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TRANSMISSION OF *PORPHYROMONAS GINGIVALIS* FROM CAREGIVERS TO CHILDREN

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SUMMARY:

Periodontal diseases are socially significant diseases, which occur in adults but in children and adolescents as well. Despite a low prevalence of aggressive periodontitis at a young age, its severity is a challenge for pediatric dentistry.

The goal of this study is to find if the prevalence of *Porphyromonas gingivalis* among children whose parents suffer from periodontal diseases is greater than among children with healthy parents.

Methods:

· Polymerase chain reaction (PCR).

· Culture method.

When PCR was used *P.gingivalis* was found in 35.5% of parents with periodontitis and in 6,5% of their children, children with healthy parents and their parents. No statistically significant relation (P>0.05) between periodontal parents and their children was found.

When culture method was used *P.gingivalis* was not detected.

Studying such correlations and standardizing methods of detection could contribute the evaluation of periodontal disease risk in adolescents.

Key words: *P.gingivalis*, transmission, periodontitis, plaque.

INTRODUCTION

Periodontal diseases often are neglected in childhood and adolescence. Although gingivitis is the most common form of periodontal disease, a localized aggressive form that causes bone loss around molars and incisors occurs in a small number of subjects usually after puberty.[1] Among the several pathogens that are known to be related to periodontal disease, *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia* and *Campylobacter rectus* can be found in individuals with different severities of periodontitis [2-4]. Umeda et al.[5] evaluated the distribution of periodontal pathogens in 56 children (from 1 to 15 years old) and their parents. A PCR-based method was used to determine the prevalence of microbial organisms in saliva and dental plaque samples. A positive correlation was observed for the presence of oral pathogens between parents and their children. Thus, the authors suggested a possible vertical familiar transmission of those periodontal pathogens. In a separate study, Kobayashi et al.[6] also investigated the progress of infection by periodontal pathogens and the role of the child's mother in this transmission process. The results indicated that the colonization of periodontal bacterial in children increased with age and that the infant's flora is acquired from his or her primary caregiver. [1]

The present study as some other[7] that evaluate the pattern of transmission is focused on the prevalence of *P. gingivalis* in parents who suffer from periodontal disease and their children.

MATERIALS AND METHODS

Subjects

Sixty-two parents-volunteers and their children participated in this clinical study. Permission for this study was obtained from the Ethical Committee of the Medical University of Sofia. The parents of the children received detailed information regarding the nature and the procedures involved in the study and signed informed consent forms. A complete periodontal examination of parents was performed by trained and calibrated examiners at least one week before microbial samples were collected to measure the probing pocket depth, the clinical attachment level, the plaque and bleeding upon probing scores. For the purpose of the present study and based on the obtained clinical record, participants were divided into four groups. Group A (n=31) included parents with periodontal disease, group B (n=31) - their children, group C (n=31) - healthy parents, group D (n=31) -

their children. The children were examined by pediatric dentists and the plaque (PI) and PSR index were determined as well as the gingival status.

Exclusion criteria

Subjects presenting with uncontrolled systemic diseases and those who were immunocompromised, who had taken antibiotics within 3 months before the clinical and microbial examination, had been undergoing periodontal treatment 3 months before the beginning of the study, were not included in the study. For control group C subjects who had no upper molars or second lower incisors were also excluded.

Specimen collection

For group A, sampled places were the deepest pockets and if they were not at the first upper molar or at the lower lateral incisor, at the closest teeth. For group C - the sulci of the first upper molar and lower lateral incisor. For group B and D sampled places were between the first and second molars in children with primary dentition; between the second primary molar and first permanent molar in children with mixed dentition; between the second premolar and first permanent molar in children with late mixed dentition or permanent dentition.[1] Before the collection procedure, cotton rolls were applied to prevent contamination of the sampling area with other oral fluids. The supragingival biofilm was gently removed with sterile cotton pellets [8] and plaque samples were collected using sterile paper points inserted in sulci or pockets during 15 seconds This procedure was done for each of the four sites previously selected and the paper points of each subject were placed in a same microtube with 1 ml of reduced transport fluid (RTF) [9]. The samples were stored at –22°C until the analyses.

