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Java project on periodontal diseases. The natural development of periodontitis: risk factors, risk predictors and risk determinants

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Abstract

Objective: To identify risk factors, risk predictors and risk determinants for onset and progression of periodontitis.

Material and Methods: For this longitudinal, prospective study all subjects in the age range 15–25 years living in a village of approximately 2000 inhabitants at a tea estate on Western Java, Indonesia, were selected. Baseline examination was carried out in 1987 and follow-up examinations in 1994 and 2002. In 2002, 128 subjects could be retrieved from the original group of 255. Baseline examination included evaluation of plaque, bleeding on probing, calculus, pocket depth, attachment loss and presence of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis, Prevotella intermedia,* spirochetes and motile microorganisms.

Results: The mean attachment loss increased from 0.33 mm in 1987 to 0.72 mm in 1994 and 1.97 mm in 2002. Analysis identified the amount of subgingival calculus and subgingival presence of *A. actinomycetemcomitans* as risk factors, and age as a risk determinant, for the onset of disease. Regarding disease progression, the number of sites with a probing depth $\geqslant 5$ mm and the number of sites with recession were identified as risk predictors and male gender as a risk determinant.

Conclusion: Screening of these parameters early in life could be helpful in the prevention of onset and progression of periodontal diseases.

Key words: periodontitis; risk factors; risk predictors; untreated disease

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During the last decades, it has become evident that severe forms of periodontitis affect only a small subset of the population (Albandar & Rams 2002). An important part of planning dental care systems is identifying those with active disease and detecting cases that are likely to develop disease in the future (Sheiham & Netuveli 2002). Therefore, studies have been carried out trying to assess the risk for periodontitis. Risk is defined as the probability that an event will occur in the future, that is, the probability that an individual develops a given disease. In this respect, three types of variables are important (Beck 1994). The first type are risk factors; i.e., characteristics that are thought to be aetiologic for the disease of interest and that have shown to increase one's odds for developing a disease, e.g. a specific bacterium. The second type of variable involves background characteristics that are not considered to be a aetiologic and are immutable to change. They may be confounders or effect modifiers for the risk factors. This variable is often referred to as a risk determinant, e.g. age, gender and race. The third type of variable are risk predictors. These are usually either biological markers that are indicative of disease or disease progression, but currently are considered not to be a aetiologic, or alternatively are historical measures of the disease being studied, such as past evidence of periodontal disease.

In order to study the value of such variables, long-lasting longitudinal investigations studying the natural history of periodontal diseases are indispensable.

Such studies should include the total sequence from onset of the disease at a young age to severe breakdown at an older age. This type of study is extremely scarce. The first and most comprehensive study was carried out by Löe and co-workers in Sri Lanka (1986). It was initiated in 1970 and had a follow-up period of 15 years. A more recent study was carried out by Baelum et al. (1997) in China and involved an evaluation period of 10 years. In both studies, the baseline evaluation was limited to clinical parameters. Therefore, these studies could only provide information on the value of clinical variables in terms of risk for periodontitis. The present study on the natural history of periodontal disease was designed both as a clinical as well as a microbiological prospective study. The baseline examination was carried out in 1987 in a study population with an age range of 15–25 years that received only emergency dental care in terms of tooth extractions. After 7 years, in 1994, the first re-evaluation was performed (Timmerman et al. 2000). In 2002, after a period of 15 years, a second re-evaluation was carried out.

The purpose of the present communication is (1) to describe the changes in the clinical condition over this 15-year period and (2) to study the value of baseline clinical, microbiological and background variables as possible risk factors, risk predictors and risk determinants for future periodontal breakdown.

Materials and Methods

Study population

For this longitudinal, prospective study, a village with approximately 2000 inhabitants at the Malabar/Poerbasari tea estate on Western Java, Indonesia, was selected. At the baseline evaluation in 1987, all inhabitants ($N = 255$) in the age range 15–25 years participated in this investigation. This particular population was selected because it had not received regular dental care and had not been exposed to preventive dental care programmes. Only emergency dental treatment, consisting of extraction of teeth, was provided by a general physician. Therefore, this study population was suitable for monitoring the natural development and progression of periodontitis. The population consisted mostly of tea labourers, receiving basic medical care, employed by a government-owned tea estate, PTP XIII.

