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

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UNTREATED PERIODONTAL DISEASE IN INDONESIAN ADOLESCENTS
Clinical and microbiological baseline data

CHAPTER 2

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During the last decades, epidemiological cross-sectional studies have suggested that periodontitis is universal, that it starts early in life and increases in serenity with age (for review see Brown & Loe 1993). The reason that in some individuals, gingivitis develops into periodontitis, is still a matter of extensive research. At present several risk factors and indicators for the initiation and progression of periodontitis have been identified (Genco 1996). Cross-sectional epidemiological surveys have shown a strong correlation between the amounts of plaque present and the extent of loss of periodontal tissue support (Mobley & Smith 1963, Ladavalya & Harris 1959, Schei et al. 1959). The same holds true for calculus (Lövdal et al. 1958). The importance of calculus has recently been reinforced by a retrospective study on juvenile periodontitis (JP) by Sjödin et al. (1993). These authors showed that 31% of the JP patients already demonstrate the presence of calculus in the primary dentition as compared to 5% in the reference group. Furthermore, it has been suggested that the composition of the subgingival microbiota may be an important risk factor for periodontitis (Genco 1996). It has been recognized that a limited number of mainly Gram negative bacteria is associated with periodontitis (Haffajee & Socransky, 1994). These bacteria are not only present in the periodontal pocket, but have also been recovered from the oral mucous membranes (Slots et al. 1980, Van Winkelhoff et al. 1986, Asikainen et al. 1991, Danser et al. 1996). Therefore colonization of the oral cavity with certain periodontal bacteria may be a risk factor for the initiation of periodontitis. In addition the degree of inflammatory response of the periodontal tissues to these etiological factors may be regarded as a risk indicator, as has been shown by Burt et al. (1990) in a longitudinal study, in which the level of gingivitis proves to be the most prominent risk indicator for tooth loss.

In order to investigate the rôle of various clinical and microbiological risk factors for periodontitis, a longitudinal study was initiated in a young population, which had not received regular dental care. The present communication describes the baseline cross-sectional data obtained in 1987 in terms of clinical periodontal condition and prevalence of periodontal bacteria in the oral cavity.
CHAPTER 2

MATERIALS AND METHODS

Study population
For this longitudinal study a village with approximately 2,000 inhabitants at the Malabar/Poerbasari tea estate on Western Java, Indonesia was selected. All subjects in the age range 15 - 25 years participated in this investigation. They amounted to a total of 255 adolescents. This population was chosen because it had not received regular dental care and had not been exposed to preventive dental care programs. Emergency dental treatment, consisting of extraction of teeth was provided by a general physician. Therefore this population was suitable to study the natural development and progression of periodontitis. The population consisted mostly of tea labourers with a low educational level employed by a government owned tea estate, PTP XIII. Most individuals had had a limited or complete primary education (N = 204) whereas 51 individuals completed further education. The subjects received basic medical care and there was no indication of obvious malnutrition.

Examination procedures
Prior to taking the clinical measurements the participants were asked about their education level, general health status and recent use of antibiotics. In addition family relationships were established (see Van der Velden et al. 1993). Subsequently all samples for bacteriological examination were taken except those from the pockets. The clinical assessments were carried out by 3 periodontists (FA, SA, EGW) and dictated to a chairside assistant. Every tenth subject was used for double scores of the clinical parameters at the Ramfjord teeth. These were carried out at the end of each morning and afternoon session. The clinical examination was performed in an office facility of the tea factory and portable dental chairs were used. After clinical measurements were completed samples from the selected pockets were taken for bacteriological examination.
Clinical Parameters

The following indices were recorded:

- Plaque (Silness & Löe 1964)
- Calculus (Björby & Löe 1967)
- Probing Depth (PD) using a force controlled probe (Brodontic® Ash/Dentsply, 240 N/cm²) with a Williams calibration.
- Bleeding on Probing (PPBI; Van der Velden 1979) using the force controlled probe on a three point scale: 0) non-bleeding sites; 1) ‘pin prick’ bleeding; 2) ‘excess’ 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 by means of a Hu-Friedy® probe (Williams calibration).

The sequence of scoring was always the same. Clinical parameters were scored on the approximal surfaces from the vestibular aspect of all teeth except third molars, as well as on the vestibular and lingual surfaces of the Ramfjord teeth (16, 21, 24, 36, 41 and 44) (Ramfjord 1959). Calculus was scored on the four surfaces of the Ramfjord teeth. Measurements, if appropriate, were rounded off to the nearest millimetre.

