Prevalence and progression of untreated periodontal disease in a young Indonesian population

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THE EFFECT OF SIBLING RELATIONSHIP ON THE PERIODONTAL CONDITION

CHAPTER 3

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CHAPTER 3

THE EFFECT OF SIBLING RELATIONSHIP ON THE PERIODONTAL CONDITION


Epidemiological studies carried out during the last decade have shown that the proportion of the population with significant destructive disease is a minority (Miyazaki et al. 1990). Potter (1990) suggested that chronic diseases that have a relatively high population frequency aggregate in families. Family studies carried out in the field of periodontology are almost exclusively dealing with families ascertained through an affected proband with juvenile periodontitis (JP). Results of these studies have indeed supported the view that in the case of JP, periodontitis aggregates in families (Benjamin & Baer 1967, Melnick et al. 1976, Saxén 1980, Ohtonen et al. 1983, Saxén & Nevanlinna 1984, Spector et al. 1985, Beaty et al. 1987, Boughman et al. 1992). Several explanations have been suggested for this aggregation of periodontitis in JP families. One explanation may be that in these families intra-familial transmission of periodontopathic micro-organisms has occurred. Strong evidence for this possibility was recently presented by DiRienzo (1991). He found, on the basis of DNA fingerprinting that children in many JP families are infected with Actinobacillus actinomycetemcomitans strains similar to those found in at least one of the parents. Another explanation for the aggregation of periodontitis in JP families may be that in these families a host response defect, e.g. in neutrophil function, is inherited, since it has been suggested that in JP patients the chemotaxis and phagocytosis by neutrophils is depressed (Van Dijke 1991).

In terms of prevalence of periodontitis, the results of the above-mentioned JP family studies differ to some extent. Especially the results of the studies of Saxén (1980) and Saxén & Nevanlinna (1984) are confusing. On the basis of their joint material, which consisted of 61 families, they found that not one single parent was affected by periodontitis. On the other hand, all above-mentioned studies show a relatively high prevalence of periodontitis in siblings of JP patients.

To our knowledge, no data are available in relation to the periodontal condition of siblings of families not ascertained on the basis of a subject with JP. Therefore the purpose of the present investigation was to study clinically as well as microbiologically the effect of sibling relationship on the periodontal condition in a population with a relatively high prevalence of periodontal disease.
CHAPTER 3

MATERIAL AND METHODS

This study is based on the baseline data of a longitudinal study on the initiation and progression of periodontal destruction in a population deprived from regular dental care. For this longitudinal study, a village with about 2000 inhabitants at the Malabar tea estate on Western Java, Indonesia was selected. All subjects in the age of 15-25 years were investigated clinically and microbiologically.

Clinical examination

The following clinical parameters were recorded.

- Plaque (Silness & Løe 1964);
- Calculus (Björby & Løe 1967);
- Probing depth (PD) using a force controlled probe (Brodontic, 240 N/cm²);
- Bleeding on probing using the force controlled probe and scored as:
  0) no bleeding;
  1) point bleeding within 30 s;
  2) immediate, overt bleeding.
- Attachment loss (AL) assessed by subtracting the distance between the gingival margin and the cemento-enamel junction (GM-CEJ) from the recorded probing depth or, in case of gingival recession, adding the GM-CEJ value to the probing depth measurement; the GM-CEJ distance was evaluated using a Hu-Friedy® probe with a Williams calibration.

All measurements were rounded off to the nearest mm.

The assessment of the clinical parameters was carried out on all interproximal surfaces from the buccal aspects as well as mid-buccally and lingually from the Ramfjord Teeth (16, 21, 24, 36, 41 and 44) with the exception of calculus which was only evaluated at the Ramfjord teeth. For the evaluation of the periodontal condition, a distinction was made between the values of the parameters at the Ramfjord teeth and the interproximal sites. The amount of plaque, calculus and bleeding at the Ramfjord teeth was used as an estimate of the gingival condition whereas the evaluation of the interproximal parameters was used as an estimate of the degree of periodontitis in the full mouth.
Microbiological examination
Samples for bacteriological examination were collected in the following order:
1) the dorsum of the tongue, from the vallate papillae to the tip of the tongue;
2) the buccal gingiva in the upper jaw, from the left to right first molar;
3) the saliva;
4) the deepest bleeding pocket without loss of attachment.