Examination procedures

At baseline in 1987, the participants were clinically and microbiologically examined. In addition, they were asked about their educational level, general health status and recent use of antibiotics. In 1994 and 2002, the clinical examination was repeated in the subjects of this group who were retrievable. Furthermore, in 2002 the self-reported smoking history in terms of number of cigarettes per day was recorded. Subjects were also asked to indicate their tooth-brushing habits in terms of the frequency of tooth brushing per week. The clinical assessments were carried out by three periodontists and dictated to a chair side scribe. All clinical examinations were performed in an office facility of the tea factory, and portable dental chairs were used.

Clinical Examination

The following indices were recorded in the subsequent order:

- Plaque (Silness & Loë 1964).
- Calculus (calculus component of the retention index, Björby & Loë 1967).
- Pocket depth (PD) using a force-controlled probe (Brodontic® Ash/Dentsply, York, UK, 240 N/cm^2) with a Williams calibration.
- Bleeding on probing (PPBI; Van der Velden 1979) using the force-controlled probe using a three-point scale: (0) non-bleeding sites; (1) ''pin prick'' bleeding; and (2) ''excess'' bleeding.

  Bleeding was scored within 30 s after probing.
- 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, by 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, Hu-Friedy, Chicago, IL, USA).

Clinical parameters were scored on the approximal surfaces of the vestibular aspect of all teeth except third molars. Calculus was scored on the approximal surfaces of the Ramfjord teeth (M1sd, I2ss, P1ss, M1 is, I1 id and P1 id) (Ramfjord 1959). Measurements were rounded off to the nearest millimetre.

Reproducibility

At baseline in 1987, every 10th subject was used for the assessment of reproducibility of the clinical parameters located at the Ramfjord teeth. At the end of each morning and afternoon session, three clinical examiners (E. G. W., F. A., S. A.) repeated the clinical assessments to establish the inter-examiner variance. Both at follow-up in 1994 and 2002, in a randomly chosen sample of 18 subjects, all clinical measurements were repeated by each of the three examiners (E. G. W., F. A., G. A. W.) in each patient.

Microbiological examination

At baseline in 1987, before the clinical examination, all samples for microbiological evaluation were obtained except those from the pockets that were taken after the clinical measurements were completed. The microbiological samples 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; and
- the deepest bleeding pocket without attachment loss.

Samples from the tongue and the buccal gingiva were obtained by sweeping a sterile swab under continuous pressure over the total surface. When sampling 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. When all clinical measurements were completed, the deepest bleeding pocket without clinical loss of attachment was selected and sampled. After carefully removing the supragingival plaque with a curette, a subgingival sample was taken using a nerve broach (Maillefer®, Ballaigues, Switzerland) wound with cotton and heat sterilized. The part of the nerve broach that had been inserted into the pocket was cut off and suspended in 0.9 ml RTFF. Specimens were vortexed for 60 s at the maximum setting. Samples were processed for phase contrast microscopy to establish the prevalence of spirochetes

(Spir) and motile microorganisms (Mot MO). The samples were fixed with formaldehyde (0.2%, v/v) for indirect immunofluorescence examination to investigate the presence and proportions of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia*. Ten microlitre 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 and subsequently further processing for indirect immunofluorescence assay (Van der Velden et al. 1993).

Data analysis

For baseline and both follow-up evaluations, the clinical parameters assessed at all approximal surfaces were computed as a mean score per patient. The number of sites showing attachment loss $\geqslant 2$ mm and the number of sites showing probing depth of $\geqslant 5$ mm were enumerated. Calculus scores were analysed as the number of approximal surfaces of the Ramfjord teeth showing subgingival calculus.

For each patient, data concerning probing depth and attachment loss at baseline and follow-up were compared at a site level. Differences between baseline and follow-up were calculated for each site individually. Subsequently, data were recalculated as mean difference scores or enumerated as a number of sites showing a given change within each subject. A site was accepted to have a change in pocket depth or attachment level when a difference of $\geqslant 2$ mm was established (Haffajee et al. 1983, Lindhe et al. 1983). Comparisons of clinical parameters between the three assessments were made using a Wilcoxon's signed rank test. Inter-examiner error was estimated by calculation of the inter-examiner standard deviation for all approximal measurements of the Ramfjord teeth in 1987 and 1994 and of the randomly selected quadrant in 2002. A mixed models analysis of variance was used at site level (inter-examiner standard deviation: in 1987; plaque: 0.11, calculus: <0.001, BOP: 0.23, PD: 0.19, AL: 0.05; in 1994; plaque: 0.07, calculus: 0.05, BOP: 0.10, PD: 0.09, AL: 0.05; in 2002; plaque: 0.11, calculus: 0.38, BOP: 0.04, PD: 0.05, AL: 0.44) and at patient mean level (inter-examiner standard deviation: in 1987; plaque: 0.11, BOP: 0.23, PD: 0.19, AL: 0.05; in 1994; plaque: <0.001, BOP: <0.001, PD: <0.001, AL: 0.09; in 2002; plaque: 0.10, BOP: 0.03, PD: 0.05, AL: 0.45). These data are within the boundaries of reproducibility as described by Haffajee & Socransky (1986).

