



## Prevalence of Periodontal Destruction and Putative Periodontal Pathogens in the Same Lebanese Family

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### ABSTRACT

**Aim:** Periodontal diseases are associated with microorganisms rich in Gram-negative species. Several studies have indicated the presence of few a periodontopathic microorganisms in the same family. A parent with severe adult periodontitis, who is infected with bacteria associated with periodontal disease, may function as a source of infection. Their children may be at a greater risk to become colonized with bacteria. The purpose of this investigation was (1) to explore the presence of three bacteria, such as *Porphyromonas gingivalis* (PG), *Prevotella intermedia* (PI), and *Aggregatibacter actinomycetemcomitans* (AA) in the same Lebanese family and (2) to study the clinical destruction in the same family and their relations as members of this family due to the presence of PG.

**Materials and methods:** A total of 10 families were screened; only 5 (13 females and 5 males) were selected for this study, and at least one member of the family had untreated periodontal disease, chronic or aggressive. Every participant signed an informed consent form. A total of 18 available deoxyribonucleic acid (DNA) samples were taken to analyze the presence of three periodontal bacteria.

**Statistics:** Multiple logistic regression was used for the exact methods.

**Results:** All 18 patients showed a positive result for PI. Also, PG. was recognized in 15 patients while AA was not detected in any of the subjects. All couples suffered from periodontitis, chronic or aggressive forms, five children suffered from gingivitis, three children had no clinical manifestation, and only one suffered from localized aggressive periodontitis.

The statistical analysis showed with each 1 year of increase in age, the odds of having periodontal disease multiply by 1.39, i.e., age as a risk factor for periodontal disease due to the presence of PG and sharing the same plate.

**Conclusion:** This investigation demonstrates a high prevalence of periodontal microorganisms in children and young adults of Lebanese periodontitis parents and a microbiological similarity between the children and their mothers. All these factors could be a high risk of developing periodontal disease in the future.

**Clinical significance:** This article shows that vertical transmission of microorganisms is a possible risk factor for developing periodontal disease in the offspring.

**Keywords:** Child, Colonization, Periodontal disease, Transmission.

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### INTRODUCTION

Periodontal diseases are associated with microorganisms rich in Gram-negative species. In recent years, certain bacteria, particularly *Aggregatibacter actinomycetemcomitans*, PG, and PI, are becoming increasingly implicated in destructive forms of periodontal disease.<sup>1</sup> Several studies have indicated the prevalence of these and other bacteria in adults, often with established disease. However, much less is known about their prevalence in adolescents or in prepubertal period. The PG is implicated as a major pathogen in adult periodontitis of the severe type.<sup>2</sup> The PG is rarely isolated in young children and adolescents with a healthy periodontium.<sup>3,4</sup> The PI has been detected in edentulous children as early as 1 month after birth, while PG and AA could not be detected in these children.<sup>5</sup> Today, we agree that there is an association of AA and PG in different forms of periodontal diseases. It has been shown based on cross-sectional studies that a high prevalence of the two organisms is observed in certain populations, particularly from developing countries.<sup>6-11</sup> Different studies have indicated that great differences

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exist in the degree of severity in periodontal disease.<sup>12,13</sup> Advanced disease was seen in 5% of Lebanese children aged 10 to 14 years and 10% of Palestinian children in refugee camps in Lebanon. Refugee girls in Lebanon as young as 12 years were found with extensive bone loss.<sup>14</sup> Severe periodontal destruction seems to be limited to only 7 to 15% uniformly.<sup>15-18</sup>

Several studies indicated the presence of few periodontopathic microorganisms in the same family. A parent with severe adult periodontitis, who is infected with bacteria associated with periodontal disease, may function as a source of infection. Their children might be at a greater risk to become colonized with bacteria, which are associated with periodontal diseases, compared with children of periodontally healthy parents.<sup>19,20</sup> There is an evidence for transmission, e.g., from parent to child or between spouses.<sup>21-29</sup> Other studies have also shown that if a child harbored periodontal pathogens, then at least one of the parents will exhibit the same genotype of bacteria.<sup>5,30-32</sup> These studies have found that various anaerobic species colonize the edentulous mouths of infants and that saliva may act as a source of some Gram-negative anaerobes.