The samples from the tongue and the gingiva were obtained by sweeping a sterile swab under continuous pressure over the total surface. In the case of the gingiva, care was taken not to touch the supragingival plaque. The sample from the tongue was suspended in 1.8 ml reduced transport fluid (RTF) supplemented with 10% Fildes extract (RTFF). Fildes extract is prepared by the action of pepsin on defibrinated sheep blood as described by Fildes (1920) and it has been shown that the addition of 10% Fildes extract to RTF preserves the motility of the micro-organisms for 48 h (Petit et al. 1991). The gingival sample was suspended in 0.9 ml RTFF. After all clinical measurements were completed the pocket was selected for subgingival sampling. Since this study was designed as a prospective study on the initiation of periodontal destruction a sample was taken from the deepest bleeding pocket without loss of attachment. After careful removal of the supragingival plaque by means of a curette a subgingival sample was obtained using a nerve broach (Maillefer®) wound with cotton and heat sterilized. During intrusion and extrusion the broach was continuously twisted. Subsequently, the sample was suspended in 0.9 ml RTFF.

All specimens were vortexed for 60 s, at the maximum setting and further dispersed by aspirating 5 times through a tuberculine syringe (1 ml Terumo syringe with a 0.45 x 11 mm Neolus needle). A few drops of the suspension were placed in a Thoma counting chamber. Samples were analyzed by a phase-contrast microscope (Micorscan) equipped with a video cassette recorder (U-matic Sony). For the evaluation 4 video recordings of the sample were made at random. Subsequently the % of spirochetes and motile micro-organisms was determined. Thereafter the samples were fixed with formaldehyde (0.2% v/v). 10 µl aliquots of the sample were transferred to multi-well slides and gently heat-fixed. Slides were stored at room temperature until further processing in the Netherlands.
Indirect immunofluorescence assay

For detection of 3 putative periodontopathic bacteria, an indirect immunofluorescence (IIF) assay was used. Strains used for immunization included: *Porphyromonas gingivalis* W 38 and 381, *Prevotella intermedia* (ATCC 25611), *A. actinomycetemcomitans* ATCC 29522, serotype b, ATCC 29523, serotype A and HCTC 9710, serotype C. The immunization was carried out according to the protocol of Mouton et al. (1981). This method has been verified by others (Slots et al. 1985).

Polyclonal rabbit sera were produced by intravenous injection of cells at a concentration of 10 mg/ml (wet weight) into the marginal ear vain of Chin Chilla rabbits (2.5 kg). The rabbits received an increasing dose of antigen as described previously (Van Winkelhoff et al. 1985). The animals were terminally bled by cardiac puncture, 7 days after the last injection. After heating at 56°C for 20 min, sera were stored at -80°C in 1 ml aliquots.

Species specificity was assessed by testing the rabbit sera against a set of 40 oral bacterial species including gram positive and gram negative obligately anaerobic and facultative anaerobic species in the indirect immunofluorescence test. A strong non-specific activity was found between the *P. gingivalis* W 83 serum and *Peptostreptococcus micros*. The *P. gingivalis* antiserum 381 did not show cross-reactivity with this species. The *P. intermedia* antiserum showed cross reactivity with *Prevotella melaninogenica*, *Prevotella loescheii* and *Prevotella corporis*. The three *A. actinomycetemcomitans* antisera reacted with *Haemophilus paraphrophilus*, *Heamophilus aphrophilus* and *Veillonella parvula*. Cross-reacting sera were absorbed with the non-specific species until they were no longer detectable in the IIF tests for *P. gingivalis*, *P. Intermedia* and *A. actinomycetemcomitans*, respectively.