Microbiological data at baseline were analysed for the prevalence of the microorganisms under study by establishing the proportion of subjects positive for the various microorganisms irrespective of the sample site.

In order to determine which background, clinical and microbiological parameters may be regarded as risk factors and/or risk determinants for the onset of periodontitis on a subject level, subjects were divided into cases and non-cases on the basis of the baseline examination. Cases ($N = 27$) were defined as the presence of an approximal attachment loss of $\geqslant 3$ mm in $\geqslant 2$ non-adjacent teeth (Tonetti & Claffey 2005). For the non-cases ($N = 101$), a multiple forward logistic stepwise regression analysis was used for subjects that did or did not develop approximal attachment loss $\geqslant 2$ mm during the first 7-year evaluation period. In the analysis, the association was determined between the dependant variable, i.e. the development of approximal attachment loss $\geqslant 2$ mm and the parameters that were entered as independent variables, i.e. age, gender, smoking, level of education and baseline values of the number of teeth, plaque index, bleeding index, mean pocket depth, the number approximal sites on Ramfjord teeth showing subgingival calculus (scores 2 and 3), number of sites with PD $\geqslant 5$ mm, presence of *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, Spir and Mot MO in the sampled pocket, on the gingiva, on the tongue and in the saliva. p values of <0.1 were required for a factor to be retained in the equation. In order to determine which background, clinical and microbiological parameters may be regarded as risk predictors and/or risk determinants for disease progression, a multiple forward linear stepwise regression model was used, analysing the association of the amount of progression of attachment loss over the 15-year period as a dependent variable with all previously mentioned variables as well as the mean pre-existent attachment loss for all retrievable subjects ($N = 128$). The essential first step in both forward stepwise analysis procedures is that each independent variable is entered into a simple equation to test for single associations between each independent variable and the dependent variable.

To examine to what extent the present study population included in 2002 severe periodontitis patients, subjects showing $\geqslant 2$ sites with a pocket depth $\geqslant 5$ mm in conjunction with $\geqslant 6$ mm attachment loss (Van der Velden 2005) were enumerated and as a group analysed in comparison with the remaining population. The mean values of the clinical parameters in 1987, 1994 and 2002 of these two groups were compared for each parameter using Mann–Whitney tests. The prevalence of micoorganisms was compared between these groups using a Fisher's exact test. Values of $p < 0.05$ were accepted as being statistically significant.

Results

In 1987, all 255 inhabitants of the village in the age range of 15–25 years participated in the study. In 2002, 128 subjects could be retrieved out of the 255 subjects originally evaluated in 1987. Analysis of the 1987 data showed that the 128 participants were older than the 127 subjects who could not be retrieved, 21.1 ± 3.0 *versus* 18.9 ± 3.0 years, respectively ($p < 0.001$). No other baseline differences in terms of clinical or microbiological parameters could be assessed. The demographic characteristics of the present study population are presented in Table 1. The number of males and females was comparable. Smoking appeared to be almost exclusively restricted to males; one female and 52 (87%) males reported to smoke. Most subjects ($>80\%$) had been smoking for $\geqslant 10$ years. The mean number of cigarettes smoked per day was 12.8 ($\pm$ 8.1); 25 subjects (47%) smoked $\leqslant 10$ cigarettes/day and 28 (53%) $\geqslant 10$ cigarettes/day. In general, subjects exhibited a low education level; 52% did not complete elementary school. In 2002, all subjects reported to brush their teeth more than once a day.