Research has indicated intrafamilial infection with periodontopathic bacteria.<sup>33</sup> It is possible that periodontopathic bacteria are transmitted from mother to child as the first step in colonization.<sup>34</sup> Tanner et al<sup>35</sup> found a similarity between the oral microbiota of preschool children and that of their caregivers.

The aim of this investigation was (1) to explore the presence of three bacterias, such as PG, PI, and AA in the same Lebanese family and (2) to study the clinical destruction in the same family and their relations as members of this family due to the presence of PG.

## MATERIALS AND METHODS

### Selection of the Families

A total of 10 families were screened, only 5 (13 females, 5 males) were selected who fulfilled the following criteria:

At least one member of the family had untreated periodontal disease defined as the presence of at least 10 pockets (10 teeth) with a pocket depth (PD)  $\geq$  5 mm and the evidence of radiographic bone loss.

The exclusion criteria were systemic diseases (diabetes mellitus, cancer, human immunodeficiency virus, metabolic disease, radiation, or immunosuppressive therapy), pregnancy or lactation, and systemic antibiotics within the previous 2 months. The total number of subjects in each family was at least 3.

### Statistics

Multiple logistic regression was used as the exact methods.

## Clinical Parameters

The plaque index<sup>36</sup> was measured on four surfaces of each tooth. The samples were taken with paper points from the deepest pocket in each quadrant; if no deep pockets were present, then from the first or second mesial molars and mesial incisors, four pooled samples were taken.

Supradental plaque was eliminated with sterile cotton before sampling, the area was isolated and dehydrated, and one paper point was inserted to the bottom of a pocket for 10 seconds. After sampling, probing PD and attachment level (AL) were scored and bleeding on probing (BOP) was determined on 4 aspects of each tooth. Clinical parameters and samples were assembled by a single analyzer (ZY). A periodontal probe (GF-W, Hufriedy11, United States) was used for periodontal examination.

## DNA Probe Analysis of Bacteria

Specification and quantification of AA, PG, and PI were performed using DNA probes (Omni Gene, MA, United States; ANAWA Laboratories, Switzerland) as detailed by French et al.<sup>10</sup> In summary, disruption of the bacteria and denaturation of the DNA molecules into two distinct entities were necessary for the processing of plaque specimens. This was attained by suspending the plaque specimens in a basic solution. The DNA specimens were then immersed in a loading buffer, bathed in 0.5 M NaCl, added to nitrocellulose filters, and kept at 80°C for 2 hours. The filters were hybridized with the matching P32-labeled DNA sample for approximately 4 hours at 65°C and rinsed for 5 minutes once with WASH I and at 65°C twice for 15 minutes with WASH II. Subsequently, the filters were air dried and subjected to Kodak X-ray film for 48 hours at -70°C. The Kodak X-ray images were filmed using a video camera and processed with image analysis software. The three filters for the microorganisms contained  $10^5$ ,  $10^4$ , and  $10^3$  control cells in triplicate, which were utilized to create a standard curve. Each sample was compared with the reference curve to obtain a numerical value representing the number of bacteria present in the samples.

## RESULTS

Table 1 shows the age, sex, the use of antibiotics drugs in the previous 5 years, and the prevalence of brushing, smoking, using the same plate, and diagnosis. The clinical and microbiological parameters for all families are summarized in Table 2.