Culture method

From each sample 50 µl aliquot was taken and plated on Brucella (Oxoid) 5% Sheep Blood Agar with Vitamin K and Hemin (BD BBL™). Plates were cultivated in anaerobic atmosphere at 37°C for 48h. To obtain pure culture black pigmented colonies were recultivated at the same conditions and then a biochemical test (REMEL, Italy) was performed. For further PCR detection bacterial suspension was prepared from the rest of bacterial colonies suspended in 100 µl 0.9% NaCl.

PCR method

Bacterial genomic DNA from direct plaque samples and from plated plaque samples was extracted by using a commercially available kit (DNA-SORB-A, Sacacae Biotechnologies Srl, Italy). The species-specific polymerase chain reaction (PCR) primers used in this study are listed in

Primer pairs (52 –32)	Amplicon length (bp)
P.gingivalis 404	
AGG CAG CTT GCC ATA CTG CG	
ACT GTT AGC AAC TAC CGA TGT	

Fig. 1. Species-specific primers for PCR

The primer sequences were selected as described by Ashimoto et al.[10] Briefly, the PCR mixture contained 1 pM/µl of each primer, 1.5 mM MgCl2, 200 µM of deoxyribonucleotide triphosphate (Invitrogen), 1x Taq polymerase buffer, 1.25 U of Taq DNA polymerase and 5 µl of template DNA in a 20 µl reaction volume. PCR reactions were carried out in a DNA thermal cycler (Gene Amp, PCR System 9700, Aplied Biosystems). The PCR profile included an initial denaturation at 94°C for 3 min followed by 36 cyclers consisting of 94°C for 45 s, 55°C for 30 s, 72°C for 45 s and a final extension at 72°C for 10 min[11]. Following amplification, 10 µl of PCR products were analyzed by electrophoresis on a 2% agarose gel. The gel was stained with 0.5 µg/ml ethidium bromide and visualized by ultraviolet light illumination. The size of the PCR products was estimated using a molecular marker Super ladder.[11] *P. gingivalis* W83 and ATCC 33277 were used as positive control and sterile distilled water as negative control for each reaction series.

Statistical analysis

The frequencies of bacteria in the study groups were analyzed using the chi-squared test. All tests were performed using statistical software (STATISTICA and SPSS19). Results were considered statistically significant at P-value <0.05.

RESULTS

In the present study the parents with periodontal disease (Group A) who were analyzed, were composed of 13 males and 18 females (Table 1). Their children (Group B) were 19 boys and 12 girls. The healthy parents (Group B) were 9 males and 22 females. Their children (Group D) were 16 boys and 15 girls (Table 2).

Table1. Distribution of the parents' population according to age and gender

Group	A		C	
Gender	Male	Female	Male	Female
n	13	18	9	22
Age	31-47	36-56	32-39	30-39
X^2	P>0,05			

No statistically significant age and gender differences between control groups C and D and experimental groups A and B were found.

Table2. Distribution of the children population according to gender

Group	Male		Female		Total	
	N	%	N	%	N	%
D	16	51,6	15	48,4	31	100
B	19	61,3	12	38,7	31	100
Total	35	56,5	27	43,5	62	100
X^2	X^2=0,590 P> 0,05					

Since gingival and oral-hygiene status are age dependent and for the purpose of the present study groups of children were divided into three age groups: first between 3 and 6 years, second between 6 and 12 and third between 12 and 16 (Table 3).

Table3. Children age groups

Age group	3-6 years		6-12 years		12-16 years		Total	
	N	%	n	%	N	%	N	%
Group D	9	29	18	58,1	4	12,9	31	100
Group B	5	16,1	20	64,5	6	19,4	31	100
Total	14	22,6	38	61,3	10	16,1	62	100
X^2	X^2=1,65 P>0,05							

Almost 80% of plated samples on Brucella 5% Sheep Blood Agar were positive for dark pigmented colonies after two days cultivation in anaerobic atmosphere but after cultivation on Blood Agar under aerobic conditions some of them survived, which means that these are not anaerobes and the other samples were negative for *P.gingivalis* when biochemical tests were applied.