The clinical condition during the 15-year observation period is presented in Table 2. The number of teeth decreased and the periodontal condition deteriorated. Gingival recession showed no increase during the first 7 years of observation, whereas thereafter a sixfold increase had occurred. Attachment loss doubled between 1987 and 1994, but

Table 1. Demographic characteristics of the study population ($N = 128$)

Number of subjects	Female	Male	All subjects
Gender	68	60	128
Smoking	1	52	53
Elementary school not completed	47	20	67
Elementary school completed	18	30	48
Junior high school	2	10	12
Senior high school	1	0	1

Table 2. Mean clinical parameters (standard deviation) at baseline (1987) and follow-up in 1994 and 2002 for all sites in 128 subjects

	1987	1994	2002
Number of teeth	27.5 (1.01)†	26.9 (1.55)†	25.9 (2.41)
Plaque index	1.01 (0.45)†	1.15 (0.38)◆	1.04 (0.39)
Bleeding on probing	0.77 (0.35)†	1.17 (0.40)	1.23 (0.39)
Number of sites with subgingival calculus‡	5.71 (3.10)	5.93 (2.74)*	5.07 (3.19)
Number of sites with gingival recession	0.59 (1.12)	0.53 (1.52)†	3.34 (6.35)
Pocket depth (mm)	3.22 (0.47)	3.33 (0.45)†	3.53 (0.58)
Attachment loss (mm)	0.33 (0.30)†	0.72 (0.49)†	1.97 (1.01)
Number of sites PD $\geqslant$5 mm	4.20 (6.11)*	5.84 (6.40)†	7.47 (7.87)

‡Score 2 and 3 as scored on approximal surfaces of Ramfjord teeth.
*$p \leqslant 0.05$.
†$p \leqslant 0.001$.
◆$p \leqslant 0.01$.

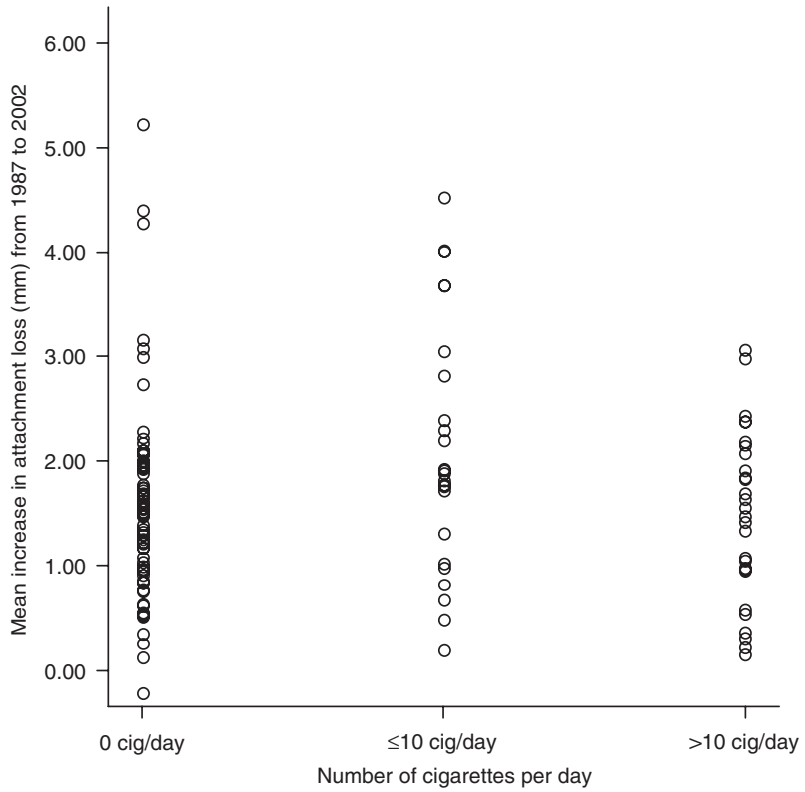

Fig. 1. Mean increase of attachment loss (mm) per subject between 1987 and 2002 in relation to the smoking status, i.e. the number of cigarettes per day.

almost tripled between 1994 and 2002. In Fig. 1, the mean increase of attachment loss between 1987 and 2002 is presented for all smokers and non-smokers. Analysis showed no difference in amount of disease progression between smokers and non-smokers. Furthermore, no effect of age on the rate of progression of attachment loss could be assessed. During the entire 15-year study period, subjects lost on average $\geqslant$2 mm attachment at 23 sites (Table 3). The highest number of sites losing $\geqslant$2 mm attachment, found in one subject, was 52. The most severe form of breakdown was found in another subject having at 19 sites $\geqslant$6 mm attachment loss.

The microbiological examination in 1987 showed a high prevalence of the putative periodontal pathogens varying between 55% for *A. actinomycetemcomitans* and 100% for *P. intermedia* and Mot MO (Table 4). *A. actinomycetemcomitans*, *P. gingivalis* and Spir were most frequently found in the pocket, whereas *P. intermedia* and Mot MO showed the highest prevalence on the dorsum of the tongue.