The amount of plaque, calculus and bleeding at the Ramfjord teeth was used as an estimate of the gingival condition. The approximal parameters were used as an estimate of the degree of periodontal breakdown (Van der Velden et al. 1993).

Rationale for selection of sampled pockets

This paper presents baseline recordings of a longitudinal survey on the natural development of periodontitis. Gathering microbiological data on pockets showing all characteristics of periodontitis except probing attachment loss, might give an opportunity to discern differences in the microbiota of pockets that during follow-up measurements prove to have suffered from periodontal breakdown and those which have not. In this respect the present data might serve as indicators for future periodontal breakdown. Therefore in all subjects
the deepest bleeding pocket without attachment loss was selected for microbiological examination of the subgingival plaque. This will be referred to as the inflamed pocket. Furthermore it was not expected that in all subjects one pocket with periodontal attachment loss would be found. The choice for a pocket without attachment loss enabled an evaluation of the subgingival flora of a comparable type of pocket in all subjects at the time of these baseline measurements. In a subset of subjects with at least one deep pocket ≥4 mm showing ≥4 mm attachment loss an additional sample for microbiological examination was taken from the deepest bleeding pocket with the greatest amount of loss of attachment. This will be referred to as the inflamed pocket with attachment loss.

Microbiological examination-
Samples for bacteriological examination were taken from various parts of the oral cavity and were collected in the following order:
- the dorsum of the tongue, from the vallate papillae to the tip of the tongue
- the buccal gingiva in the upper jaw, from the left to the right first molar
- the saliva
- the inflamed pocket
- when present: the inflamed pocket with ≥4 mm attachment loss.

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 disturb the supragingival plaque. The sample from the tongue was suspended in 1.8 ml reduced transport fluid supplemented with 10% Fildes extract (RTFF, Petit et al. 1991). The gingival sample was suspended in 0.9 ml RTFF. Saliva was sampled by adding approximately 1 ml of unstimulated saliva to 0.9 ml RTFF. After all clinical measurements were completed the ‘inflamed pocket’ was selected and sampled. When present the ‘inflamed pocket with attachment loss’ was sampled as well. After careful removal of the supragingival plaque a subgingival sample was taken using a nerve broach (Maillefer®) wound with cotton and heat sterilized. During insertion and removal the broach was continuously twisted. The part of the nerve broach, which had been inserted in the pocket was cut off and suspended in 0.9 ml RTFF (Van Winkelhoff et al. 1988). Samples were processed for phase contrast
microscopic and immunofluorescence examination of periodontal pathogens. All specimens were vortexed for 60 s at the maximum setting. They were further dispersed by aspirating 5 times through a tuberculin syringe (1 ml Terumo syringe with a 0.45 x 12 mm Neolus needle). A few drops of the suspension were placed in a Thoma counting chamber and analyzed with a phase-contrast microscope (Microscan) equipped with a video cassette recorder U-matic (Sony). For evaluation, video recordings of the samples were made including random shots of at least 100 micro-organisms. The tapes were examined upon return in The Netherlands to assess the % of spirochetes and motile rods.

Subsequently the samples were fixed with formaldehyde (0.2%, v/v). Ten μl aliquots of the sample were transferred to multi-well slides, air-dried and gently heat-fixed. Slides were stored at room temperature until transportation to The Netherlands. Upon arrival in Amsterdam slides were stored at -20°C until further processing for indirect immunofluorescence assay.

**Indirect Immunofluorescence assay**

For the detection of 3 putative periodontal pathogens, an indirect immunofluorescence (IIF) assay was developed. Strains used for immunization included: *Porphyromonas gingivalis* W 83 and FDC 381, *Prevotella intermedia* ATCC 25611, *Actinobacillus actinomycetemcomitans* ATCC 29523 (serotype a), ATCC 29522 (serotype b) and NTCC 9710 (serotype c). The immunization was carried out according 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 vein 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. This included 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 FDC 381 did not show cross-reactivity with this species. *P. intermedia* antiserum showed
cross reactivity with *Prevotella melaninogenica*, *Prevotella loescheii* and
*Prevotella corporis*. The three *A. actinomycetemcomitans* antisera reacted with
*Heamophilus 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 assay.
The samples were reacted with 20 µl of species-specific antisera, which were
diluted to a working titre (highest dilution with brilliant fluorescence) in
phosphate-buffered saline (PBS, pH 7.2) containing 0.05% Tween 20 (PBS-T)
for 10 minutes. The specimens were rinsed in PBS-T, washed in PBS and
subsequently rinsed with distilled water. Specimens were then incubated with
20 µl 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 with a Zeiss Axioskop, equipped with
a 100 W halogen light source for both phase contrast illumination and light
fluorescence. Cells were considered positive if they had well-defined cell outline
with dark centres. Phase-contrast was used for total cell count.