The samples were reacted with 20 μl of species-specific antisera, which were diluted to a working titre (highest 2-fold dilution with brilliant fluorescence) in phosphate-buffered saline (PBS-T) for 10 min. The specimens were rinsed in PBS-T, washed in PBS and subsequently rinsed with distilled water. Specimens were then incubated with 20μl with affinity-purified goat anti-rabbit IgG conjugated to fluorescein isothiocyanate (Nordic Immunology). Slides were mounted with glycerol in PBS 2:1 v/v, pH 9.0. No cross-reactivity of the conjugate was observed with the specific bacterial species.

Positive and negative controls were used each time an IIF assay was performed.
The positive controls consisted of the homologous reactions of each test species. Negative controls included staining of plaque smears with only the second antiserum (conjugate).

Examination of the specimen was carried out with a Zeiss Axioskop, equipped for phase contrast illumination and for light fluorescence. The light source was 100 W halogen. Cells were considered positive if they had well-defined cell outlines and dark fluorescing centres. Relative proportions of specific bacteria were determined by counting the total number of bacteria in a certain area on a microscopic slide by phase contrast microscopy. Then the number of fluorescent cells was counted in the same area resulting in a % of a particular species.

Statistical analysis
The determination of a sibling effect on the clinical and microbiological parameters was carried out with a one-way random effect analysis of variance. This analysis determines whether the variation between families is larger than can be expected on the basis of the variation within families. In order to obtain homogeneity of variances a square root transformation was carried out. When a significant sibling effect was found the influence of other parameters was investigated by means of an analysis of covariance. The relationship between 2 parameters was determined by calculating Pearson’s correlation coefficients.

RESULTS

In total, 255 subjects were examined. Since these subjects represented all individuals in the age range of 15 to 25 years of one and the same village, a great number of siblings was present in this material. In order to investigate a possible sibling effect, only those family units were selected for analysis, which consisted of three or more siblings. Thus the data analysis of this study was carried out on 23 sibships, consisting of 78 subject.

The mean values of the clinical parameters are presented in Table 1. The mean plaque score at the Ramfjord teeth and interproximally were equally low, whereas the mean bleeding score was somewhat higher interproximally. The mean interproximal amount of LA in this population was 0.29 mm. The
individual mean LA ranged from 0 to 1.27 mm. For this study, we defined periodontitis as a site with a PD of 5 mm or more in conjunction with 2 mm LA or more. The mean % of sites showing this condition was 5%. Further analysis revealed that 33% of the subjects had such a site. On an individual basis this value ranged from 0 to 39.2%. As can be seen in Table 1, the statistical analysis revealed a significant sibship effect for plaque, calculus, LA and an almost significant effect for periodontitis. It can be seen that the mean amount of LA per family varies to a considerable extent. In Fig. 1, the families are presented on the basis of an increasing amount of LA. In Fig. 2, the mean interproximal plaque scores per family are presented. This figure illustrates the significant sibship effect for plaque.

Table 1. The mean values and standard deviations (SD) for the clinical parameters at the Ramfjord teeth and the interproximal sites and the sibship effect

<table>
<thead>
<tr>
<th></th>
<th>Mean(SD)</th>
<th>Sibship effect p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ramfjord teeth</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plaque</td>
<td>0.88 (0.42)</td>
<td>0.001</td>
</tr>
<tr>
<td>Calculus</td>
<td>0.91 (0.39)</td>
<td>0.014</td>
</tr>
<tr>
<td>Bleeding</td>
<td>0.66 (0.34)</td>
<td>0.626</td>
</tr>
<tr>
<td><strong>Interproximal sites</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plaque</td>
<td>0.87 (0.43)</td>
<td>0.006</td>
</tr>
<tr>
<td>Probing depth (PD)</td>
<td>3.20 (0.53)</td>
<td>0.566</td>
</tr>
<tr>
<td>Bleeding</td>
<td>0.74 (0.35)</td>
<td>0.407</td>
</tr>
<tr>
<td>Loss of attachment (LA)</td>
<td>0.29 (0.29)</td>
<td>0.004</td>
</tr>
<tr>
<td>%sites with PD≥5mm and LA≥2mm</td>
<td>4.72 (8.24)</td>
<td>0.057</td>
</tr>
</tbody>
</table>
Fig. 1. Mean value and standard deviation (SD) per family unit for loss of attachment and number of sites with a probing pocket depth of 5 mm or more in conjunction with 2 mm or more loss of attachment.

