolerance tests at 80°C for 10 min. The species was determined using characterization tests, i.e. requirement of fermentable carbohydrate, hydrosis of esculin and starch, liquefaction of gelatin, the reduction of nitrate, production of indole, catalase reaction, growth in 40% bile, production of lecithinase and lipase, and mobility. Capnophiles were identified according to the method described by Leadbetter et al. (11) based on the aforementioned characterization tests. Identification procedures of facultatives were performed with the method described in the Color Atlas and Textbook of Diagnostic Microbiology (12) except for streptococci. They were identified according to the method of Facklam (13, 14). These data were analyzed with Student's *t* test.

RESULTS

Bacteriological Examination

The number of cases in each class and the results of the bacteriological examinations are shown in Table 1.

TABLE 1. Number of cases with positive cultures

Class	No. of Cases	No. of Positive Cultures	Positive Cultures (%)
1	11	11	100†
2	13	13	100†
3 (control)	12	7*	58.3

* Three of seven cases had few colonies (1 to 15 colonies from original plate).
† Significant level of difference from control (class 3, *t* test), $p < 0.05$.

All of the patients in classes 1 and 2, who had percussion pain, had positive cultures. Seven of 12 patients in class 3 had positive cultures. Only a few colonies were isolated from three of these patients (1 to 15 from the original plates). The proportional distribution of bacteria isolated from the 31 patients who had positive cultures were further studied.

Isolates from Class 1 Cases

The proportional distribution of bacteria isolated from patients in class 1 is shown in Table 2. Anaerobes were found predominantly (more than 79%), while facultatives were not isolated as major constituents except in cases 5 and 11.

Among anaerobes, Eubacterium, Bacteroides, and Peptostreptococcus species were found frequently in class 1. Especially, *Peptococcus magnus* which was isolated from six cases and predominated in three cases (more than 60%). Exudate from root canals of these teeth was mucous in nature. Among the facultatives, *Streptococcus faecalis* and *Streptococcus MG-intermedius* were isolated from three cases.

Isolates from Class 2 Cases

Bacteria isolated from patients in class 2 are shown in Tables 3 and 4. In these cases, anaerobes such as *P. magnus*, Peptostreptococcus, Eubacterium, Actinomyces, and *Bacteroides* species were isolated. In one case, pure *Bacteroides praeactus* was recovered. A high isolation frequency of facultatives was noted in these cases (Table 4). Among facultatives, *S. MG-intermedius*, *S. anginosus-constellatus*, *S. faecalis*, and enteric bacteria such as Acinetobacter, Citrobacter, and Arizona were isolated. The *S. faecalis* and Acinetobacter species each were isolated in pure form from one case.

Isolates from Class 3 Cases

In comparison to cases in class 2, facultatives were more prevalent as shown in Table 5. Facultatives isolated were oral streptococci, D-group streptococci, and enteric bacteria. The number of isolated bacteria was less in class 3 than in other classes.

Clinical Symptoms and Microorganisms

The percentage of cases with no growth or that were predominantly facultatives is summarized in Table 6.

TABLE 2. Proportional distribution of bacterial species from root canals classified as class 1

	1	2	3	4	5	6	7	8	9	10	11
Anaerobes											
Peptococcus											
magnus	85.0	75.0	68.5	30.5	13.4	2.2					
prevotii					4.7						
Peptostreptococcus											
productus	15.0	2.8					1.6				
parvulus				10.5							
sp.						2.2	3.3				
Gafkya											
anaerobia								18.8			
Eubacterium											
alactolyticum			14.8					14.1	93.1		
lentum						37.8					
tenue				14.4							
combesii								17.2			
saburreum								18.7			
sp.								23.2			
Actinomyces											
israelii		5.6									
naeslundii										100.0	
Propionibacterium											
acnes							33.4				
sp.			4.4					1.6			
Bacteroides											
asaccharolyticus						29.5					
melaninogenicus									6.9		
ss. intermedius											
praeactus			53.4								
sp.						0.9					
Veillonella											
parvula							61.7				
Facultatives											
Streptococcus											
MG-intermedius		16.6			9.3						2.2
anginosus-constellatus											97.8
morbillorum					40.0						
sp.					32.6	0.9					
faecalis			16.7			13.3		3.2			

The percentage was larger in asymptomatic teeth, class 3, than with the symptomatic classes. Class 1, with spontaneous pain, showed the smallest percentage. There was no significant difference between class 1 and 2, but there was a significant difference between class 3 and class 1 or class 2. From this study it is clear that symptomatic teeth are associated more frequently with predominately anaerobic microorganisms.

DISCUSSION

Microorganisms were demonstrated in cultures from root canals in all cases with severe clinical symptoms, especially percussion pain which often persists during endodontic procedures (classes 1 and 2). The results of this study confirm the findings of Sundqvist (2).

In the asymptomatic class 3, only 7 of 12 cases exhibited positive cultures. However, a small number of colonies were isolated from these positive plates. These results suggest that lack of clinical symptoms related to low bacterial growth in the root canal and periapical region.