**Data analysis**
Clinical parameters assessed at all approximal surfaces were calculated as mean
scores per patient. The same procedure was followed for all measurements
concerning Ramfjord teeth. The number of sites showing attachment loss > 2
mm was enumerated. Calculus scores were analyzed as mean frequencies per
score and relative frequency of the scores found per surface measured. For each
tooth the prevalence of attachment loss in the population was calculated.
Microbiological data were analyzed for prevalence of the micro-organisms
included in the study and for relative proportions of the organisms found. Three
patient categories were defined on the basis of the maximum amount of
approximal attachment loss measured at one or more sites within each subject
(adapted from Brown et al. 1990):
CLINICAL AND MICROBIOLOGICAL BASELINE DATA

-no or minor periodontitis: 0-2 mm maximum attachment loss,
-moderate periodontitis: 3-4 mm maximum attachment loss,
-advanced periodontitis: ≥5 mm maximum attachment loss.

Inter-examiner error was estimated by calculation of the inter-examiner variance using a repeated measures model of the analysis of variance both at site level (Plaque: 0.25, Calculus: 0.32, PPBI: 0.30, PD: 0.23, AL: 0.37) and at patient mean level (Plaque: 0.07, Calculus: 0.05 PPBI: 0.09, PD: 0.06, AL: 0.07).

Comparisons between the 3 defined groups were made using analyses of variance, chi-square and Mann-Whitney tests where appropriate. All comparisons concerning prevalence of micro-organisms on several sampled sites and between groups were made using chi-square tests. Data concerning percentages of recovery were tested with non-parametric tests (Kruskal-Wallis and Mann-Whitney tests). For within-patient comparisons McNemar, Friedman and Wilcoxon tests were used. Values of p < 0.05 were accepted as statistically significant.

RESULTS

Mean values and distribution of clinical parameters

In total 130 males and 125 females with a mean age of 20.0 yrs (SD 3.2) were included in the sample. Age and gender were relatively equally distributed throughout all age categories. In Table 1 the clinical data are presented as mean values for the whole population as well as for the 3 patient categories according to the maximum amount of attachment loss, as observed at one or more sites in a subject. The mean number of teeth present per subject was 27.4. The mean approximal loss of attachment in the whole population was 0.36 mm. The individual mean loss of attachment ranged from 0 to 3.07 mm. The mean approximal pocket depth was 3.23 mm with an individual mean pocket depth ranging from 2.15 to 5.03 mm. No difference was observed between males and females for any of the clinical parameters scored on approximal sites. In 169 subjects no or minor attachment loss (0 - 2 mm) was observed, whereas of the remaining 86 subjects with periodontal disease, 65 had moderate (3 - 4 mm) and 21 had advanced (≥ 5 mm) periodontal breakdown. The mean number of teeth for these three patient categories was 27.5, 27.2 and 27.3 respectively.
The mean loss of attachment was 0.20, 0.56, 1.00 for the three categories respectively. The mean pocket depth was 3.09, 3.49 and 3.58 for these three categories. The mean number of sites with attachment loss ≥3 mm present was 2.08 for the ‘moderate’ and 7.29 for the ‘advanced’ category. The Mann-Whitney test showed statistical significance of this difference between the two categories (p = 0.0002) (Table 1). Thirteen subjects showed gingival recession at approximal sites (mean 1.42 mm for patients showing recession; range 1 to 5 mm), of whom 4 showed moderate periodontitis and 9 belonged to the category of advanced disease.