Fig. 2. Mean and standard deviation (SD) of the interproximal plaque index per family unit.
Table 2 shows the results of the microbiological evaluation. It can be seen that all 4 investigated bacteria show a high prevalence in this population. *P. intermedia* was found in all subjects, with the highest % on the dorsum of the tongue. *P. gingivalis* was present in about 70% of the subjects with the highest % in the pocket. Spirochetes were found in about 55% of the subjects and showed also the highest % in the pocket. *A. actinomycetemcomitans* showed the lowest prevalence. Nevertheless, this bacterium was found in about 43% of the subjects in equally low percentages at the 4 sampling sites. Further data analysis on the prevalence of micro-organisms in families showed a statistically significant sibship effect for spirochetes on the tongue and in the pocket, *P. gingivalis* on the gingiva and in the saliva an *P. intermedia* in the saliva. These findings are illustrated by Figs. 3-7 in which the mean % of micro-organisms per family unit can be seen. In addition, an almost significant sibship effect was found for *P. intermedia* in the pocket (*p = 0.074*) and *A. actinomycetemcomitans* in the saliva (*p = 0.093*).

Table 2. Prevalence of micro-organisms as well as mean %’s in positive subjects and standard deviations (SD) for positive subjects

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>Tongue prevalence mean(SD)</th>
<th>Gingiva prevalence mean(SD)</th>
<th>Saliva prevalence mean(SD)</th>
<th>Pocket prevalence mean(SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. actinomycetemcomitans</em></td>
<td>28.2 0.3 (0.4)</td>
<td>19.2 0.7(0.7)</td>
<td>19.2 0.3(0.2)</td>
<td>43.6 0.7(0.5)</td>
</tr>
<tr>
<td><em>P. gingivalis</em></td>
<td>70.5 1.1 (1.9)</td>
<td>25.6 1.0(1.5)</td>
<td>47.4 1.4(1.7)*</td>
<td>62.8 4.6(5.7)</td>
</tr>
<tr>
<td><em>P. intermedia</em></td>
<td>100.0 19.1(16.0)</td>
<td>70.5 2.4(6.8)</td>
<td>84.6 3.8(6.6)</td>
<td>79.5 2.3(2.3)</td>
</tr>
<tr>
<td>Spirochetes</td>
<td>46.2 4.2 (5.9)*</td>
<td>15.4 5.7(7.5)</td>
<td>55.1 5.5(4.0)</td>
<td>37.7 14.5(9.1)*</td>
</tr>
</tbody>
</table>

* significant sibship effect (*p<0.05*)
Fig. 3. Mean % and standard deviation (SD) of spirochetes on the tongue per family unit.

Fig. 4. Mean % and standard deviation (SD) of spirochetes in the pocket per family unit.
Next on an individual basis, independent from the presence of sibships, the relationship was determined between LA and the clinical parameters plaque, calculus and bleeding as well as with the microbiological parameters. In Table 3, it can be seen that all three clinical parameters show a highly significant correlation with LA. For the microbiological data only a significant correlation was found for spirochetes on the gingiva and for *A. actinomycetemcomitans* and spirochetes in the saliva.