### Periodontal Condition

All couples suffered from periodontitis, chronic or aggressive forms as shown in Table 2. The mean of PL (I) for all

**Table 1:** Age, sex, and exposure to antibiotics in the past 5 years, frequency of toothbrushing, and smoking habits of the families

<i>Clinical parameters</i>								
	<i>Patient no</i>	<i>Age</i>	<i>Sex</i>	<i>Antibiotics past 5 years</i>	<i>Same plate</i>	<i>Brushing</i>	<i>Smoking (1 pack/day)</i>	<i>Diagnosis/type</i>
I	1	42	Female	No	Yes	1/week	Yes	AG.P/ii
	2	23	Female	Yes	Yes	5/week	No	AG.P/ii
	3	22	Female	No	Yes	1/day	No	G
	4	19	Male	No	Yes	1/week	No	G
II	5	37	Male	No	Yes	3/week	No	AG.P/ii
	6	35	Female	No	Yes	1/day	No	AG.P/ii
	7	6	Female	Yes	Yes	1/day	No	NO.D
	8	5	Male	No	Yes	1/day	No	NO.D
III	9	45	Female	Yes	Yes	3/week	Yes	C.P/I
	10	25	Female	No	Yes	4/week	No	G
	11	12	Female	No	Yes	1/day	No	G
IV	12	49	Male	No	No	1/day	Yes	AG.P/ii
	13	45	Female	Yes	No	1/day	No	C.P /I
	14	17	Female	Yes	No	1/day	No	NO.D
	15	13	Female	Yes	No	1/day	No	G