Table 1. Mean clinical parameters for the total population and for different patient categories of severity of attachment loss

<table>
<thead>
<tr>
<th></th>
<th>Total Population</th>
<th>no/minor 0-2 mm AL</th>
<th>moderate 3-4 mm AL</th>
<th>advanced ≥5 mm AL</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>255</td>
<td>169 (66.3%)</td>
<td>65 (25.5%)</td>
<td>21 (8.2%)</td>
</tr>
<tr>
<td>θ</td>
<td>125</td>
<td>82 (32.2%)</td>
<td>30 (11.8%)</td>
<td>13 (5.1%)</td>
</tr>
<tr>
<td>σ</td>
<td>130</td>
<td>87 (34.1%)</td>
<td>35 (13.7%)</td>
<td>8 (3.1%)</td>
</tr>
<tr>
<td>Number of teeth</td>
<td>27.4(1.3)</td>
<td>27.5(1.0)</td>
<td>27.2(1.9)</td>
<td>27.3(1.1)</td>
</tr>
<tr>
<td>Ramfjord teeth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleeding on probing</td>
<td>0.70(0.33)</td>
<td>0.63(0.29)</td>
<td>0.84(0.37)</td>
<td>0.80(0.31)</td>
</tr>
<tr>
<td>Plaque index</td>
<td>0.99(0.42)</td>
<td>0.92(0.39)</td>
<td>1.07(0.42)</td>
<td>1.30(0.50)</td>
</tr>
<tr>
<td>Approximal surfaces</td>
<td>3.23(0.49)</td>
<td>3.09(0.37)</td>
<td>3.49(0.59)</td>
<td>3.58(0.57)</td>
</tr>
<tr>
<td>Attachment loss</td>
<td>0.36(0.40)</td>
<td>0.20(0.17)</td>
<td>0.56(0.32)</td>
<td>1.00(0.86)</td>
</tr>
<tr>
<td>Bleeding on probing</td>
<td>0.78(0.33)</td>
<td>0.71(0.30)</td>
<td>0.93(0.37)</td>
<td>0.86(0.36)</td>
</tr>
<tr>
<td>Plaque index</td>
<td>0.99(0.44)</td>
<td>0.90(0.42)</td>
<td>1.10(0.44)</td>
<td>1.28(0.50)</td>
</tr>
<tr>
<td>Number of sites with AL ≥3 mm</td>
<td>0</td>
<td>2.08(1.51)</td>
<td>7.29(8.44)</td>
<td></td>
</tr>
</tbody>
</table>

% of total population in [] parentheses.
Standard deviation in ( ) parentheses.

In the advanced periodontitis category the mean distance of the cemento-enamel junction to the gingival margin was 2.56 mm in contrast to the moderate and no or minor periodontitis categories, in which this distance amounted to 2.89 and 2.92 mm respectively (p = 0.039). In the present study population, loss of attachment was mostly not manifest as clinically visible recession.
Fig. 1. Number of subjects with ≥3 mm attachment loss for each tooth type.

Fig. 1 shows for each tooth type the number of subjects with ≥3 mm approximal attachment loss at these teeth. The first upper molar and the upper and lower incisors appeared to be the sites most frequently affected with periodontal breakdown.

Calculus scores on Ramfjord teeth are shown in Fig. 2. The upper molar showed the highest scores of calculus on all surfaces. Lingual surfaces of the teeth in the lower jaw showed calculus in the vast majority of cases. In Table 2 mean frequencies of different calculus scores per patient are shown for the different categories of disease severity. The number of teeth showing only supragingival calculus (score 1) is comparable for all categories. The absence of calculus (score 0), the presence of subgingival calculus (score 2), and an abundance of calculus (score 3) showed a difference between the category no/minor periodontitis and the other 2 categories.
Fig. 2. Calculus scores on Ramfjord teeth shown as relative frequency distributions of scores on approximal (AP), vestibular (V) and lingual (L) surfaces.

Table 2. Mean frequencies of calculus scores on the 24 surfaces of Ramfjord teeth for total population and for different patient categories of severity of attachment loss

<table>
<thead>
<tr>
<th>Patient categories</th>
<th>Calculus scores</th>
<th>Total Population Number of surfaces</th>
<th>no/minor 0-2 mm AL</th>
<th>moderate 3-4 mm AL</th>
<th>advanced &gt;5 mm AL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>9.6</td>
<td>10.5*</td>
<td>8.1</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3.9</td>
<td>4.1</td>
<td>3.3</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9.8</td>
<td>9.9*</td>
<td>11.4</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.7</td>
<td>0.5*</td>
<td>1.1</td>
<td>1.4</td>
</tr>
</tbody>
</table>

* Significantly different from the other two categories using the analysis of variance; number of surfaces with each calculus score entered as dependent variable.