From a clinical point of view it is interesting to see that both interproximal plaque and bleeding are significantly correlated with LA. Whereas only the plaque showed a sibship effect. Therefore, an analysis of covariance was carried out in order to determine the relative effect of interproximal bleeding and plaque on the sibship effect of LA. The results showed that the main sibship effect of LA remains ($p = 0.028$) when the bleeding was included as a covariable, whereas the main sibship effect disappears ($p = 0.128$) when the plaque was included as a covariable. This implies that the main sibship effect in LA can be explained by differences in amount of plaque between families.

Further analysis showed that the bacteria which were significantly correlated with LA (spirochetes on the gingiva, *A. actinomycetemcomitans* and spirochetes in the saliva) were also significantly correlated with the amount of interproximal plaque (Table 4).

### Table 3. Correlations between loss of attachment and clinical and microbiological parameters

<table>
<thead>
<tr>
<th>Correlation coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ramfjord teeth</strong></td>
<td></td>
</tr>
<tr>
<td>plaque</td>
<td>0.51</td>
</tr>
<tr>
<td>calculus</td>
<td>0.56</td>
</tr>
<tr>
<td>bleeding</td>
<td>0.40</td>
</tr>
<tr>
<td><strong>Interproximal sites</strong></td>
<td></td>
</tr>
<tr>
<td>plaque</td>
<td>0.53</td>
</tr>
<tr>
<td>bleeding</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>Gingiva</strong></td>
<td></td>
</tr>
<tr>
<td>spirochetes</td>
<td>0.34</td>
</tr>
<tr>
<td>salivary</td>
<td>0.33</td>
</tr>
<tr>
<td><strong>Saliva</strong></td>
<td></td>
</tr>
<tr>
<td>A. actinomycetemcomitans</td>
<td>0.45</td>
</tr>
</tbody>
</table>
Table 4. Correlations between interproximal plaque and the microbiological parameters

<table>
<thead>
<tr>
<th></th>
<th>Correlation coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gingiva spirochetes</td>
<td>0.21</td>
<td>0.03</td>
</tr>
<tr>
<td>Saliva spirochetes</td>
<td>0.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Saliva <em>A. actinomycetemcomitans</em></td>
<td>0.36</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Fig. 5. Mean % and standard deviation (SD) of *P. gingivalis* on the gingiva per family unit.
Fig. 6. Mean % and standard deviation (SD) of *P. gingivalis* in the saliva per family unit.

Fig. 7. Mean % and standard deviation (SD) of *P. Intermedia* in the saliva per family unit.
DISCUSSION

In the present study, the effect of a sibling relationship on the periodontal condition was investigated in a population with a relatively high prevalence of destructive periodontal disease. This relatively high prevalence may be illustrated by the result that 33% of the subjects had ≥ 1 site with a probing pocket depth of 5 mm or more in conjunction with 2 mm of LA, whereas in 15 years olds in Amsterdam (The Netherlands), this was only found in 4% (Van der Velden et al. 1989). This high prevalence of periodontitis in the population at the Malabar tea estate made this group of subjects with a relatively great amount of siblings highly useful for a familial analysis of the periodontal condition.

In this study, a significant sibling effect was found for plaque, calculus, LA and a number of microbiological parameters. One explanation for the sibship effect on plaque may be that some families are more dental-health oriented than others. In other words, in some families, the teeth are brushed better compared to others. However, there are several reasons why this explanation seems questionable. First, the village under investigation was chosen because it was deprived from regular dental care. Only extractions were carried out by the general physician. Second, it was a small, remote village where toothbrushes were rarely seen when visiting the living condition of the subjects; on the other hand, the individual possession of a toothbrush was not included in the evaluation. Third, the mean plaque index for this group is rather low. Although this may also point towards an effective use of the tooth-brush, the low mean interproximal plaque score originates from a great number of sites with a plaque score of zero. These sites are usually difficult to clean without proper oral hygiene instructions. Another explanation for the low plaque score may be the diet. For example, this population seldom uses sugar, and the tea, the most popular drink, contains approximately 0.45 PPM of fluoride.

