Periodontal disease in mothers indicates risk in their children

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Introduction. It is well established that severe periodontitis clusters in families, but there are no data about the relationship between mothers with chronic periodontitis and their children's periodontal status.

Objective. To evaluate a risk for periodontal diseases in children of periodontally diseased and healthy mothers.

Methods. Four study groups were included: (I) 20 female patients with untreated generalized severe chronic periodontitis, (II) their children (34), (III) 13 periodontally healthy mothers and (IV) their children (13). Material was collected from years 2004–2006. The clinical examination included registration of visible plaque index, modified gingival index and, bleeding sites on probing. Periodontal microbiological samples were obtained from all study subjects and the isolates were identified according to morphology and biochemical profiles; similar interfamilial pathogens were compared by PCR-technique.

Results. The children of diseased mothers more frequently had periodontal diseases, especially gingivitis. In addition, clinical parameters of gingival inflammation were more expressed and oral hygiene was worse in this group of children. VPI and VPI% of the diseased and healthy mothers differed significantly. The most common oral pathogens were *P. intermedia/nigrescens* and *A. actinomycetemcomitans*. The children of healthy mothers harboured pathogens less frequently than the children of diseased mothers. The sharing of *P. intermedia/nigrescens* was more frequent (5 families) than *A. actinomycetemcomitans* (2 families).

Conclusion. Maternal indicators, such as periodontitis, hygiene habits, and periodontal microflora are risk factors for childhood periodontal diseases, and might be predictive of future childhood and adolescent periodontitis.

Introduction

Periodontitis is a chronic inflammation of the periodontium that results in periodontal tissue destruction and alveolar bone loss. Tissue destruction occurs as a consequence of the host's attempt to eliminate bacteria from the gingival sulcus by evoking an immunoinflammatory response. Left untreated, it may lead to complete loss of dental attachment structures, and subsequent loss of teeth.

A number of different forms of periodontal disease can present in children and adults[1]. The exact cause of each of these disease forms is still unclear but is likely to be influenced by the composition of the periodontal microbiota and the competency of the host response. Environmental and genetic factors will also influence the balance between the two opposing factions, microbe and host[2].

Although the most important pathogens are well-known in the literature (e.g., *Prevotella intermedia/nigrescens*, *Micromonas micros* and *Aggregatibacter actinomycetemcomitans*), the spectrum of pathogens may vary. Different spectrums of pathogens have been associated with different forms of periodontal disease[3,4]. Also, the distribution of periodontal microbes varies according to geography and region, as well as age and socio-economic status[5–9]. Among different populations, the presence of the pathogens may be more reflective of the local environment; conversely, the presence of suspected pathogens may not be strictly related to the disease[10,11].

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It is well established that severe periodontitis clusters in families[12,13]. This suggests that susceptibility factors for the onset and progression of the disease may be transferred from person to person in families. The transfer of genetic susceptibility, common behavioural factors, and exposure to environmental agents including transmission of putative pathogenic bacteria may be involved.

On the other hand, there are no data about the relationship between mothers with chronic periodontitis and their children's periodontal status although chronic periodontitis is one of the most common among all forms of periodontal disease.

The aims of this study were to (i) compare the periodontal status and oral hygiene of mothers with generalized severe chronic periodontitis with the periodontal status of their children, periodontally healthy mothers and their children; and (ii) determine the spectrum of obligatory and potential pathogens of mothers and children by phenotyping and genotyping, including their possible spread in the family.

Materials and methods

Study groups

The following four study groups were included in this study; the first group included 20 female patients with untreated generalized severe chronic periodontitis (median age 35, ranges 31–44 years) and the second group was composed of their children (21 female and 13 male; median age 12, range 5–17 years). The third group included 13 periodontally healthy mothers (median age 36, range 29–43 years) and the fourth group was comprised their children (nine female and four male; median age 12, range 10–16 years). The age of periodontally ill and healthy mothers and their children was not significantly different ($P > 0.05$).

All patients had no history of systemic disease or antibiotic therapy within the 6 months prior to sampling. Main inclusion criteria of the study were the following; healthy and periodontally diseased mothers with their children (fully erupted first incisors

and first molars) with first permanent teeth, up to 18-year old.

Severe cases of generalized chronic periodontitis were diagnosed based on gingival inflammation; periodontal breakdown with pocket depth >6 mm in all sextants; minimum radiographic marginal alveolar bone loss less than one-third of the root length in at least two quadrants, and a Community Periodontal Index of Treatment Needs (CPITN) score of 4 in at least three sextants. All the patients had at least 22 natural teeth. Gingivitis was defined as gingival redness, swelling, and loss of contour, marginal bleeding, and pseudo-pockets in the absence of bone loss[1]. Healthy individuals were defined as having no radiographic or clinical evidence of inflammation and attachment loss. All patients were recruited consecutively from new referrals to the Policlinic of the Tartu University Dental Clinic from January 2004 to December 2006.