In 1987, 101 subjects were identified as non-cases. In this group, 72 subjects developed attachment loss during the first 7 years. The logistic regression analysis identified the number of sites with subgingival calculus and the subgingival presence of *A. actinomycetemcomitans* as risk factors, and age as a risk determinant for the onset of disease, with odds ratios 1.4, 4.3 and 1.3, respectively (Table 5). In order to determine which background, clinical and microbiological parameters as assessed in 1987 may be regarded as risk predictors or risk determinants for disease progression, a multiple linear stepwise regression model was carried out for the data over the 15-year evaluation period. The results of this analysis are presented in Table 6. It can be seen that three variables showed a statistically significant effect and were predictive for the amount of future attachment loss. This signifies that the number of sites with a pocket depth $\geqslant$5 mm and the number of sites with recession were identified as risk predictors and male gender as a risk determinant. The number of sites with a pocket depth $\geqslant$5 mm explained 10.9% of the variance, the number of sites with recession 2.8% and male gender 3.5%.

The prevalence of severe periodontitis patients, i.e. subjects showing $\geqslant$2 sites with a pocket depth $\geqslant$5 mm in conjunction with $\geqslant$6 mm attachment loss (Van der Velden 2005) amounted to 20% in 2002 (26 subjects). The mean values of the clinical parameters at baseline and at follow-up in 1994 and 2002 of these subjects were compared with the remaining population (Table 7). Analysis showed that already at baseline

Table 3. Mean number (standard deviation) and range of sites that lost or gained attachment between 1987 and 2002 per subject ($N = 128$); four subjects did not develop attachment loss $\geqslant 2$ mm

Attachment level change (mm)	1987–2002					
	number of sites with attachment loss			number of sites with attachment gain		
	N	mean (SD)	range	*N*	mean (SD)	range
$\geqslant 2$	124	23.1 (13.8)	0–52	19	0.3 (1.3)	0–12
$\geqslant 3$	114	10.3 (10.7)	0–46	—		
$\geqslant 4$	91	4.4 (7.5)	0–37	—		
$\geqslant 6$	29	1.0 (2.8)	0–19	—		

N, number of subjects.

Table 4. Prevalence, in percentage, of microorganisms in the study population at baseline in 1987 for sample sites and subjects ($N = 128$)

$N = 128$	Pocket	Gingiva	Tongue	Saliva	Subjects
Actinobacillus actinomycetemcomitans					
Prevalence	36	16	20	9	55
Proportion	0.9 (0.7)	1.5 (2.5)	0.3 (0.3)	0.5 (0.5)	
Porphyromonas gingivalis					
Prevalence	66	36	63	49	88
Proportion	4.4 (6.9)	0.8 (1.3)	1.0 (2.6)	1.1 (1.6)	
Prevotella intermedia					
Prevalence	81	63	100	82	100
Proportion	2.7 (4.0)	1.4 (1.7)	16.6 (14.5)	2.6 (6.1)	
Spirochetes					
Prevalence	65	13	48 4.0	56	90
Proportion	12.4 (7.7)	4.6 (3.1)	(3.2)	4.8 (4.3)	
Motile microorganisms					
Prevalence	60	23	91 18.5	87	100
Proportion	15.5 (11.7)	9.6 (11.6)	(12.5)	21.3 (16.9)	

In subjects who are positive for a given microorganism, the mean proportions (standard deviations) are given.

Table 5. Odds ratios and confidence intervals of significant risk factors and risk determinants for the onset of attachment loss $\geqslant 2$ mm in the period from 1987 to 1994 as assessed by means of a multiple logistic stepwise regression analysis ($N = 101$)

Variable	Regression coefficient	SE	*p*-value	Odds ratio	Confidence interval
# sites with subgingival calculus	0.34	0.124	0.006	1.40	1.10–1.79
Subgingival presence *Actinobacillus actinomycetemcomitans*	1.46	0.662	0.028	4.30	1.17–15.75
Age	0.28	0.093	0.003	1.32	1.10–1.58
Model constant	− 6.62	2.14	0.002	0.001	

SE, standard error.

and throughout the study period, the periodontal condition in these 26 subjects was more severe in terms of plaque, bleeding on probing, the number of sites with gingival recession, pocket depth, the number of sites with a pocket depth $\geqslant 5$ mm and the amount of attachment loss. However, no difference in the prevalence of the studied microorganisms could be assessed between these 26 subjects and the rest of the population.

