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Direct microscopic features of subgingival plaque in localized and generalized juvenile periodontitis

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Abstract

Subgingival dental plaque from 12 untreated patients with juvenile periodontitis (JP) was examined undispersed in wet-mount preparations with direct phase-contrast microscopy. Observations of the bacterial morphotypes present revealed high numbers (≥125/field) of spirochetes and large, spinning, motile rods in all but one of the localized and generalized types of JP patients. Generalized JP patients tended to have a more complex and organized microflora, with brush formations, amoebae, and a wider variety of motile rods than in localized juvenile periodontitis (LJP) cases. All JP subjects examined also had high levels (≥125/field) of accumulated crevicular polymorphonuclear leukocytes associated with the subgingival dental plaque samples. These findings are related to previous bacteriologic studies of JP dental plaque. The possible role of direct microscopy as a diagnostic tool to supplement clinical examinations in evaluating bacteriologic risk factors in JP patients is discussed.

Juvenile periodontitis (JP) is characterized by a rapid loss of alveolar bone and periodontal attachment in otherwise healthy adolescents. It generally is localized to the permanent first molars and incisors, with minimal gingival inflammation and almost no clinically detectable dental plaque or calculus on the affected teeth.[1,2] Most patients with this form of periodontal disease have been shown to have host cell-mediated immunodeficiencies in the form of impaired neutrophil chemotaxis and rate of migration,[3,4] which are likely to contribute to the early and rapid onset of periodontal deterioration.

Studies have shown clearly that the clinical destruction also is related to the presence of a subgingival plaque and an inflammatory process. Waerhaug[5] demonstrated that loss of periodontal attachment in JP patients always is associated with a thin 20-200 μm) and unmineralized subgingival dental plaque that is difficult to detect clinically. This subgingival plaque was shown to be capable of unusually rapid advancement in an apical direction (up to 5 μm/day or 1.8 mm/year),[6] and always was associated with a cellular inflammatory infiltrate seen histopathologically in areas of attachment loss.[5] Use of continuous anaerobic culturing techniques have revealed that a distinct bacterial microbiota dominated by gram-negative anaerobic and microaerophilic rods (63% of the cultivable flora) is present in subgingival plaque from JP patients.[7,8]

Direct darkfield or phase-contrast microscopy have been employed recently to assess rapidly and inexpensively bacterial populations present in dental plaque specimens.[9,10] The authors reported observations on the variety of bacterial morphotypes and organizational patterns in adult patients having different states of periodontal health and disease (using wet-mount preparations of undispersed subgingival plaque viewed with direct phase-contrast miscroscopy).[10] In order to investigate further the microbiologic features of JP dental plaque, as well as the potential utility of direct microscopy as a diagnostic tool in evaluating JP patients, these same approaches were used in gathering cross-sectional, descriptive observations on subgingival plaque from both localized and generalized types of JP patients.

Methods and Materials

Patient Selection

As a part of ongoing clinical therapeutic studies on human periodontal diseases conducted between 1974 and 1982 at the National Institute of Dental Research in Bethesda, Maryland, 12 untreated subjects younger than 22 years of age with a diagnosis of idiopathic juvenile periodontitis (using criteria defined by Baer[1]) were evaluated (Table 1). All subjects were in good general health and presented with classical molar-incisor involvement (radiographic evidence of greater than 50% bone loss associated with the permanent first molars and/or incisors, and minimal clinical evidence of inflammation).

Table 1. Juvenile Periodontitis Subjects Examined

Males 3; *Females* 9; *Black* 9; *Caucasian* 3.
Age Range 12-21 years, *Mean Age* 17.8 years.

Type of JP	*Age*	*Race*	*Sex*	*Site Sampled and Pocket Depth*
1. Localized	12	B	F	#30D - 10 mm
2. Localized	15	C	F	#14M - 8 mm
3. Localized	16	B	F	#19M - 8 mm
4. Localized	16	B	F	# 3M - 10 mm
5. Localized	19	B	F	#30D - 10 mm
6. Localized	20	B	F	#19D - 8 mm
7. Localized	21	B	M	#18M - 7 mm
8. Localized	21	C	F	#30D - 8 mm
9. Generalized	16	B	F	#14M - 10 mm
10. Generalized	18	B	M	# 3D - 10 mm
11. Generalized	18	B	F	# 3M - 8 mm
12. Generalized	21	C	M	#19D - 10 mm

Subjects having any systemic disorder reported to be associated with periodontal manifestations in adolescents, such as diabetes mellitus, sarcoidosis, Down's syndrome, cyclic neutropenia, agranulocytosis, Papillon-Lefévre syndrome, and Chédiak-Higashi syndrome, were excluded as were subjects receiving any type of periodontal prophylaxis or systemic antibiotic therapy in the previous six-month period.