The Ethics Review Committee on Human Research of the University of Tartu approved the study protocol (Protocol No. 118/72, 25.08.2003).

Clinical examination

The baseline examination included the registration of dental plaque, gingival inflammation, and presence of suppuration on probing at four sites, periodontal probing depth (PPD), and attachment level at six sites. The third molars were excluded from oral examination.

Oral hygiene status of an individual was represented by two indicators; the visible plaque index percentage – VPI%, and the VPI by Silness and Löe[14]. Gingival inflammation was given as a modified gingival index (MGI; 0 – healthy with no bleeding on probing; 1 – pinprick, bleeding on probing, 2 –immediate and overt bleeding on probing, 3 – spontaneous bleeding) and as the frequency of bleeding sites on probing (BOP), expressed as a percentage of all sites. PPD was measured to the nearest millimetre from the gingival margin to the bottom of the gingival sulcus/pocket and relative attachment level (RAL) from the cemento-enamel junction to the bottom of the periodontal pocket using WHO periodontal probes. Measurements were

performed at six probing points per each tooth. The mean values for PPD and attachment levels sites were calculated for each patient.

Microbiological investigations

In patients with periodontitis, the six deepest inflamed periodontal pockets, three in upper jaw and three in lower jaw, were selected for sampling. In healthy persons and persons with gingivitis, six sulci (first molars and first incisors in opposite jaws) were selected.

The pooled samples were obtained with a sterile Gracey 11/12 M and 13/14 M curette. The plaque was transferred to the 2 mL VMGA III medium vials and the samples were processed to the laboratory within 4 h[15].

For the detection of common periodontal pathogens[3,16], bacteria were cultivated as follows. The collected samples were serially diluted in Brucella broth (Oxoid, Basingstoke, Hampshire, UK), and 100 lL aliquots from the dilutions were inoculated onto the Brucella agar (Oxoid), enriched with 5% horse blood and 1% menadione, and TSBV (Oxoid) agar. The Brucella Agar plates were incubated in an anaerobic chamber (Sheldon Manufacturing Inc.) and on TSBV plates under microaerobic (CampyPak; Oxoid) conditions. After incubation at 35 °C for 5–7 days, the isolates were identified according to colonial and cellular morphology, the potency disc pattern (Vancomycin, Kanamycin, Colistin, Brilliant Green, and Oxgall), catalase, oxidase and spot indole reactions, long-wave UV light fluorescence, and MUG assay[16]. All anaerobic microorganisms were tested for absence of growth under microaerobic conditions.

Genotyping of pathogens

Three- to four-day-old cultures on Wilkins-Chalgren Blood Agar (Oxoid) were suspended in 500 mL of lysis buffer [50 mM Tris–HCl (pH 8.0), 5 mM EDTA, 50 mM sodium chloride, 1 mg of pronaseB (Roche Diagnostics GmbH, Mannheim, Germany) per ml, 1% sodium dodecyl sulphate], and the mixture was incubated for 60 min at 56 °C. After centrifugation ($12,000 \times g$) for 10 min, DNA was extracted twice with 70% phenol–water–chloroform, adjusted to pH 7.6 with 1 M Tris–HCl (pH 8.3), and precipitated with a 0.8 volume of isopropyl alcohol and 0.1 volume 3 M potassium acetate. A DNA pellet was rinsed with ice-cold 70% ethanol, and was resuspended in distilled water.

Microbiologically similar interfamilial species of pathogens (*P. intermedia/nigrescens* and *A. actinomycetemcomitans*) were determined by comparison using arbitrary PCR. For genotyping, AP-PCR (arbitrary primed polymerase chain reaction) was carried out with the primer set of ERIC1R (5′-ATGTAAGCT CCTGGGGATTCAC-3′) and ERIC2 (5′-AAG-TAAGTGACTGGGGTGAGCG-3′). DNA amplification was performed for 35 cycles, with each cycle comprising 45 s at 95 °C, 1 min at 45 °C, and 1 min at 65 °C, with a single final extension step for 5 min at 72 °C. DNA amplification was done in a DNA thermal cycler Mastercycler Personal (Eppendorf) In brief, DNA amplification was performed in a 50 lL reaction mixture consisting of 7.5 mM Tris–HCl (pH 8.8), 20 mM ammonium sulphate, 0.1% (v/v) Tween 20, 4 mM magnesium chloride, deoxynucleoside triphosphate at a concentration of 400 mM, and 2.5 U of *Taq* DNA polymerase (Solis Biodyne, Tartu, Estonia). The reaction products were subjected to agarose gel electrophoresis with a 1.5% agarose (SeaKem® GTG® Agarose, FMC Bioproducts, Rockland, Maine, USA) gel and were visualized under UV transillumination following ethidium bromide staining.