V	16	55	Female	No	Yes	never	No	C.P/I
	17	16	Female	Yes	No	1/day (2 years)	No	C.P/I
	18	14	Male	Yes	No	1/day (5 months)	No	AG.P/ii

AGP: Aggressive Periodontitis; CP: Chronic Periodontitis; NOD: No disease; G: Gingivitis

**Table 2:** Clinical and microbiological description of the families

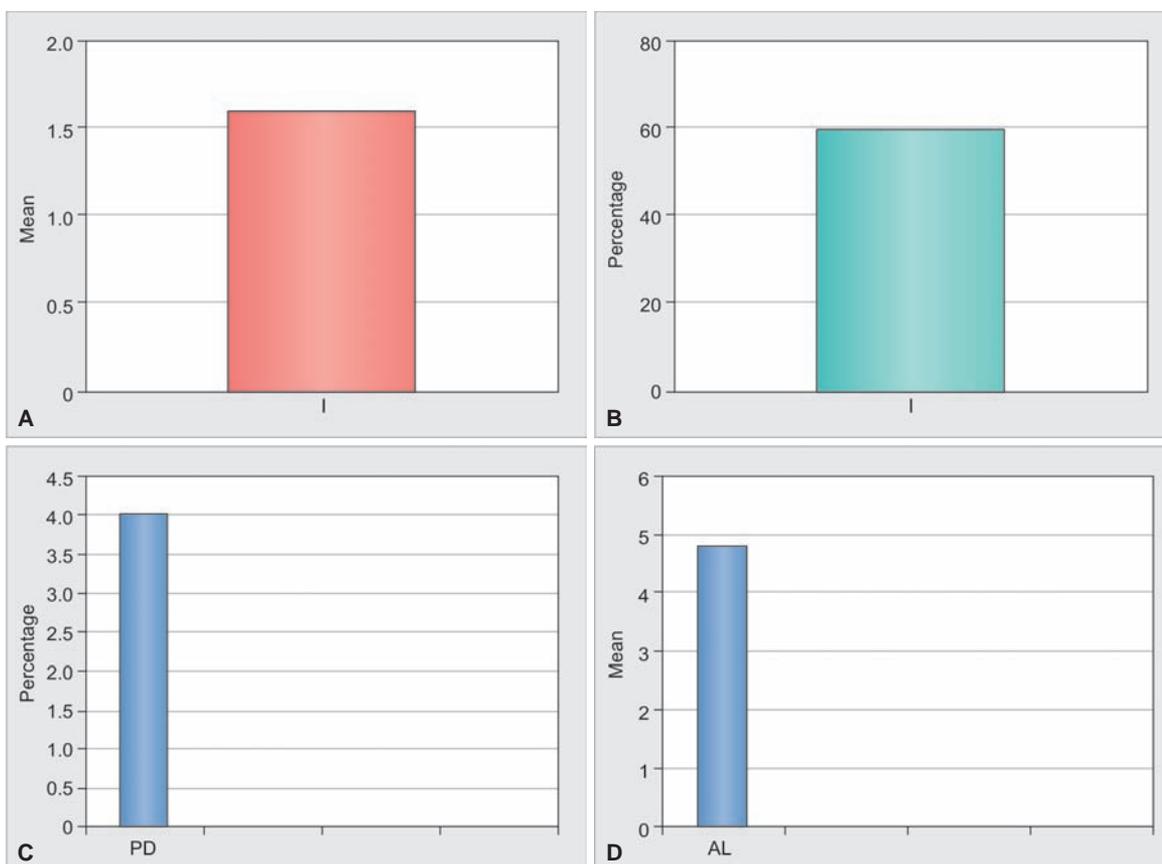
<i>Family No</i>	<i>Patient No</i>	<i>Age</i>	<i>Sex</i>	<i>Diagnosis/type</i>	<i>Presence or absence</i>		
					<i>AA</i>	<i>PG</i>	<i>PI</i>
I	1	42	Female	A.P	(-)	(+)	(+)
	2	23	Female	A.P	(-)	(-)	(+)
	3	22	Female	G	(-)	(+)	(+)
	4	19	Male	G	(-)	(+)	(+)
II	5	37	Male	A.P	(-)	(+)	(+)
	6	35	Female	A.P	(-)	(+)	(+)
	7	6	Female	No disease	(-)	(-)	(+)
	8	5	Male	No disease	(-)	(+)	(+)
III	9	45	Female	C.P	(-)	(+)	(+)
	10	25	Female	G	(-)	(+)	(+)
	11	12	Female	G	(-)	(+)	(+)
IV	12	49	Male	A.P	(-)	(+)	(+)
	13	45	Female	C.P	(-)	(+)	(+)
	14	17	Female	Healthy	(-)	(+)	(+)
	15	13	Female	G	(-)	(-)	(+)
V	16	55	Female	C.P	(-)	(+)	(+)
	17	16	Female	L.A.P	(-)	(+)	(+)
	18	14	Male	L.A.P	(-)	(+)	(+)