JP subjects also were subgrouped as being localized (first molars, incisors, and additional teeth <14 total teeth) or generalized cases (≥14 total teeth), based on the number of affected teeth.[11]

Bacteriological Procedures

After prior removal of any supragingival plaque, subgingival plaque samples were taken from the most apical portion of involved periodontal pockets (Table 1) with a sterile currette and immediately placed undispersed into approximately 0.02 ml of physiological saline on a microscopic slide. The specimens were coverslipped and only lightly compressed without fixing or staining. The wet-mount preparations then were examined within 10 minutes of plaque removal with phase-contrast microscopy at 400 and 1000x. At least 10 fields containing the greatest concentration of motile forms and accumulated crevicular polymorphonuclear leukocytes were assessed quantitatively at 400x, with qualitative assessments of other biologic features obtained from any area of the slide.

Results

Subgingival plaque from the JP patients was sparse and had a slimy consistency. Extra care in handling was necessary during sampling and preparation of the wet-mount slides. On examination, all of the plaque samples contained complex bacterial populations; a distribution of the various bacterial morphotypes seen in the patients examined with direct phase-contrast microscopy is presented in Table 2.

Table 2. Frequency of Bacterial Forms Found in Subgingival Plaque of JP

	Localized JP N=8		*Generalized JP* N=4		*Total JP* N=12	
Bacterial Forms	No.	%	No.	%	No.	%
Spirochetes						
≥125/field	7	87.5	4	100	11	91.7
100⁺/field	1	12.5	0	0	1	8.3
<100/field	0	0	0	0	0	0
Brush Formations	4	50	3	75	7	58.3
Motile Rods						
Gliding (2.5 x 5-10 μm)	2	25	3	75	5	41.7
Spinning* (2 x 6-10 μm) ≥125/field	7	87.5	4	100	11	91.7
Spinning* (2 x 3-5 μm) ≥125/field	5	62.5	3	75	8	66.7
Spiral (2 x 10-20 μm)	1	12.5	2	50	3	25
"Clock arms"	2	25	0	0	2	16.7
Coccobacilli	1	12.5	0	0	1	8.3
Nonmotile Filaments and Coccoid Cells	8	100	4	100	12	100
Entamoebae gingivalis	1	12.5	2	50	3	25
Yeast Cells	1	12.5	0	0	1	8.3
Crevicular Leukocytes						
0-100/field	0	0	0	0	0	0
≥125/field	8	100	4	100	12	100

0 – Indicates none observed.
* – Rods with a corkscrew motion at one end of cell.
No significant differences were found between generalized and LJP cases.

Nonmotile filamentous rods and coccoid cells were seen in all wet-mount preparations. The direct microscopic techniques used in this study did not enable further taxonomic classification or identification of these cells.

Motile bacterial cells were prevalent in all of the JP patients examined, with spirochetes being the most predominant form. Eleven of 12 cases had high levels of spirochetes present (≥ 125/field, Figure 1). In seven subjects, aggregated masses of spirochetes were seen organized with branching nonmotile rod complexes into brush formations.[10] The motility of densely packed spirochetes associated with brush formations was coordinated collectively and synchronized in phase with each other. These configurations were seen more commonly in generalized JP cases (Table 2), although no statistically significant differences between generalized and localized juvenile periodontitis (LJP) were found.

Motile rods also were observed in all patients, with high levels (≥ 125/field) of large spinning rods (2 x 6-10 μm) being present in all patients (Figure 2). These rods characteristically moved in the wet-mount preparations with a corkscrew or rotary motion. Spiral rods[10] and cigar-shaped gliding rods (2.5 x 5-10 μm),[10] which translocated with minimal flexing of cell walls, were

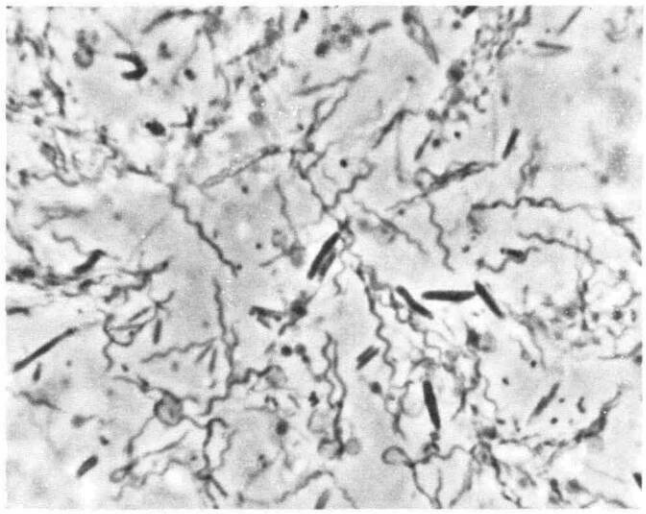

Figure 1. Subgingival spirochetes and small spinning rods associated with an LJP patient (1000x, phase-contrast).