When PCR method was used 11(35,5%) patients from group A were positive for *P.gingivalis*, while in each of other three groups the positive cases were 2 (6,5%) (Table4).

Table4. Prevalence of P.gingivalis in study groups detected with PCR method

	Group A	Group B	Group C	Group D
Male	5(16.1%)	2(6.5%)	0(0%)	2(6.5%)
Female	6(19.4%)	0(0%)	2(6.5%)	0(0%)
Total	11(35.5%)	2(6.5%)	2(6.5%)	2(6.5%)

Prevalence of *P.gingivalis* is greater in Group A ($P<0.05$) compared to other groups. There was no statistically significant difference between male and female patients.

According to periodontal status 17 were patients with periodontitis chronica localisata, 6 of them were positive, from 8 patients with periodontitis chronica generalisata 3 were positive and from 6 patients with gingivitis catarrhalis 2 were positive (Table5).

Table 5. Prevalence of P.gingivalis in Group A according to periodontal status

Group A	Positive		Negative		Total	
Periodontitis chronica localisata	6	35,3%	11	64,7%	17	100%
Periodontitis chronica generalisata	3	37,5%	5	62,5%	8	100%
Gingivitis catarrhalis	2	33,3%	4	66,7%	6	100%
Total	11	35,5%	20	64,5%	31	100%

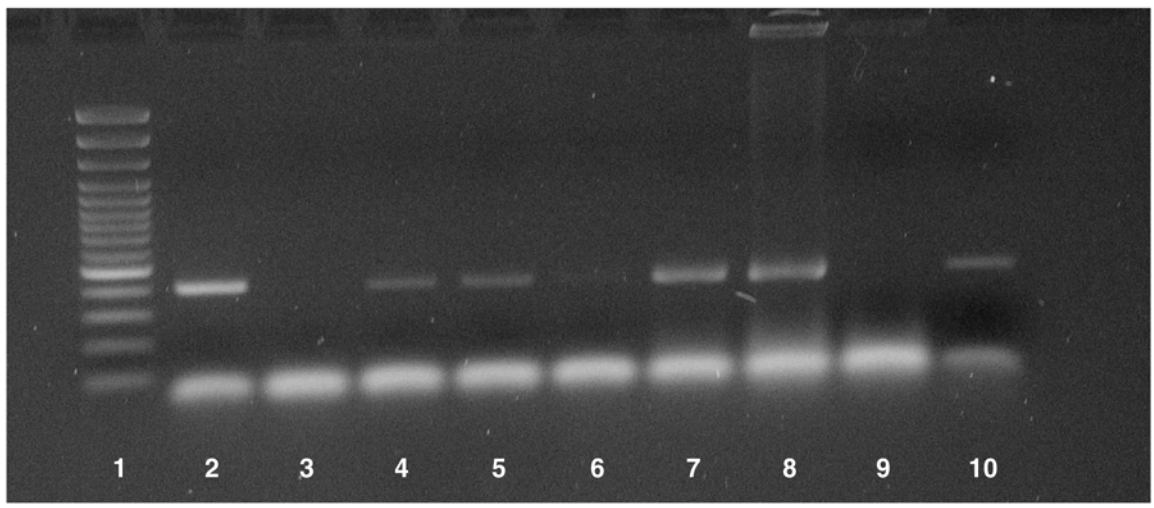
The present study did not find statistically significant difference (P>0.05) in the prevalence of *P.gingivalis* between children with healthy parents and children whose parents were with periodontal disease (Table 6).

Table 6. Prevalence of P.gingivalis in groups B and D

Group	Children negative for *P.gingivalis*		Children positive for *P.gingivalis*		Total	
	n	%	n	%	N	%
Group B	29	93,5	2	6,5	31	100
Group D	29	93,5	2	6,5	31	100
X^2	$X^2=0$ P>0,05					

The results above were obtained when PCR was applied to direct plaque samples (Fig.2). PCR detected no positive for *P.gingivalis* samples, when these were prepared from bacterial suspensions obtained from culture media.