Statistical analysis

The characteristics of healthy and diseased mothers and their children were compared with Mann–Whitney *U*-test and Fisher's test. Percentages were compared with proportion test. *P*-values < 0.05 were considered as statistically significant. The ʀ version 2.4 (The R Foundation for Statistical Computing) was used for statistical analysis.

Results

Comparing children of healthy mothers and mothers with periodontitis we found that

Table 1. Periodontal status of children of diseased and healthy mothers.

Periodontal status	Children of diseased mothers (*n* = 34) % (*n*)	Children of healthy mothers (*n* = 13) % (*n*)	*P*-value
Healthy	29 (10)	85 (11)	<0.001
Gingivitis	56 (19)	15 (2)	0.02
Periodontitis	15 (5)	None	0.313

Table 2. Comparison of clinical parameters of children of healthy and diseased mothers.

Clinical parameters		Groups		
		CHM	CMP	*P*-value
BOP	Median	23.6	35.9	<0.001
	Ranges	10.8–38.3	12.1–67.5	<0.001
MGI	Median	0	2	NM
	Ranges	0	2–3	NM
VPI	Median	0.9	1.3	0.006
	Ranges	0.3–1.8	0.9–2.3	0.006
VPI%	Median	28.1	45.3	<0.0001
	Ranges	15.8–42.5	24.4–73.5	<0.001

CHM, children of healthy mothers; CMP, children of mothers with periodontitis; BOP, bleeding sites on probing; MGI, modified gingival index; VPI%, visible plaque index percentage.

children of diseased mothers had more frequently periodontal diseases, especially gingivitis (Table 1).

Furthermore, clinical parameters of gingival inflammation (BOP, MGI) were more expressed and oral hygiene (VPI, VPI%) was worse in this group of children (Table 2). Mean and ranges of clinical pocket depth (CPD) and RAL values of the diseased mothers were following 5.2 (3.9–6.8) and 6.0 (4.0–7.2). The CPD data of their children with periodontitis were 3.6 (3.2–3.8) and those of RAL 3.6 (3.2–3.9). We found that such oral hygiene parameters as VPI and VPI% differed significantly when the diseased and healthy mothers were compared ($P < 0.001$).

Most commonly isolated oral pathogens in our study were *P. intermedia/nigrescens* (21 isolates) and *A. actinomycetemcomitans* (16 isolates). *Micromonas micros* was isolated in three cases. Also, coexistence of two pathogens occurred in six events. The children of healthy mothers' harboured pathogens less frequently than the children belonging to the diseased mothers study group (Table 3).

In nine families with diseased mothers, we found pathogen of the same species in two or more family members. Healthy mothers and their children did not share similar pathogens. Using genotyping we found that from nine suspected cases, the interfamilial

Table 3. The distribution of pathogens among different study groups.

Presence of pathogens	Status of patients/number of pathogens (%)					
	HM (*n* = 13)	MP (*n* = 20)	*P*-value	CHM (*n* = 13)	CMP (*n* = 34)	*P*-value
Actinobacillus actinomycetemcomitans	0 (0)	6 (30)	0.06	0 (0)	10 (29)	0.043
Prevotella intermedia/nigrescens	2 (15)	9 (45)	0.132	1 (8)	9 (26)	0.244
Micromonas micros	0 (0)	1 (5)	1	0 (0)	2 (6)	1
Any pathogen	2 (15)	13 (65)	0.011	1 (8)	18 (53)	0.007
Two or more pathogens	0 (0)	3 (15)	0.261	0 (0)	3 (9)	0.55

HM, healthy mothers; MP, mothers with periodontitis; CHM, children of healthy mothers; CMP, children of mothers with periodontitis.
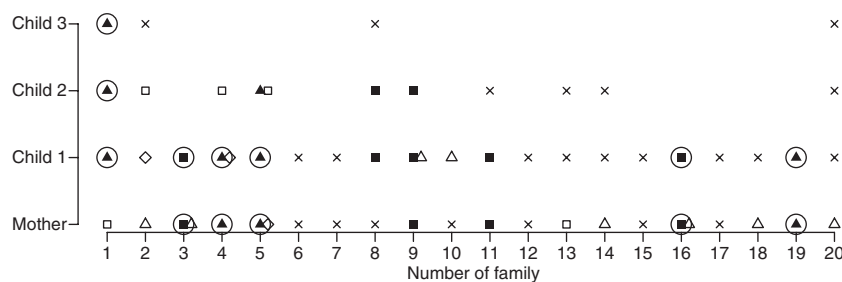
Fig. 1. The interfamilial spread of pathogens of mothers with periodontitis and their children. □, *Actinobacillus actinomycetemcomitans*; Δ, *Prevotella intermedia/nigrescens*; ◇, *Micromonas micros*; ×, no pathogens; dark dots – similar pathogens among family; ○, same clone among family.

spread of the same genotype was confirmed in six cases (Fig. 1). The sharing of *P. intermedia/nigrescens* was more frequent (five families) than *A. actinomycetemcomitans* (two families). However, the prevalence of *P. intermedia/nigrescens* in studied families was quite similar to the frequency of *A. actinomycetemcomitans*.