Discussion

The present investigation dates back to the 1980s. In those days, several epidemiological studies suggested that the prevalence of periodontitis may be much lower than previously assumed. In this perspective, a pilot study was carried out in several villages on the same tea estate before the initiation of the present study. In these villages, a number of subjects $\geqslant 50$ years were randomly selected and screened for their periodontal condition. The purpose of this pilot study was to investigate whether subjects in this rural area did indeed develop severe periodontitis. As several subjects were identified with severe periodontitis, it was decided that it would be appropriate to establish a longitudinal research project. The present data confirm these initial observations as 20% of the study population (mean age 37 years; SD = 4.37) showed the presence of deep pockets with severe attachment loss in 2002. In 2002, we were able to retrieve 128 subjects from the initial study population. The reasons for the dropout of 127 subjects who could not be examined in 2002 are most likely the changing conditions on and around the tea estate. In 1987, the area was remote and relatively isolated from the outside world. In 2002, there was rapid communication to the city of Bandung by public transport and almost all inhabitants of the village now had a television set. The increased awareness of the outside world by the people in the village is most likely responsible for the fact that especially younger subjects moved to the city. This is reflected in the observation that the non-retrievable subjects were on average 2 years younger than those re-examined in 2002.

The results of the present study show a mean annual attachment loss of 0.05 mm during the first 7 years of observation and increased to 0.15 mm during the following 8 years. The initial progression rate of 0.05 mm/year is comparable with values reported in other longitudinal studies with long-lasting observation periods, e.g. Papapanou et al. (1989) and Norderyd et al. (1999) in Swedish populations, Ismail et al. (1990) in an American population and Löe et al. (1986) in the non-progressive group of a Sri Lanka population. The higher annual rate of attachment loss of 0.15 mm of the present study during the second evaluation period is comparable with the rate of progression as observed in subjects with early onset periodontitis (Albandar et al. 1998). It is also comparable with the progression rate in a Chinese population (Baelum et al. 1997) and, on average, in the moderate progressive group of the Sri Lanka study after the age of 20. However, 0.15 mm is considerably less in comparison with the rapidly progressive group of the Sri Lanka population after the age of 20, which showed a

Table 6. Significant risk predictors and risk determinants for disease progression between 1987 and 2002 as assessed by means of a multiple linear stepwise regression analysis ($N = 128$)

Variable	Unstandardized coefficient B	SE	Correlation coefficient β	p-value	% variance explained
# of PD $\geqslant$ 5 mm	0.050	0.013	0.331	<0.0001	10.9
# of Recessions	0.150	0.061	0.169	0.015	2.8
Male gender	-0.371	0.137	-0.187	0.008	3.5
model constant	2.008	0.300		<0.0001	

SE, standard error.

mean annual attachment loss of 0.5 mm or more (Löe et al. 1986). This value is also much higher than the mean annual attachment loss of 0.27 mm of the 26 subjects with severe periodontitis in the present study during the second evaluation period. This suggests that the rate of attachment loss in the rapidly progressive group of the Sri Lanka population may be exceptionally high.

In the present study, age had no influence on the rate of progression of attachment loss. However, a higher rate of progression was observed during the second evaluation period of 8 years compared with the first 7 years. The reason why the attachment loss accelerated irrespective of age is difficult to explain. It may be hypothesized that an environmental factor was introduced that affected the host defence and resulted in a reduced resistance to periodontal breakdown. In this respect, it may be suggested that the economic crisis in the late 1990s that severely hit the Indonesian economy could be responsible. In 2002, the income of tea labourers was almost the same as in 1994, whereas prices had more than doubled over this period. This could have resulted in a change of diet towards less expensive food containing fewer vitamins. Further investigation is ongoing to substantiate this hypothesis.

The results of the microbiological examination at baseline showed a high prevalence of putative periodontal pathogens. These data are in line with the results of other studies in Asian populations. For example, *A. actinomycetemcomitans* showed a prevalence of 62–83% in Chinese populations (Papapanou et al. 1997, Mombelli et al. 1998) and 92% in a Thai population (Papapanou et al. 2002). These values were, for *P. gingivalis*, 55–100% (Chinese population) and 100% (Thai population), respectively.