Fig. 2. Gel electrophoresis of PCR products



After running the gel the DNA fragments can be seen with a UV light. They are separated according to their weight and positive samples are at the same level as the positive controls

1 - marker Super Ladder
2, 10 - positive control for *P.gingivalis*
3 - negative control
4, 5, 7, 8 - positive samples
6, 9 - negative samples

DISCUSSION

The prevalence (35.5%) of *P.gingivalis* in patients with chronic periodontitis according to our study is lower than the prevalence (54%) according to other study[13] using real-time PCR as detecting method. *P.gingivalis* was detected in 77.3% of samples from patients with early periodontitis using culture method and in 85,6% using immunofluorescence.[14] Some studies report higher levels in gingivitis than in early periodontitis.[15] Our study detected same prevalence in patients with periodontitis and gingivitis.

The positive samples from group D were from two eight year old boys with gingivitis localisata and mixed dentition and from group B were from two sixteen-year old boys with gingivitis ulceronecroticans and permanent dentition. The prevalence in both groups was 6.5%. Similar studies investigated the detection of periodontal pathogens by studying dental plaque and/or saliva obtained from children at different age, gender and ethnic group and reported frequency between 6.5% and 21%.[11]

Furthermore the parents of positive for *P.gingivalis* children were negative and there was no evidence for

transmission of the latter bacteria from caregivers to their children. Some authors using BANA-test found higher prevalence of BANA-positive bacteria (including *P.gingivalis*) in children whose caregivers were BANA-positive as well.[1] According to other study no *A.actinomycetemcomitans* transmission was found between Brazilian women with severe chronic periodontitis and their children.[16]

The discrepancy between PCR-based and culture-based studies could be explained by the lower detection limit of PCR. For PCR, the detection limit is typically 25–100 cells, whereas, for culturing 103–104 bacteriaare needed before detection. The sensitivity of bacterial culturing is rather low, especially for non-selective media, and therefore, low numbers of a specific pathogen in a subgingival sample will remain undetected.[17] Another potential source of error in the culturing procedure for anaerobic bacteria resides in the processing of samples, including transport media[9]. The agreement between culture and PCR method in detecting the absence of P.gingivalis, when PCR was performed with the bacterial suspension obtained after cultivating of plaque samples, supposed that there were no viable bacteria. Additionally, PCR will detect not only viable but also moribund and dead cells (Sanz et al. 2004)[17]. All these factors explain why in our study PCR detected P. gingivalis and microbial culturing failed, even though the same samples had been used. The same observation of a lower prevalence by culture technique was found when analysing Enterococcus faecalis in endodontic lesions and oral rinse samples.[17] Some studies (Riviere et al., 1996)[15]detected *P.gingivalis* more frequently in early periodontitis than in health, while other (Tanner et al., 1998)[18] did not find such association, using culture method.

CONCLUSION

The primery etiology of periodontitis is the presence of specific bacteria in the subgingival plaque biofilm. Thus it seems likely that indentificationof certain species could aid in the periodontal risk assessment. Some studies found positive relations between oral microbiota in children and their parents with periodontal diseases, and some as the present study did not detect such relation. Obviously this topic depends on many factors, such as geographic population, research methods, clinical status e.t.c. Studying such correlations and standardizing methods of detection could contribute the evaluation of periodontal disease risk in adolescents.

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REFERENCES:

1. Lee Y, Tchaou WS, Welch KB, Loesche WJ. The transmission of BANA-positive periodontal bacterial species from caregivers to children. *J Am Dent Assoc.* 2006 Nov;137(11):1539-46. [PubMed]

2. Tanaka S, Murakami Y, Ogiwara T, Shoji M, Seto K, Nagasaki M, et al. Frequency of reactivity for Porphyromonas gingivalis and Prevotella spp. in supra- and subgingival plaques, and periodontal clinical parameters according to subject age. *J Periodontol.* 2002 Aug;73(8):877-85. [PubMed] [CrossRef]

3. Cortelli JR, Cortelli SC, Jordan S, Haraszthy VI, Zambon JJ. Prevalence of periodontal pathogens in brazilians with aggressive or chronic periodontitis. *J Clin Periodontol.* 2005 Aug;32(8):860-6. [PubMed] [CrossRef]