couples was 1.06, the % of sites of BOP was 60%, and the mean of PD and AL for all couples was 4 and 4.83 mm respectively (Graph 1). The mean of PL (I) for all children and young adults was 1.03, the percentage of sites of BOP was 46%, and the mean PD and AL were 2.81 and 3.21 mm respectively (Graph 2). In the age group of 5 to 16 years, only two brothers were affected by localized periodontitis, the first was a male aged 14 years old with the mean PD of 3.35 mm and AL 3.55 mm, and the percentage of sites

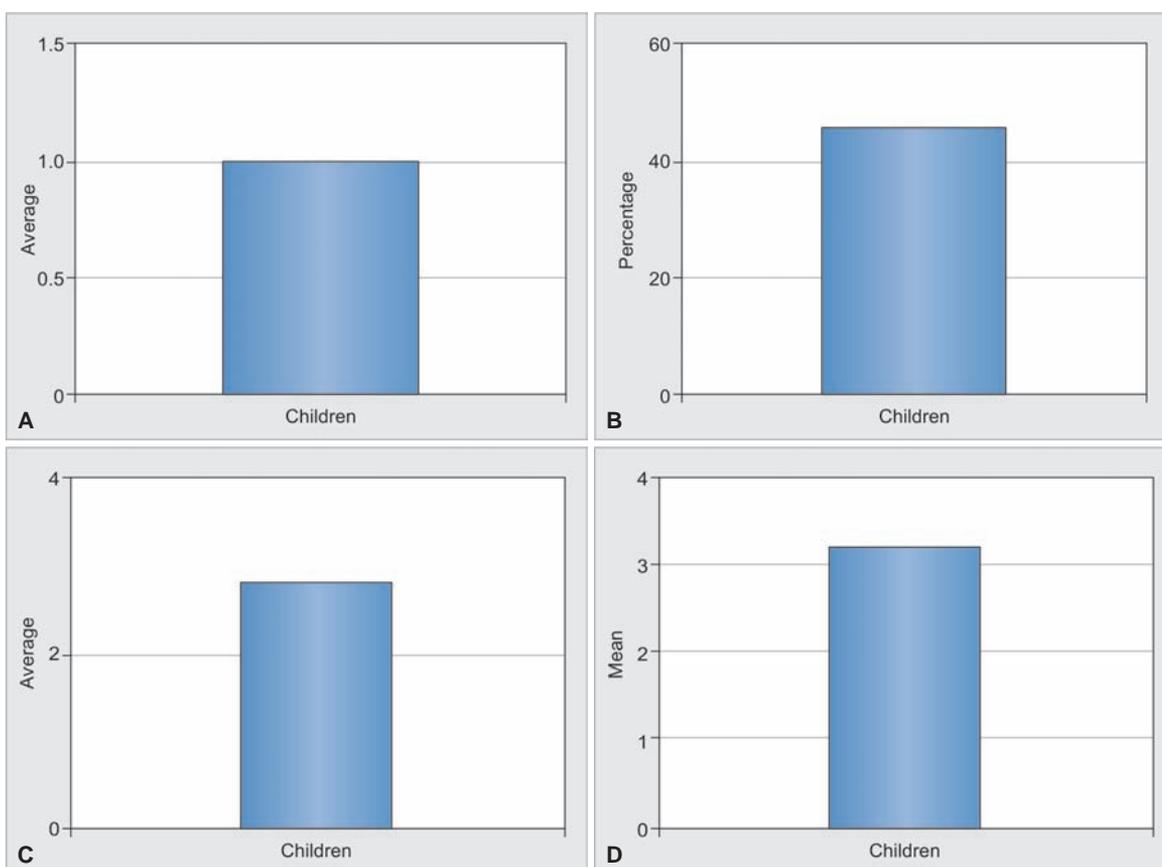
of BOP was 75%. The sister was 16 years old with the mean of PD as 4 mm and AL as 4.37 mm and the level of BOP was 55%.

### Microbiological Evaluation

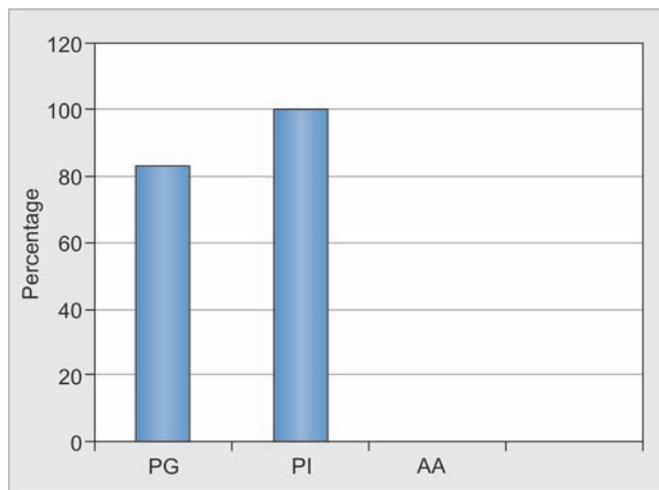
The prevalence of the three putative periodontal pathogens in the couples is listed in Table 2. All subjects were positive for PI, 15 out of 18 were also positive for PG, but AA was not detectable in any of the subjects (Graph 3).



