

PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.

<http://hdl.handle.net/2066/20645>

Please be advised that this information was generated on 2017-12-05 and may be subject to change.

Microscopic spirochete counts in untreated subjects with and without periodontal tissue destruction

Bakari S. Lembariti¹,
Frans H. M. Mikx² and
Wim H. van Palenstein Helderma³

Departments of ¹Restorative Dentistry and
²Community and Preventive Dentistry, Muhimbili
University College of Health Sciences, Dar es
Salaam, Tanzania, ³Department of
Periodontology and Preventive Dentistry,
University of Nijmegen, The Netherlands

Lembariti BS, Mikx FHM, van Palenstein Helderma WH: Microscopic spirochete counts in untreated subjects with and without periodontal tissue destruction. *J Clin Periodontol* 1995; 22: 235-239. © Munksgaard, 1995.

Abstract. The purpose of the study was to determine microscopically the %s of spirochetes at sites without periodontal destruction in subjects with destructive periodontal diseases (cases) and in subjects free of it (controls), who had not received professional prophylaxis. From a sample of 164 individuals aged between 30-44 years living in rural and urban areas of Tanzania, cases and controls were selected. Cases ($n=25$) were selected who exhibited at least 3 teeth with pocket depth of >5 mm. The controls ($n=28$) had no pockets deeper than 3 mm. From each subject, 1 subgingival plaque sample was taken at the mid point of the lingual surface of 1 of the upper premolars which showed bleeding on probing but no calculus and no pockets. In addition, one subgingival sample was obtained from a pocket. Pockets contained the highest %s of spirochetes, which confirms earlier findings. A significant difference in % of spirochetes between cases and controls was found at non-destructive sites, indicating a host effect on the subgingival microflora. However, the spirochete counts at non-destructive sites did not provide a reliable measure to identify subjects with destructive periodontal disease.

Key words: periodontal disease; subgingival microbiota; spirochetes

Accepted for publication 15 March 1994

Periodontal diseases are bacterial in origin. Several micro-organisms as well as groups of micro-organisms are found, either by cultural techniques or by microscopic counts, to be associated with periodontal diseases (Van Palenstein Helderma 1981, Slots & Genco 1984, Loesche 1988, Socransky & Haffajee 1992). Because of their shape, particularly spirochetes can be recognized and counted in subgingival plaque samples with a microscope (Listgarten & Helldén 1978). The % of spirochetes in subgingival plaque is found to be associated with the degree of inflammation at the site of sampling (Lindhe et al. 1980, Mousques et al. 1980, Armitage et al. 1982, Singletary et al. 1982, Savitt & Socransky 1984, Mikx et al. 1986), with the depth of the

pocket (Mousquès et al. 1980, Listgarten & Levin 1981, Armitage et al. 1982, Savitt & Socransky 1984, Wolff et al. 1985) and with the amount of calculus at the site of sampling (Africa et al. 1985). The results of these studies clearly indicate that the number of spirochetes is influenced by the clinical variables of the sampling site. In addition, Evian et al. (1982) indicated the presence of a subject effect on the spirochete numbers in periodontal pockets.

The present study addressed the question of to which extent spirochete numbers are subject associated, and furthermore attempted to assess whether spirochete counts at sites not affected by periodontal destruction could serve as a marker for the selection of subjects with destructive periodontal

disease. In order to reduce the effects of sample-site-related variables on the spirochete numbers, a case-control experimental design was chosen. Sites with comparable clinical conditions were sampled both in subjects with destructive periodontal disease (cases) and in subjects without destructive periodontal disease (controls) who had not received professional prophylaxis. The latter condition was chosen to rule out the possible bias by treatment of spirochete numbers (Listgarten 1984).