Among bacterial species isolated in this experiment, obligate anaerobes predominated, supporting the usefulness of the continuous anaerobic system (6). Sundqvist (2), using a similar system, has reported that more than 90% of the bacteria isolated from necrotic pulps were anaerobic. In his report, *B. melaninogenicus* was isolated from all seven cases where exacerbation occurred after endodontic treatment, suggestive that acute inflammation in the periapical region was induced by specific bacteria such as *B. melaninogenicus*. Griffee et al. (15) also isolated *B. melaninogenicus* from purulent infectious periapical diseases and indicated that the presence of this organism was closely related to feelings of discomfort and swelling in the involved area. In acutely symptomatic cases in this study Bacteroides and *P. magnus* were frequently isolated. This might suggest that Bacteroides and Peptococcus species exert an important influence on exacerbation of chronic

TABLE 3. Proportional distribution of bacterial species from root canals classified as class 2

	12	13	14	15	16	17	18	19	20	21
Anaerobes										
Peptococcus										
magnus	54.6	53.7								
Peptostreptococcus										
parvulus	7.3		8.7							
anaerobius	5.5		21.7	45.0	12.8					
productus		1.9				7.7				
asaccharolyticus						25.6				
sp.		5.5				2.6				
Gafkya										
annaerobia					11.7					
Eubacterium										
alactolyticum		1.9	69.6	35.0	1.1		60.0			
limosum				18.3						
aerofaciens				1.7						
sp.	3.6									
Actinomyces										
odontolyticus	14.5									
israelii		35.4					40.0			
naeslundii						20.5				
Arachnia										
propionica						43.6				
Propionibacterium										
sp.								46.0		
Bacteroides										
asaccharolyticus	12.7									
praeactus									100.0	
sp.										100.0
Fusobacterium										
sp.	1.8									

TABLE 4. Proportional distribution of bacterial species from root canals classified as class 2

	13	16	19	22	23	24
Facultatives						
Streptococcus						
MG-intermedius		5.0	46.0			
anginosus-constellatus		67.0				
faecalis				100.0	13.2	
sp.	1.9					
Proteus						
mirabilis			8.0			
Acinetobacter						
sp.						100.0
Citrobacter						
freundii					81.6	
Arizona						
hinshawii					2.6	
Enterobacter						
aggalomerans					2.6	

periapical lesions. *P. magnus* was isolated from almost all cases with mucous exudation. On the other hand, Bacteroides, Eubacterium, and Veillonella species were major isolates from cases with serous exudation, while *P. magnus* and facultatives were not found. These results indicate the possibility that *P. magnus* lacks the ability to produce DNAse. Regardless of the reason, these findings are useful in endodontic treatment.

Among those bacteria related to acute inflammations, Bacteroides species were frequently isolated from the oral cavity, especially gingival crevices. Some Bacteroides species possess a high potential for protein hydrolysis (collagenase, gelatinase and other proteases) and amino acid hydrolysis (decarboxylase and deaminase) and produce various cytotoxic factors. Furthermore, some Bacteroides species can adhere to the tooth surface and gingiva. From these pathogenic properties, Bacteroides species are regarded among the causative bacteria in advanced adult periodontitis (16, 17).

TABLE 5. Proportional distribution of bacterial species from root canals classified as class 3 (five had no growth)

	25	26	27	28	29	30	31
Anaerobes							
Peptostreptococcus							
parvulus	30.8						
anaerobius		6.3					
Actinomyces							
israelii			100.0				
Propionibacterium							
sp.		9.5		100.0			
Facultatives							
Streptococcus							
MG-intermedius		44.5			100.0	12.2	
anginosus-constellatus		39.7				84.8	
D-group streptococcus	61.5						
sp.						3.0	
Gram-negative bacilli							
Proteus							
morganii							100.0

TABLE 6. No growth or predominantly facultative versus predominantly anaerobic growth

Class	No. of Cases	No Growth or Facultative	%
1	11	2	18.2*
2	13	5	38.5*
3 (control)	12	10	83.3

* Significant level of difference from control (class 3, *t* test): $p < 0.05$.

On the other hand, *P. magnus* has frequently been isolated from purulent infectious inflammations such as abscesses of the breast and other soft tissues, surgical wounds, osteomyelitis, and infections involving the neck and dentoalveolar tissues, respiratory tract, and female genital tract (18). Although this organism constitutes a minor group in the oral microbial flora, it was frequently isolated from our cases of acute inflammation. These facts indicate that *P. magnus* as well as *B. melaninogenicus* is comparatively pathogenic among the endogenous bacteria.

CONCLUSIONS

It may be concluded that growth of a mixture of several mainly anaerobic bacterial species in the root canal is closely related to the presence of clinical symptoms. Furthermore, relatively pathogenic species such as *B. melaninogenicus* and *P. magnus* may play an important role in the development of clinical symptoms. However, in this study, only 36 cases were included and the clinical classes comprised few cases. Therefore, further studies should be carried out.

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