Besides a sibship effect for plaque, a significant sibship effect was found for calculus. It can be supposed that this effect may be due to an inherited predisposition for calculus in certain families. This supposition is supported by the fact that in general there are individual differences in the degree of calculus formation (Genco 1990). The variation in amount of calculus between families may also explain the sibship effect for plaque because it is well-known that
calculus is a retention factor for plaque (Lindhe 1989).

In relation to the microbiological parameters, also significant sibship effects were found. This was established for spirochetes on the tongue and in the pocket, for *P. gingivalis* on the gingiva and in the saliva as well as for *P. intermedia* in the saliva. An explanation for this phenomenon may be that in some families intra-familial transmission of the bacteria has occurred. The most recent studies investigating the possibility of transmission of periodontopathogenic micro-organisms within families are using the DNA fingerprinting method. On the basis of this method, DiRienzo (1991) suggested that *A. actinomycetemcomitans* can be transmitted between family members at a relatively early age and that the parents can be the source. By using the DNA method in a family study, evidence could be presented for possible transmission of *A. actinomycetemcomitans* (Petit et al. 1993) and *P. gingivalis* (Van Steenbergen et al. 1992). Since these studies strongly suggest that transmission of *A. actinomycetemcomitans* and *P. gingivalis* can occur between family members, it seems likely that the same holds true for other oral bacteria including spirochetes and *P. intermedia*. This is supported by the finding in the present study of a significant sibship effect for spirochetes (tongue and pocket) and *P. intermedia* (saliva).

The most interesting result of the study is the finding of a significant sibship effect for LA in a population with a relatively high prevalence of destructive periodontal disease. From a microbiological point of view the high prevalence of the disease can be easily explained: the prevalence of suspected periodontal pathogens is extremely high in this population. However, the three bacteriological parameters which were significantly correlated with LA (spirochetes on the gingiva and in the saliva and *A. actinomycetemcomitans* in the saliva) showed no sibship effect whereas the bacteriological parameters which showed a sibship effect were not significantly correlated with LA. Nevertheless, the 3 bacterial parameters which were correlated with LA were also correlated with the amount of interproximal plaque, which in itself showed both a correlation with LA as well as a sibship effect. In all, the sibship effect for LA can not easily be explained by the microbiological data only.

Another explanation for the sibship effect on LA may be that periodontitis has a genetic background. Studies which may give information in this respect are family studies. Up to now, most family studies are dealing with JP families. The
results of these studies strongly suggest a genetic background for this disease (Melnick et al. 1976, Saxén 1980, Ohtonen et al. 1983, Saxén & Nevanlinna 1984, Spector et al. 1985, Beaty et al. 1987, Boughman et al. 1992). Recently, an extremely powerful method was published in order to investigate the heredity of periodontal diseases (Michalowicz et al. 1991). In this study, a comparison was made between monozygous twins who were separated at birth and reunited in adulthood and monozygous and dizygous twins reared together. The results show that between 38 to 82% of the population variance in terms of probing depths, attachment loss and plaque may be attributed to genetic factors. An explanation for the genetic background in general is rather difficult. For JP, it has been suggested that defects in the immune system such as defects in neutrophil function (Van Dyke 1991) may play a role in the inheritance of JP. However it is questionable whether in all periodontitis patients such a defect is present. Another explanation for the genetic background of periodontitis may be the possession of particular histocompatibility antigens. For example, it has been shown that HLA-A9 is involved in the susceptibility to periodontal disease (Klouda et al. 1986, Amer et al. 1988).

The results of the present study suggest another factor which may be genetically controlled and contributes to the development of periodontitis: calculus. It was found in this population where calculus was never removed that calculus was significantly correlated with LA and also had a sibship effect. Therefore presence of calculus as a retention factor for plaque in conjunction with certain microbes as well as predisposition to periodontal disease by a reduced function of neutrophils and/or a given HLA combination may explain the familial aggregation of periodontitis.

In conclusion, our results of present study support the hypothesis that periodontitis in general aggregates in families.

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