Material and Methods

Subjects

164 individuals aged between 30-44 years living in rural and urban areas of Morogoro which is situated 200 km

west of Dar es Salaam, were examined for periodontal conditions. None of the subjects had received any regular professional dental care. All subjects were examined at the place of residence on a portable dental chair fitted with an artificial source of light. Using a graduated Williams periodontal probe and mirror, probing depth was scored on 6 surfaces of each tooth excluding the 3rd molars. Subjects that experienced signs of periodontal disease, that is 3 teeth with pocket depth >5 mm, were assigned as cases. Those who did not have pockets deeper than 3 mm served as controls. 25 cases and 28 controls stratified according to age, sex and residence, were selected. Table 1 shows the distribution of the sample according to sex, age and residence. Assessment of bleeding at the gingival margin was at the midpoint of the lingual surface of the tooth. The tip of the pressure-sensitive probe was placed on the crevicular site, 1 mm under the margin of the gingiva. Bleeding was provoked by exerting a constant force (0.75 N) perpendicular away from the tooth at an angle of 45°. A force of 0.75 N is clinically well tolerated (Van der Velden 1979).

Plaque samples

From each subject, cases and controls, 1 subgingival sample was taken at the

midpoint of the lingual surface of 1 of the upper premolars which showed bleeding on probing but no periodontal pocket and no calculus. This site was chosen because of its low incidence of periodontal destruction, increasing the chance of measuring a subject effect. In addition, in subjects with destructive periodontal disease (cases), 1 subgingival sample was collected from a periodontal pocket of >5 mm showing bleeding at the marginal gingiva after probing. Before the plaque sample was collected, the site was isolated with cotton rolls and supragingival plaque was carefully removed with a curette. The subgingival plaque sample was then collected with a clean curette. The curette was inserted to the bottom of the gingival crevice or pocket, placed in contact with the root surface and subsequently moved in a coronal direction in a scaling stroke. The sample obtained was suspended into 1 ml 4% phosphate buffered formaldehyde and stored at room temperature before examination.

Microscopy

Microscopic examination was carried out after homogenization of the plaque suspension by ultrasonic treatment, 10 s at 10% output power of the homogenizer (Kontes USA), and spreading of 5 μ l of the plaque suspension on a multi-

test slide (Flow laboratories USA). After heat fixation and staining with crystal violet and iodine, the sample was evaluated by light microscopy at 1200 \times magnification using a Zeiss Ph3 Plan 100/1.25 oil objective (Zeiss, Germany). In randomly-selected microscopic fields, all spirochetes and all other bacteria were counted until a total number of at least 200. Samples with a density of more than 100 units per microscopic field were diluted 5 \times . In the group of spirochetes, "large" spirochetes were separately counted. The "large" spirochetes were irregularly winded, stained dark blue and had a width of about 0.5 μ m. They resembled the "large" spirochetes defined by Listgarten & Helldén (1978) which we confirmed by darkfield microscopy of the formalin-fixed suspensions. The investigation was conducted on a double-blind basis. One investigator performed all the clinical examinations and the collection of subgingival plaque samples, whereas another investigator performed the microscopic counts, without awareness of the origin of the coded samples.

Statistics

Repeated counts of 5 samples gave a standard error of the mean spirochetes count of 10%. Differences in % of spirochetes between cases and controls were tested with ANOVA after square root transformation. A three-way ANOVA was applied for assessment of confounding, since in the present sample, sex and age were not evenly distributed over cases and controls (Table 1). Age and sex effects on spirochete count were not presented separately. Differences in % of spirochetes between destructive and non-destructive sites in cases were tested by the paired *t*-test. Odds ratios and confidence intervals as a measure of risk of destructive periodontal disease based on the presence of spirochetes were calculated according to Woolf's method (Schlesselman 1982).

Results

Gingivitis sites without periodontal destruction in case subjects exhibited a statistically significant higher % of spirochetes than in control subjects (Table 2). Sites with pockets, only present in case subjects, showed significantly higher %s of spirochetes than the gingivitis sites without pockets. These sig-

Table 1. Distribution of cases and controls according to sex, age and residence

	Sex		Age (years)		Residence	
	male	female	30-36	37-44	urban	rural
cases	21	4	11	14	15	10
controls	19	9	19	9	18	10

Table 2. Median and interquartile range of the %s of total and large spirochetes in subgingival plaque samples from destructive and non-destructive sites in subjects with periodontal destruction (cases) and from non-destructive sites in subjects without periodontal destruction (controls)