The present clinical and microbiological prospective study was undertaken in order to identify possible risk factors and risk determinants for the onset of disease and risk predictors and risk determinants for progression of disease. As far as we are aware, no studies are available that investigated risk factors for the onset of disease in an untreated population. Most prospective studies determine risk factors for disease progression and/or onset of new disease on a site level rather than on a subject level (e.g. Beck et al. 1995, Muller et al. 2002, Kocher et al. 2005, Neely et al. 2005). This is not in line with the definition of risk factors as commonly used in medicine, as for risk factors it has to be shown that they increase a person's chances of developing a disease (MedicineNet 2006). In order to be able to identify risk factors for the onset of disease, subjects must be classified at baseline into cases and non-cases. For this classification, the criteria as proposed by 5th European Workshop in Periodontology were applied (Tonetti & Claffey 2005). Therefore, non-cases may have sites with an attachment loss measurement of up to 2 mm. For development of disease, the criterion $\geqslant$2 mm attachment loss was used as, e.g. an additional loss of 2 mm reflects a site with 4 mm attachment loss. Such an individual has obviously become a case. The results demonstrated significant odds ratios for the onset of disease with age, amount of subgingival calculus and the subgingival presence of *A. actinomycetemcomitans*. In previous reports, the outcome of the first evaluation period of 7 years has been presented for 167 retrievable subjects showing comparable results as in the present evaluation, although a slightly different analysis was used. The fact that in the present smaller group of subjects again subgingival calculus and *A. actinomycetemcomitans* were identified as significant risk factors for the onset of disease strengthens the previous observation. Age came out as a risk determinant. One could argue that due to the fact that with ageing a subject is exposed to a longer period of time to the bacterial challenge, the chance for disease development increases. However, the logistic regression analysis does not corroborate

Table 7. Mean values of the clinical parameters at baseline in 1987 and follow-up in 1994 and 2002 for 26 subjects with severe periodontitis in 2002, in comparison with the remaining population ($N = 102$)

	1987		1994		2002	
	severe periodontis	remaining population	severe periodontitis	remaining population	severe periodontitis	remaining population
Number of teeth	27.4 (1.06)*	27.5 (1.00)	26.8 (1.68)	27.0 (1.52)	25.5 (2.84)	26.0 (2.29)
Plaque index	1.19 (0.52)◆	0.96 (0.41)	1.32 (0.39)◆	1.09 (0.37)	1.15 (0.39)	1.01 (0.39)
Bleeding on probing	0.93 (0.41)†	0.74 (0.32)	1.31 (0.35)*	0.74 (0.32)	1.33 (0.41)	1.20 (0.38)
Number of recessions	1.50 (1.86)†	0.35 (0.67)	1.31 (1.76)†	0.33 (1.40)	11.12 (9.31)†	1.35 (3.12)
Pocket depth	3.48 (0.64)†	3.15 (0.39)	3.70 (0.47)†	3.23 (0.41)	4.07 (0.81)†	3.39 (0.41)
Attachment loss	0.56 (0.41)†	0.28 (0.23)	1.19 (0.57)†	0.28 (0.23)	3.37 (1.29)†	1.61(0.67)
Number of sites PD $\geqslant$ 5	7.85 (6.11)†	3.33 (4.77)	10.65 (6.40)†	4.62 (5.52)	15.23 (10.94)†	5.49 (5.36)

*p-value $\leqslant 0.05$.
◆p-value $\leqslant 0.001$.
†p-value $\leqslant 0.01$.

this mechanism as age was identified as a significant factor, even though all microbiological parameters were entered into the analysis. Regarding calculus, it may be not surprising that it came out as a risk factor for onset of disease as calculus and periodontal disease are inextricably intertwined with each other (Roberts-Harry & Clerehugh 2000). A major finding of the present study was that *A. actinomycetemcomitans* could be identified as a risk factor for onset of disease. This microorganism has frequently been implicated in the aetiology of early periodontitis, however, only on the basis of associations between presence of this microorganism and the presence and progression of periodontal disease in cross-sectional and longitudinal studies (Slots & Ting 1999). An interesting aspect with regard to *A. actinomycetemcomitans* in the present study is the finding that only the subgingival presence had a predictive value for the onset of disease and not the presence of *A. actinomycetemcomitans* on the mucous membranes. Apparently, the mere colonization of the mucous membranes by *A. actinomycetemcomitans* is not enough to predict onset of disease. This finding possibly reflects the incapability of the susceptible host to resist subgingival colonization by *A. actinomycetemcomitans*.