Microbiological results

Table 3A shows the prevalence of *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, spirochetes and motile rods on the tongue, gingiva, in saliva, and in the inflamed pockets. Since only 37 subjects had one pocket with attachment
loss (AL ≥4 mm) the data for this sample site were not included in the comparative analysis between different sample sites. P. intermedia was the most frequently detected micro-organism and was found on the dorsum of the tongue in all but 1 subject. When comparing the results concerning the different sampled sites, the highest prevalence of P. gingivalis was found in the pocket (66%) and on the dorsum of the tongue (61%). The highest prevalence of A. actinomycetemcomitans was found in the inflamed pocket (37%).

Phase-contrast microscopic analysis revealed that motile rods were most frequently observed on the tongue (92%) and in saliva (89%) (p<0.01). Spirochetaes were most frequently found in the inflamed pocket (p<0.01).

Table 3A. Prevalence of micro-organisms per sampled site expressed as the % of individuals with detectable levels of each micro-organism at each site

<table>
<thead>
<tr>
<th></th>
<th>Tongue</th>
<th>Gingiva</th>
<th>Saliva</th>
<th>Pocket without AL</th>
<th>Pocket with AL</th>
<th>All sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunofluorescence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. actinomycetemcomitans</td>
<td>25%</td>
<td>18%</td>
<td>15%</td>
<td>37%</td>
<td>59%</td>
<td>57%</td>
</tr>
<tr>
<td>P. gingivalis</td>
<td>61%*</td>
<td>33%</td>
<td>50%</td>
<td>66%*</td>
<td>86%</td>
<td>87%</td>
</tr>
<tr>
<td>P. intermedia</td>
<td>99%</td>
<td>63%</td>
<td>85%</td>
<td>79%</td>
<td>81%</td>
<td>100%</td>
</tr>
<tr>
<td>Phase Contrast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spirochetes</td>
<td>47%</td>
<td>12%</td>
<td>51%</td>
<td>63%</td>
<td>84%</td>
<td>89%</td>
</tr>
<tr>
<td>Motile rods</td>
<td>92%*</td>
<td>23%</td>
<td>89%*</td>
<td>59%</td>
<td>70%</td>
<td>100%</td>
</tr>
</tbody>
</table>

All sites: % of individuals being positive irrespective of the sites where the micro-organisms were found. Prevalence of the micro-organisms at the sampled site, tested between sites, within patients, using multiple McNemar's tests: • Site with the highest prevalence.

In total 37 subjects were selected. The inflamed pocket with attachment loss was not included in the analysis.

The mean % of A. actinomycetemcomitans, P. gingivalis and P. intermedia for subjects positive for each micro-organism are presented in Table 3B. Data are presented for the mucosal sites (tongue, gingiva), saliva, and pockets without and with attachment loss. Again the data from the pocket with attachment loss were not included in the analysis. The proportion of A. actinomycetemcomitans did not exceed 1%, whereas the mean % of P. gingivalis ranged from 1 to 4%. The highest % of P. gingivalis was observed in the inflamed pocket (4.37, p<0.0001). The % of P. intermedia was the highest on the dorsum of the tongue (17%, p<0.0001).
Phase-contrast data revealed that the % recovery of spirochetes from the inflamed pocket is comparable to that of motile rods. This was in contrast with the data of the other sampled sites, where motile rods formed the larger part of the motile micro-organisms (p < 0.001).

Table 3B. Proportion of micro-organisms by sampled site expressed as the mean % of each micro-organism at each site in patients with detectable levels of the micro-organism at the specific site

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Tongue Mean (SD)</th>
<th>Gingiva Mean (SD)</th>
<th>Saliva Mean (SD)</th>
<th>Pocket without AL Mean (SD)</th>
<th>Pocket with AL Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase Contrast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spirochetes</td>
<td>4.35 (4.60)</td>
<td>5.10 (5.39)</td>
<td>64.85 (4.05)</td>
<td>12.90 (8.91)</td>
<td>14.29 (10.70)</td>
</tr>
<tr>
<td>Motile rods</td>
<td>17.97 (12.38)</td>
<td>8.90 (10.64)</td>
<td>21.02 (16.76)</td>
<td>15.01 (11.11)</td>
<td>15.03 (10.55)</td>
</tr>
</tbody>
</table>