4. Bodet C, Chandad F, Grenier D. Pathogenic potential of Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia, the red bacterial complex associated with periodontitis. *Pathol Biol (Paris).* 2007 Apr-May;55(3-4):154-62. (in French) [PubMed] [CrossRef]

5. Umeda M, Miwa Z, Takeuchi Y, Ishizuka M, Huang Y, Noguchi K, et al. The distribution of periodontopathic bacteria among Japanese children and their parents. *J Periodontal Res.* 2004 Dec;39 (6):398-404. [PubMed] [CrossRef]

6. Kobayashi N, Ishihara K, Sugihara N, Kusumoto M, Yakushiji M, Okuda K. Colonization pattern of periodontal bacteria in Japanese children and their mothers. *J Periodontal Res.* 2008 Apr;43(2):156-61. [PubMed] [CrossRef]

7. Greenstein G, Lamster I. Bacterial transmission in periodontal diseases: a critical review. The impact of the maternal's periodontal status on the detection of periodontal pathogens in newborn children. *J Periodontol.* 1997 May;68(5):421-31. [PubMed]

8. Cortelli SC, Cortelli JR, Aquino DR, Holzhausen M, Franco GC, Costa Fde O, et al. Clinical status and detection of periodontopathogens and *Streptococcus mutans* in children with high levels of supragingival biofilm. *Braz Oral Res.* 2009 Jul-Sep;23(3):313-318. [PubMed] [CrossRef]

9. Syed SA, Loesche WJ. Survival of human dental plaque flora in various transport media. *Appl Microbiol.* 1972 Oct; 24(4):638-644. [PubMed]

10. Ashimoto A, Chen C, Bakker I, Slots J. Polymerase Chain Reaction

detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. *Oral Microbiol Immunol.* 1996 Aug;11(4):266-273. [PubMed]

11. Kulekci G, Leblebicioglu B, Keskin F, Ciftci S, Badur S. Salivary detection of periodontopathic bacteria in periodontally healthy children. *Anaerobe.* 2008 Feb; 14(1):49-54. [PubMed] [CrossRef]

12. Van Assche N, Van Essche M, Pauwels M, Teughels W, Quirynen M. Do periodontopathogens disappear after full-mouth tooth extraction? *J Clin Periodontol.* 2009 Dec;36(12):1043-1047. [PubMed] [CrossRef]

13. Byrne SJ, Dashper SG, Darby IB, Adams GG, Hoffmann B, Reynolds ES. Progression of chronic periodontitis can be predicted by the levels of *Porphyromonas gingivalis* and Treponema denticola in subgingival plaque *Oral Microbiol Immunol.* 2009 Dec;24(6):469-477. [PubMed] [CrossRef]

14. Kamma JJ, Nakou M, Gmьr R, Baehni PC. Microbiological profile of early onset/aggressive periodontitis patients. *Oral Microbiol. Immunol.* 2004 Oct;19(5): 314-21. [PubMed] [CrossRef]

15. Riviere GR, Smith KS, Tzagaroulaki E, Kay SL, Zhu X, DeRouen TA, et al. Periodontal status and detection frequency of bacteria at sites of periodontal health and gingivitis. *J Periodontol.* 1996 Feb;67(2):109-115. [PubMed]

16. Rego RO, Spolidorio DM, Salvador SL, Cirelli JA. Transmission of Aggregatibacter actinomycetemcomitans between Brazilian women with severe chronic periodontitis and their children. *Braz Dent J.* 2007; 18(3):220-4. [PubMed] [CrossRef]

17. Van Assche N, Van Essche M, Pauwels M, Teughels W, Quirynen M. Do periodontopathogens disappear after full-mouth tooth extraction? *J Clin Periodontol.* 2009 Dec;36(12):1043-1047. [PubMed] [CrossRef]

18. Tanner A, Maiden MF, Macuch PJ, Murray LL, Kent RL Jr. Microbiota of health, gingivitis, and initial periodontitis. *J Clin Periodontol.* 1998 Feb;25(2):85-98. [PubMed] [CrossRef]

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