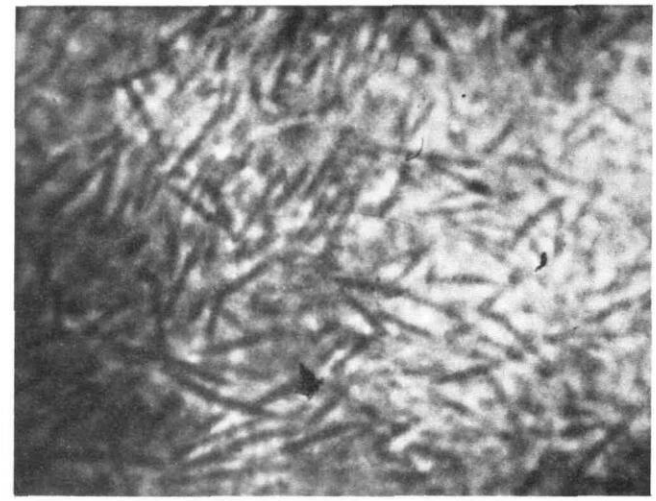

Figure 2. A field entirely filled with large spinning rods (1000x, phase-contrast).

more prevalent in generalized JP patients, along with the oral protozoan *Entamoeba gingivalis* (Table 2).

High levels ($\geq$ 125/field) of accumulated crevicular polymorphonuclear leukocytes were associated with all subgingival bacterial plaque samples.

Discussion

Phase-contrast microscopic examinations of undispersed subgingival plaque revealed that large numbers of spirochetes, large motile rods, and crevicular polymorphonuclear leukocytes were associated with both LJP and generalized JP patients. The microflora of the generalized JP subjects were found to be more complex and organized, with brush formations, *Entamoeba gingivalis,* and a wider variety of more prevalent motile rods.

These observations are consistent with previous studies of plaque from generalized JP (or post-JP) patients, where high proportions of spirochetes (from 34 to 56% of microscopic count) and motile rods were found.[12-14]

However, our findings with LJP are in contrast to several studies which reported only very low or negligible levels of spirochetes and motile rods in dispersed LJP plaque examined with direct microscopy,[8,12,15,16] and electron microscopic evaluations where spirochetes and flagellated rods were not found.[17]

On the other hand, our LJP findings are in agreement with other investigations where numerous small and intermediate-sized spirochetes were seen in LJP plaque analyzed with electron microscopy,[18] and in direct microscopic examinations where proportions of spirochetes and motile rods were elevated significantly (>44% of count) relative to healthy periodontal sites.[19-21] Also, histopathologic studies of gingival biopsies from LJP subjects have uncovered spirochetes within gingival connective tissues,[22] and in direct contact with the surface of alveolar bone in advanced LJP lesions.[23]

The reasons why these differences among LJP patients exist is not entirely clear, but may be related in part to geographical differences in the patients studied. Liljenberg and Lindhe[12] have suggested that spirochetes consistently are absent in younger JP patients ($\leq$ 16 years) studied in Sweden. However, all U.S. JP subjects $\leq$ 16 years of age examined in the present study harbored high levels of both spirochetes and motile rods.

Another source of variation may stem from difficulties in obtaining representative plaque samples from the advanced periodontal sites. Dispersion procedures used prior to microscopic examinations of plaque may be important factors, as gram-negative organisms (i.e., spirochetes are particularly sensitive to sonic oscillation techniques.[24] Plaque dispersion procedures used by other investigators probably explain why the organized spirochetal motility seen in conjunction with brush formations have not been reported previously in JP patients (although portions of brush formations have been noted in electron micrographs of fixed JP plaque).[18]

In contrast to JP patients, adolescents with healthy periodontal conditions or marginal gingivitis have been found to harbor predominantly nonmotile coccoid cells ($\geq$75%) in their subgingival plaque, whereas negligible or low levels of spirochetes (1%) or motile rods (<10%) were present.[25] A relative absence of motile bacteria in periodontal health also has been reported in adult patients.[9,10,17,20]