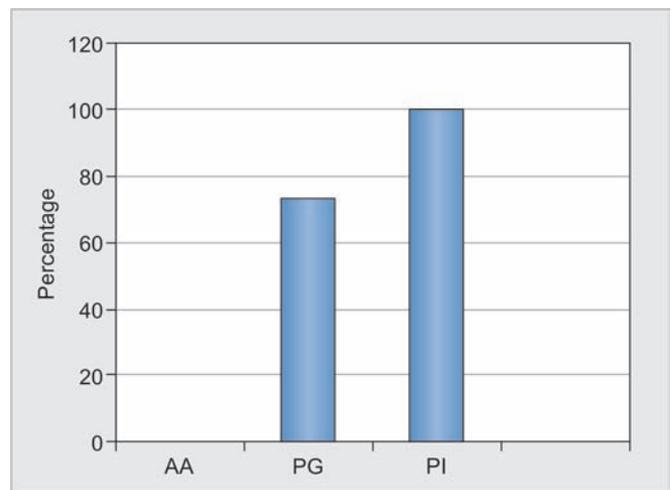
**Graphs 1A to D:** (A) The mean of PL (I) for all couples was 1.6%; (B) The percentage of sites BOP in all couples was 60%; (C) The mean PD for all couples was 4 mm; and (D) The mean AL for all couples was 4.83 mm



**Graphs 2A to D:** (A) Average PL (I) in all children; (B) Percentage of sites bleeding in all children; (C) The average of PD for all children and young adults; and (D) The mean AL in all children and young adults



**Graph 3:** The percentage of subgingival bacteria in all samples



**Graph 4:** The percentage of subgingival bacteria in all children and young adults

The presence or absence of these microorganisms in the children and young adults is listed in Table 2. The PG was present in 83% of the subjects. All subjects were positive for PI, and AA was not detectable in any one (Graph 3). All children were positive for PI, PG was present in 72%, but AA was not detectable in any of the children and young adults (Graph 4).

Table 2 shows the microbiological data for the children and young adults. The youngest, in whom PG and PI were present, was a 5-year-old boy. His sister was 6 years old and she was also PI positive.

### Distribution of the Microorganisms in Relation to Clinical Parameters

Five families were selected for the study forming five spouses. Only two probands could participate in this study and the other three could not for several reasons (2 edentulous, 1 died).

One young couple of the five families suffered from aggressive periodontitis, with one child of 5 years positive for PG and PI. The other child, a 6-year-old girl, showed positive for PI with the absence of any clinical manifestation for the disease. The other couple, both parents, suffered from periodontitis of the aggressive and chronic forms. Both subjects were positive for PG and PI with high levels. The two young daughters of this family were positive for PI, and one harbored both microorganisms PG and PI with slight signs of the disease.

Table 3 shows the periodontal destruction according to age, presence of PG, and eating from the same plate. Statistically, the association between these factors (age, PG, and same plate) and periodontal destruction was highly significant ( $p = 0.002^*$ ) and statistically significant at  $p < 0.05$ . The statistical analysis showed for each 1 year of increase in age, the odds of having periodontal disease multiplied by 1.39, i.e., age is a risk factor for

**Table 3:** Multivariate analysis showing associations between the presence of periodontal destruction and selected variables (n = 18)

Associated variables	Periodontal destruction <sup>†</sup>			
	OR	Suff.	p-value	95% CI
Age (years)	1.39	361	0.002 <sup>*</sup>	[1.049; 3.554]
Presence of PG (No)	1.00	9	1.000	[0.025; 39.976]
Eating from same plate (No)	0.71 <sup>§</sup>	5	0.667	[0; 4.416]

( ) Base outcome; CI: Confidence interval; Suff.: Sufficient statistic for the test of the null hypothesis that OR = 1; <sup>§</sup>Median unbiased estimates (MUE); <sup>\*</sup>Statistically significant at  $p < 0.05$ ; <sup>†</sup>Model score = 8.901;  $p \geq 0.014$ ; OR: Odds ratio

periodontal disease with the presence of PG and sharing the same plate.