**Immunofluorescence**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Tongue Mean (SD)</th>
<th>Gingiva Mean (SD)</th>
<th>Saliva Mean (SD)</th>
<th>Pocket without AL Mean (SD)</th>
<th>Pocket with AL Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. actinomycetemcomitans</td>
<td>0.40 (0.53)</td>
<td>1.09 (1.86)</td>
<td>0.40 (0.36)</td>
<td>0.78 (0.69)</td>
<td>1.07 (1.19)</td>
</tr>
<tr>
<td>P. gingivalis</td>
<td>1.03 (2.18)</td>
<td>1.09 (1.49)</td>
<td>1.28 (1.82)</td>
<td>4.37 (6.02)</td>
<td>8.20 (10.37)</td>
</tr>
<tr>
<td>P. intermedia</td>
<td>16.55 (14.39)*</td>
<td>1.75 (4.46)</td>
<td>4.35 (4.60)</td>
<td>4.37 (6.02)</td>
<td>17.97 (12.38)</td>
</tr>
</tbody>
</table>

**Standard deviation in parentheses.**

% of the micro-organisms at the sampled sites, tested between sites, within patients with a detectable level of the specific micro-organism at at least one of the sampled sites, using a Friedman test, and Wilcoxon tests for post-testing: *= Site at which the highest % of a micro-organism was recovered.

% in total 37 subjects were selected. The inflamed pocket with attachment loss was not included in the analysis.

Relationship clinical and microbiological findings

Table 4 shows the prevalence of the studied micro-organisms in the subgingival plaque in relation to the three different categories of attachment loss. No statistically significant relationship was found between the prevalence of *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, spirochetes, motile rods on all selected sample sites and the severity of disease as analyzed for the different periodontitis patient categories.
Table 4. Prevalence of micro-organisms in sampled sites for different patient categories of severity of attachment loss

<table>
<thead>
<tr>
<th>Patient categories</th>
<th>A. actinomycetemcomitans</th>
<th>P. gingivalis</th>
<th>P. intermedia</th>
<th>Motile rods</th>
<th>Spirochetes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tongue</td>
<td>gingiva</td>
<td>saliva</td>
<td>pocket without AL</td>
<td>pocket with AL</td>
</tr>
<tr>
<td>no/minor 0-2mm AL n=169</td>
<td>27%</td>
<td>17%</td>
<td>15%</td>
<td>37%</td>
<td>76%</td>
</tr>
<tr>
<td>moderate 3-4mm AL n=65</td>
<td>22%</td>
<td>20%</td>
<td>17%</td>
<td>37%</td>
<td>78%</td>
</tr>
<tr>
<td>advanced ≥5mm AL n=21</td>
<td>14%</td>
<td>24%</td>
<td>10%</td>
<td>43%</td>
<td>42%</td>
</tr>
</tbody>
</table>

Inflamed pockets without attachment loss in comparison with inflamed pockets showing attachment loss

The 37 patients in which a pocket with attachment loss was sampled, belonged to the categories ‘moderate periodontitis’ (n = 18) and ‘advanced periodontitis’ (n = 19). Due to technical reasons data of 2 of the 21 subjects of the latter category were unable to be analyzed.

Table 5 shows the clinical and microbiological data of the 37 subjects in the inflamed pocket with and without attachment loss. No difference in mean plaque and bleeding index was observed between the 2 types of pockets. As expected, the mean pocket depth was larger in pockets showing loss of attachment.
Analysis of the prevalence of micro-organisms in the 2 types of pockets showed a higher prevalence of *P. gingivalis* and spirochetes in inflamed pockets with attachment loss than in inflamed pockets without attachment loss (p = 0.0143, p = 0.0013 respectively).

With respect to the % of micro-organisms recovered from the 2 different pockets *P. gingivalis* and spirochetes were once again found in higher percentages in the pockets showing attachment loss (p=0.0042, p = 0.0126, respectively).