With regard to risk predictors for disease progression, the present study identified three variables that had a predictive value for the amount of future attachment loss, i.e. the number of sites with a pocket depth $\geqslant$5 mm, the number of sites with recession and male gender. One may argue that both the number of sites with a pocket depth $\geqslant$5 mm and the number of sites with recession might affect the stability of the analysis. However, the correlation between these parameters was only 0.172 and not significant. This accounts only for 3% of the explained variance. The finding that approximal recession and pockets $\geqslant$5 mm are a risk predictor for the rate of disease progression may indicate that subjects who develop periodontal breakdown early in life are also more prone to develop further progression. This suggestion is supported by the finding that the 26 subjects who developed severe disease already had a worse periodontal condition in 1987 in comparison with the remaining population. The fact that the presence of pockets $\geqslant$5 mm has a predictive value is in itself not surprising. The presence of deep residual pockets has been shown to be predictive of further disease progression (Renvert & Persson 2002). Empirically, periodontal treatment is aimed at creating shallow pockets. In addition, in studies evaluating the effect of periodontal treatment the most important outcome measure is pocket depth reduction to the range of shallow pockets (Lindhe & Palmer 2002). Furthermore, in children with healthy gingivae the probing depth varies between 1 and 2 mm (Tenenbaum & Tenenbaum 1986, Srivastava et al. 1990). Even in the presence of some degree of gingivitis, the majority of sites in the primary dentition still have probing depths $\leqslant$2 mm. However, in a small number of children probing depths up to 4.5 mm can be found (Rosenblum 1966, Kleiner & Garcia-Godoy 1982). As in the present study it was shown that in a population of 15–25 years of age the number of sites with a pocket depth $\geqslant$5 mm was related to the amount of future attachment loss, it may be hypothesized that the development of sites with a deeper probing depth early in life may be a sign of susceptibility to periodontitis.

It is a well-known finding that males have more severe periodontal disease than females and that smokers have more disease than non-smokers (Baelum et al. 1997, Chen et al. 2001, Albandar & Rams 2002, Calsina et al. 2002, Hyman & Reid 2003, Susin et al. 2005). In the present study, male gender was found to be related to disease progression whereas smoking was not. This finding is not in accordance with the above-mentioned literature. In the present study population, only one female reported to be a smoker whereas 52 out of the 60 males were smokers. From a statistical point of view, the numbers of one female smoker and eight male non-smokers are too small to state with enough power that smoking had no influence on the periodontal condition in this population. In other words, it is possible that in the statistical analyses the entire effect of smoking as an explanatory variable has been carried by the gender variable. However, the data as shown in Fig. 1 show that the range in the amount of disease progression in smokers was smaller as compared with non-smokers. This may indicate that smoking in this population indeed had no influence on the progression of disease. One explanation for the absence of a relationship between smoking and disease progression could be that the approximal pockets were assessed only from the vestibular aspect. It has been shown that smokers especially have deeper pockets, on the lingual sites of teeth (Haffajee & Socransky 2001, Van der Weijden et al. 2001). Another explanation may be that the smokers in this population commonly smoke kretek cigarettes. Kreteks are also known as clove cigarettes, as they typically contain 40% cloves and 60% tobacco. The cloves would serve to provide aroma while also contributing eugenol, a natural component of cloves and a sensory deadening agent to numb the airways while smoking (Fowles 2004). Eugenol belongs to the group of essential oils that possess relatively strong antimicrobial properties (Kalemba & Kunicka 2003), possibly compensating for the negative impact of tobacco smoking on the periodontal tissues. To date there no data are available about the relationship between kretek smoking and periodontal disease.

In conclusion, the present study identified the amount of subgingival calculus and the subgingival presence of *A. actinomycetemcomitans* as risk factors, and age as a risk determinant, for the onset of disease. With regard to disease progression, the number of sites with a probing depth $\geqslant$5 mm and the number of sites with recession were identified as risk predictors and male gender as a risk determinant. It may be suggested that the evaluation of these parameters early in life could be helpful in order to be able to prevent and/or treat periodontal disease at an early stage. In addition, prevention of colonization by *A. actinomycetemcomitans* might contribute to the reduction of the odds to develop periodontitis. This hypothesis can be tested in a longitudinal prospective treatment study.

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Clinical Relevance

Scientific rationale for the study: For the prevention and treatment of periodontal diseases, it is important to know which factors are related to the onset and progression of the disease. Identification of such factors can be obtained in longitudinal prospective studies. Such studies should include the total sequence from initial onset at a young age to severe breakdown at an older age.

Principal findings: Age and subgingival presence of calculus and *A. actinomycetemcomitans* were associated with the onset of disease, while the presence of pockets with probing depths $\geqslant 5$ mm, gingival recession and male gender were associated with disease progression.

Practical implications: During routine dental check-ups of children and young adults, dentist and dental hygienists should always include pocket probing and the assessment of subgingival calculus formation in order to be able to prevent and/or treat periodontal disease at an early stage. The data might support a strategy to screen children for *A. actinomycetemcomitans,* and in case of presence to take measures to control for it.