### DISCUSSION

It is well known that human periodontal disease has a multifactorial etiology and not all subjects are equally susceptible for the development of periodontitis. Colonization with specific periodontal pathogens at a young age may be one of the factors that influences the genetic contribution when present and the initiation of periodontal destruction.<sup>19</sup> Several microbiological studies in children mentioned the presence of PI in edentulous children as early as 1 month after birth.<sup>4,5</sup> In a previous study, Petit et al<sup>20</sup> indicated that PG was isolated from a 5-year-old boy from the cheeks, the tonsillar area, and saliva, out of 36 children, all having PG-positive parents and for AA, they reported that only 5 out of 26 children had it. They concluded that colonization with PG usually does not occur at an early age, but for AA, if a child was positive, it implies that at least one of the parents was also positive for this microorganism. The youngest child in our study had a similar age as Petit et al's<sup>20</sup> study, with the only difference that PG and PI were isolated from the subgingival pockets, not from the tonsillar or saliva. In a more recent study, in Japan Kobayashi et al<sup>37</sup> observed

the presence of AA and PG in 104 Japanese children aged between 2 and 12 years using polymerase chain reaction (PCR) method. Toothbrushing has been used to collect plaque samples. The prevalence of AA in healthy subjects was 4.8%, those with gingivitis and periodontitis was 6.8% and 20.0% respectively, the prevalence of PG was 4.8% in healthy subjects, and 9.6 and 20.0% in those with gingivitis and periodontitis respectively. A total of 3 of all 104 subjects were found to be positive for both microorganisms, AA, and PG. The minimum age of a healthy subject positive for AA and PG was 3 years and 5 months and 5 years and 3 months old respectively. They concluded that the prevalence of these species is rarely present in the oral cavity of healthy children. In the current investigation, 100% of the children and young adults aged between 5 and 25 years old were positive for PI and 73% of these subjects were also PG-positive. The AA was not detectable in any of these subjects.

About 5 children under the age of 15 were positive for PI and 3 for PG with clinical attachment loss of 2 to 4 mm with at least one member of the parent having severe periodontal disease and positive for PG and PI.

This phenomenon may be due to transmission of microorganisms between family members.

This difference in the prevalence of PI and PG between both studies could be due to the different techniques used in each study and also to the difference in age of each group.

This investigation has shown a high prevalence of two putative periodontal microorganisms in both groups even with low number of samples, and PI and PG were found in 18 and 15 samples out of a total of 18 respectively. On the contrary, in Europe, the oral distribution of these microorganisms seems to be different, as also in North European patients with moderate to advanced periodontal disease.<sup>38,39</sup> A total of 927 subgingival samples were collected in this study, and 84 to 102 specimens were available from each subject. The PG was not detected in three patients, and only one site was positive in one patient. Intrafamilial transmission of AA in families with a high prevalence of aggressive periodontitis has been suggested by several authors.<sup>34,35</sup> In the present study, AA was not detectable in any of our groups. Many research groups have shown a close relationship between specific pathogens and lesions in children and gingivitis.<sup>36,40</sup> Relatively little is known about when such colonization takes place in the oral flora. Intrafamilial transmission of periodontal bacteria has been demonstrated, and significant positive association has been found in species detection between caregiver and child.<sup>35</sup>

In more recent research, Kobayashi et al<sup>37</sup> studied the colonization of 11 periodontal bacterial species in 78 Japanese children aged from 3 to 9 years and 68 mothers

using the PCR method, and statistical analysis revealed that the detection of several bacterial species in children was consistent with that in their mothers and these microorganisms increased in number with age. This statement could explain in part that, in our study, all children were not only negative for AA, but so also were their mothers. The PI was present in all children and mothers at the same time.

A recent epidemiological investigation from Syria (data not published) studied the prevalence of aggressive periodontitis. In a group of 250 subjects from different areas in Syria, aged between 14 and 25 years, only 20 patients were selected for microbiological study using the DNA probe technique.

All the above patients were diagnosed with aggressive periodontitis, the localized or generalized form. All patients were positive for PI and PG. The AA was detectable only in 7 patients out of 20. The low prevalence of AA even with diseased subjects leads to the question: Is AA rarely detectable in this area?

Could the ethnic background play a role in the prevalence of several bacteria in this area also? To answer these questions, we need further research to clarify this point.

## CONCLUSION

This study shows a high prevalence of putative periodontal microorganisms in children and young adults of Lebanese periodontitis parents and a microbiological similarity between the children and their mothers. All these factors could indicate a high risk of developing periodontal disease in the future.

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