Table 5. Comparison between inflamed pocket with and without attachment loss within individuals of mean clinical parameters, prevalence and proportion of cultivable subgingival microbiota

<table>
<thead>
<tr>
<th></th>
<th>without attachment loss (n=37)</th>
<th>with attachment loss (n=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pocket depth</td>
<td>3.84 (0.87)</td>
<td>7.27 (1.90)</td>
</tr>
<tr>
<td>Attachment loss</td>
<td>0.00 (0.00)</td>
<td>5.27 (2.83)</td>
</tr>
<tr>
<td>Plaque</td>
<td>1.54 (0.90)</td>
<td>1.62 (0.79)</td>
</tr>
<tr>
<td>Bleeding</td>
<td>1.68 (0.47)</td>
<td>1.51 (0.69)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>Prevalence % recovery</th>
<th>Prevalence % recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. actinomycetemcomitans</em></td>
<td>49%[18] 0.73 (0.77)</td>
<td>59%[22] 1.07 (1.19)</td>
</tr>
<tr>
<td><em>P. gingivalis</em></td>
<td>70%[26] 2.99 (2.95)</td>
<td>86%[32]* 8.20(10.37)*</td>
</tr>
<tr>
<td><em>P. intermedia</em></td>
<td>84%[31] 2.36 (3.65)</td>
<td>81%[30] 4.10 (3.85)</td>
</tr>
<tr>
<td>Spirochetes</td>
<td>51%[19] 11.12 (7.82)</td>
<td>84%[31]* 14.29(10.70)*</td>
</tr>
<tr>
<td>Motile rods</td>
<td>54%[20] 15.94(12.76)</td>
<td>70%[26] 15.03(10.55)</td>
</tr>
</tbody>
</table>

Number of subjects in [] parentheses
Standard deviation in () parentheses
Prevalence of the micro-organisms tested between pockets with and without attachment loss, within patients, using McNemar's tests: • p<0.02.
% of micro-organisms tested between pockets with and without attachment loss, within patients, using the Wilcoxon test: • p<0.02.

**DISCUSSION**

The present study describes the periodontal condition and the prevalence of selected micro-organisms in all young adolescents between 15-25 years of a rural Indonesian village. The clinical data show a large number of subjects with moderate periodontitis (26%), whereas 8% had advanced attachment loss. To compare the present results to other epidemiological data on periodontitis in rural area's in Africa and Asia is difficult, since most of these studies used the...
CPITN index system (Miyazaki et al. 1991, Wibowo et al. 1987). In the present study full mouth approximal measurements are presented at subject level. Other authors present their material as the % of sites exhibiting a certain level of attachment loss (Löe et al. 1986, Baelum et al. 1988). In their classical study amongst Sri Lankan tea labourers, Löe et al. (1986) demonstrated that 8% of the population showed rapidly progressive periodontal disease (RPP) and approximately 80% moderate progression of periodontal disease (MP). In the age group 15-25 years of the Sri Lankans within the RPP group, 16.3% of the sites showed advanced disease. This was 3.8% within the MP group. Beyond the age of 35, all subjects in the study population showed periodontal attachment loss (> 2 mm). In a rural Kenyan population, Baelum et al. (1988) found in the 15-24 years age group approximately 1% of sites showing ≥ 4 mm. attachment loss. Despite the limitations set by attempting to compare the Kenyan and Sri Lankan studies, the present data indicate that the extent of periodontal attachment loss in this Indonesian study population can be considered high.

As shown in Fig. 1 the first upper molar and the upper and lower incisors appeared to be the sites most frequently affected by attachment loss. Baelum et al. (1988) show a similar pattern for the Kenyan population. Löe et al. (1978a, 1978b) calculated the mean attachment loss on buccal and mesial surfaces per tooth type for different age cohorts in the Sri Lankan population. They found that the first upper molar and the upper and lower incisors were the sites showing the highest mean attachment loss. Although these results are presented differently, they do suggest, together with the present results and those shown by Baelum et al. (1988), that periodontitis which develops without the interference of oral hygiene measures and dental treatment, affects upper molars and both upper and lower incisors more frequently than other teeth.

In a previous report on this study population, the analysis showed a significant effect of sibling relationship on the periodontal condition (Van der Veijen et al. 1993). This suggests that a communal genetic influence could in part be responsible for the high prevalence of periodontitis in this population. A further explanation for this high prevalence may be the observation that putative periodontal pathogens are present in abundance at various oral mucosal sites. The latter could be attributed to transmission of these bacteria. Spread of bacteria between members of an isolated population, who live in the same
village for generations may have occurred. This suggestion is based on findings showing intra-familial transmission of putative periodontal pathogens (Petit et al. 1994). In addition, the prolonged accumulation of supragingival plaque and calculus may have provided the ecological conditions favourable to the permanent establishment of periodontal bacteria (Gmür & Guggenheim 1994) and thus forming a source for transmission.