Discussion

We found significant relation between severe periodontitis in mothers and the presence of periodontal disease in their children. Although other studies have shown severe forms of periodontitis clusters in families[17,18] no correlation between mothers and children carrying periodontal diseases has been demonstrated. Our suggestion is that maternal severe periodontitis can predispose future disease of their child. In most cases, children of diseased mothers had gingivitis but not periodontitis. However, gingivitis has been shown as an important factor predicting further development of periodontitis[19–21]. Thus, a longer observation period is probably needed to show a stronger relation between severe generalized periodontitis in mothers and in children.

We found worse oral hygiene in diseased mothers (as compared with healthy ones) and their children (as compared to children of healthy mothers). It is possible that mothers' oral hygiene habits can influence habits of their children and thus predispose them to the development of periodontitis. However, the role of VPI and VPI% as indicators of oral

hygiene and risk factors of periodontitis are not fully understood yet. According to van der Velden[22], the amount of supragingival plaque does not adequately reflect personal oral hygiene habits whereas the people with abundant inflammation develop massive plaque. In contrast, Axelsson and Alabander[23,24] established the importance of dental plaque as the primary aetiological factor in the development of gingival inflammation and chronic periodontitis. Based on this finding, Alabandar *et al.*[23] concluded that secondary prevention of periodontitis in children is of prime importance and may be achieved through early detection of high-risk patients. The weakness of VPI indices is the fact that some patients may brush their teeth directly before the dental visit. Measurement of supragingival plaque might provide data about local factors present in the subject's mouth and can evaluate their habits and attitudes. However, based on present knowledge VPI indices alone are insufficient to predict periodontis.

This study revealed sharing of periodontal pathogens of the same species and in some cases also the sharing of similar genotypes among families with diseased mothers. Detailed knowledge regarding the acquisition and transmission of infectious agents facilitates a more comprehensive approach towards prevention. One difficulty in investigating the influence of parental sulcular microflora on the child's periodontal health is that periodontitis is not a single bacterial infection; it displays heterogeneity even in the same mouth. In addition, oral and family

environmental factors, immunological and inflammatory host profiles may modulate colonization and establishment of periodontal pathogens in early childhood.

Children and young adults with chronic periodontal disease were previously studied along with patients having localized aggressive periodontitis and generalized aggressive periodontitis. In most studies, interfamilial spread of periodontal diseases was subjected to investigation of aggressive periodontitis and to single specific pathogens, but there are no data available about the spread of sulcular microflora in the case of chronic periodontitis[25,26].

Some studies have shown that if children harbour *A. actinomycetemcomitans*, usually one or two parents harbour the same strain. However, identical genotypes in family members are not 100% proof of transmission, as there is not an infinitive number of genotypes and finding identical genotypes may have occurred by chance[25,26]. The frequency of vertical transmission of *A. actinomycetemcomitans* is between 30% and 60% based on detection of identical genotypes in children and parents.

We found that acquisition of periodontal pathogens in different age groups was statistically not different. There was a trend that colonization/infection with periodontal pathogens occurs in children before or at the age puberty. We expected that the colonization will occur in the younger age period when the mother–child contact was stronger. However, the limited size of this study does not allow of such speculation.

In conclusion, this study provides evidence that such maternal indicators as periodontitis, hygiene habits, and periodontal microflora are risk factors for periodontal diseases in children. We can conclude that maternal disease might be predictive for periodontitis in children and adolescents. Although additional studies are needed, we can state that the above indicators are useful in identifying mothers whose children will be at high risk of developing periodontitis. The identification of high-risk children and their early treatment may help to reduce the development of periodontal disease in the future.

What this paper adds
- We found significant relation between severe periodontitis in mothers and the presence of periodontal disease in their children.

Why is this paper important to paediatric dentists
- Maternal indicators, such as periodontitis, oral hygiene habits, and periodontal microflora are proved to be risk factors for childhood periodontal diseases, and might be predictive of future childhood and adolescent periodontitis.
- The identification of high-risk children and their early treatment may help to reduce the development of periodontal disease in the future.

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