Can direct microscopy be useful as a diagnostic tool to supplement clinical findings in evaluating the efficacy of therapeutic measures and the prognosis of treated JP patients? Studies monitoring motile bacteria in treated adult periodontitis patients have shown that those left with high levels of spirochetes and motile rods after the completion of periodontal therapy experience a higher incidence of disease-relapse and clinical deterioration in the following 12-month period, including continued loss of periodontal attachment.[26,27] These findings suggest

that motile bacteria seen in plaque with direct microscopy are relevant diagnostically as indicator organisms for evaluating subgingival bacteriologic risk factors in adult patients. Similar studies involving JP patients have not been conducted, although Lindhe[16] has reported from his longitudinal studies that six periodontal sites in four treated LJP patients experiencing clinical signs of disease-relapse did not have an associated increase in the proportions of motile bacteria.

It is likely that these sites were affected by increased numbers of *Actinobacillus actinomycetemcomitans (A.a.)*, a nonmotile rod whose presence in subgingival plaque cannot be determined with direct microscopic examinations alone. This gram-negative, facultative organism has been implicated as a suspected periodontopathogen in JP patients based on its presence in high numbers in 90-95% of deep LJP pockets,[7,8,28,29] and its ability to: elaborate collagenase,[30] inhibit fibroblast proliferation,[31] lyse human polymorphonuclear leukocytes and monocytes with a leukotoxin,[32,33] and promote bone resorption in vitro.[34]

Levels of motile bacteria and *A.a.* do not always coincide, as *A.a.* species can persist subgingivally in high levels, leading to continued loss of periodontal attachment,[35] even when proportions of spirochetes are decreased significantly in the same sites by repeated periodontal scaling and root planing.[36] *A.a.* apparently repopulates these LJP pockets following mechanical debridement from adjacent gingival tissues infected with the organisms.[23,37]

This suggests that additional bacteriologic assessments are needed with JP patients in addition to determination of spirochete and motile rod levels. It has been proposed that routine isolation and identification of *A.a.* can be achieved with use of recently developed culture media[38,39] or immunological approaches, such as monoclonal antibodies to the organisms.[40]

Also, accumulated crevicular polymorphonuclear leukocyte levels, which have been suggested as providing diagnostically valuable insight into local immunopathologic conditions in periodontal pockets of adult patients,[10] may be useful in evaluating JP patients. Histopathologic studies have found large, dense areas of inflammatory cell accumulations in connective tissue and epithelium close to the bottom of JP pockets,[5,12,41] consistent with our findings of high crevicular leukocyte levels ($\geq$ 125/field) in untreated JP lesions. These observations are in contrast to the low levels ($<$ 10/field) associated with healthy periodontal sites.[10]

It is likely that the elevated numbers of *A.a.* in subgingival JP plaque contribute to these increased crevicular leukocyte levels, since its biologically potent endotoxin has been associated with promoting inflammatory changes in vitro (i.e., bone resorption).[34] Thus, elevated crevicular leukocyte levels remaining in subgingival JP sites following suppression or elimination of motile bacteria by therapeutic measures may reflect persistence of high numbers of nonmotile periodontopathogens in the subgingival plaque, such as *A.a.* However, further clinical research on this point is indicated.

Conclusions

1. Subgingival plaque from both localized and generalized forms of JP as seen with direct phase-contrast microscopy contains high levels ($\geq$ 125/field) of spirochetes, large motile rods, and accumulated crevicular polymorphonuclear leukocytes.
2. Organized brush formations, *Entamoeba gingivalis*, and other types of motile rods are associated more commonly with generalized JP cases.
3. Direct phase-contrast microscopic evaluations of JP subgingival plaque for key indicator organisms (such as spirochetes, motile rods, and brush formations), may be useful as a supplement to clinical examinations in assessing some of the bacteriologic risk factors associated with JP patients. However, additional monitoring of crevicular leukocyte levels with direct microscopy and/or *A.a.* with cultural or immunological techniques also appears necessary.

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Quotable quote: dietary carcinogens

The view that dietary practices might be a causative factor in cancer is not new. Epidemiologists have noted marked differences in cancer rates between population groups. Effects from changes in diet following migration also have been observed. Results of current studies are beginning to delineate more sharply specific causative agents. When more definitive information is available, it should be possible for prudent persons to choose fruits and vegetables that present minimal hazards. In the meantime, there is persuasive evidence that charred meats and rancid fats should not be part of the diet.

Abelson, P.H. Dietary carcinogens. Science 221:1,249, 1983.