The microbiological data of the present study show that the prevalence of *A. actinomycetemcomitans, P. gingivalis* and *P. intermedia* in the subgingival plaque is 37%, 66%, and 79% respectively. The pockets sampled were sites without attachment loss, the purpose of which has been explained in the material and methods. This makes comparison with other microbiological investigations complicated, because generally a diseased site with loss of attachment is used as the sample unit.

In Western populations *A. actinomycetemcomitans, P. gingivalis* and *P. intermedia* are found infrequently in periodontal health (Mandell & Socransky 1981, Lie et al. 1994, Riggio et al. 1996, Furcht et al. 1996, Conrads et al. 1996). A relatively high prevalence of these micro-organisms was found in Arabic and African adults in the absence of pockets deeper than 3 mm (AL-Yahfoufi et al. 1994, Dahlén et al. 1992). The data of the present study also show a high prevalence of *A. actinomycetemcomitans, P. gingivalis* and *P. intermedia* in the relatively healthy part of the study population (0 - 2 mm maximum attachment loss). A study amongst adult Kenyans (Dahlén et al. 1989), using samples from 2 pre-selected sites, most likely to suffer from attachment loss, showed a prevalence of *A. actinomycetemcomitans, P. gingivalis, P. intermedia* (40%, 70%, 100% resp.), which was comparable to the data of the present study. Wolff et al. (1993) also investigated the prevalence of these 3 micro-organisms in a western (U.S.A.) population, having primarily gingivitis and early periodontitis. They found 38%, 32%, and 42% respectively (separate mesial and distal samples of all molars and premolars in a randomly chosen quadrant). This indicates that the prevalence of *P. gingivalis* and *P. intermedia* in both rural populations (Kenya and Indonesia) is higher compared to a western population.

The data obtained from the analysis of the pocket without attachment loss, presented in Table 4, suggest a slight trend of increasing prevalence of the 3 micro-organisms with increasing disease severity. This could however not be
statistically substantiated. No such trend could be discerned for the other sample sites. Furthermore within subjects with extensive periodontal destruction, a significant difference was observed between pockets with or without attachment loss for \textit{P. gingivalis} and spirochetes (Table 5). Wolff et al. (1993) also observed an association of a high prevalence of \textit{P. gingivalis} with the presence of $\geq 5\text{mm}$ pockets. In the population of Sri Lankan tea labourers, that was earlier investigated by Loe et al. (1986), Preus et al. (1995) showed, that the prevalence of \textit{P. gingivalis} and \textit{P. intermedia} in sites with moderate to advanced periodontitis was higher than in sites showing no disease or gingivitis; the prevalence in advanced sites was higher than in moderate sites. Dahlén et al. (1992) compared sites with and without attachment loss in a diseased adult Kenyan subpopulation. The prevalence of \textit{P. intermedia} was 86\% in both sites, which was in agreement with the present results. \textit{A. actinomycetemcomitans} was observed less frequently although the difference in prevalence between sites with and without attachment loss was of comparable magnitude as in the present study. As seen in the present population, \textit{P. gingivalis} was the only micro-organism, which was significantly more prevalent in inflamed pockets with attachment loss than in inflamed pockets without attachment loss. As was suggested by these authors, the relatively undisturbed plaque in their subjects, comparable to the situation in the present population, may favour the establishment of \textit{P. gingivalis} and therefore provide an explanation of the finding that this micro-organism was frequently recovered from sites without attachment loss.

In conclusion a high prevalence of periodontitis was found in the present population. \textit{A. actinomycetemcomitans, P. gingivalis, P. intermedia}, motile rods, and spirochetes were observed in abundance in the saliva, on the dorsum of the tongue and gingiva as well as in the pocket, both in sites with and without attachment loss. No significant association between the clinical periodontal parameters and the studied micro-organisms was observed at a patient level. At a site level, both \textit{P. gingivalis} and spirochetes were more prevalent in sites with attachment loss. The actual role of these putative periodontal pathogens may be better understood, when longitudinal data on the present population become available.
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