	Cases (n=25)		Controls (n=28)
	destructive sites	non-destructive sites	non-destructive sites
<i>Total spirochetes</i>			
median	12.4%	7.5%	2.7%
interquartile range	(6.9-28.7)	(2.1-14.0)	(1.4-9.6)
<i>p</i> -value	0.04 (paired-test)		0.04 (ANOVA)
<i>Large spirochetes</i>			
median	6.6%	2.7%	2.3%
interquartile range	(1.7-14.6)	(1.4-9.6)	(1.0-6.3)
<i>p</i> -value	0.20 (paired-test)		0.07 (ANOVA)

Table 3. Decision matrices and odds ratios based on different screening values and presence of destructive periodontal disease as validation criterion

validation criterion	Screening criterion, % of spirochetes in subgingival samples from non-destructive sites											
	>3%		>4%		>5%		>6%		>7%		>8%	
	-	+	-	+	-	+	-	+	-	+	-	+
controls*	14	14	16	12	17	11	20	8	22	6	23	5
cases**	9	16	11	14	11	14	11	14	12	13	13	12
odds ratio	1.8		1.7		2.0		3.2		4.0		4.2	
95% confidence limits	0.6-5.3		0.6-5.0		0.7-5.9		1.0-9.9		1.2-13		1.2-14	

* Without destructive disease. ** With destructive disease.

nificant differences were not observed for the large spirochetes.

The probability of encountering a subject with destructive periodontal disease on the basis of spirochete %s was low, but reached a significant value, odds ratio 3.2 at a screening level of >6% of spirochetes (Table 3). The sensitivity and specificity values of the screening for destructive periodontal disease on the basis of spirochete %s varied between 0.48 and 0.82 (Fig. 1).

Discussion

An advantage of using a curette in preference to paperpoints in sampling of the subgingival plaque is the larger sample size. The fixation of the plaque

samples in formalin enabled us to include microbial monitoring in this field study. The fixed suspensions could be used for repeated counts and for estimation of the reproducibility. The standard error of 10% is in accordance with the findings of MacFarlane et al. (1986) who found in fixed and stained smears a reproducibility of 90%. The fixation of the samples has the disadvantage that no motility could be estimated. However this is not a problem for the estimation of the number of spirochetes which were easily visible amongst the other stained bacteria. The problems in sampling, preparation and microscopic examination of specimens of subgingival plaque have been discussed by others (Omar & Newman 1986, MacFarlane et al. 1986) and multiple sampling at a time interval has been advocated.

In the present field study, we were restricted to one sample moment and a limited number of samples. In order to maintain the representativeness of the study, we chose to sample many subjects instead of sampling many sites in the same subject and accepted the error of single sampling. In spite of this limitation, it was found that subjects with destructive periodontal disease (cases) harboured significantly higher %s of spirochetes than subjects without destructive periodontal disease (controls) and that destructive sites harboured significantly higher %s of spirochetes than non-destructive sites. These findings are in accordance with previous publications (Listgarten & Helldén 1978, Armitage et al. 1982, Savitt & Socransky 1984, Africa et al. 1985). However, where the cited publications reported differences in spirochetes between subjects, it is not clear to which extent the differences are attributed to subject ef-

fects or site effects. In periodontal pockets, Evian et al. (1982) found a greater variance in % of spirochetes between subjects than between pocket sites. This indicates the presence of a subject effect on spirochete numbers at sites with periodontal destruction. In the present study, a significant difference in spirochete counts at non-destructive sites between periodontally diseased and non-diseased subjects was found, which indicates a subject effect on the subgingival spirochetes.

Dahlén et al. (1992) followed a comparable experimental design in the estimation of 7 "putative periodontal pathogens", but found no difference between non-destructive sites in "diseased" and "non-diseased" subjects. The observed differences in the subgingival microflora between pockets and non-destructive sites suggest the existence of a sampling-site effect rather than a subject effect on the microflora. Unfortunately spirochetes were not considered in the study of Dahlén et al. (1992).

The character of the subject effect on the spirochete counts in the present study is unknown. Host defence mechanisms, components of the gingival crevice fluid or members of the indigenous microflora might account for the observed host effect and should be further investigated (Ter Steeg et al. 1988, Simonson et al. 1992).

The finding that subjects with destructive periodontitis at non-destructive sites harbour higher %s of spirochetes than subjects without destructive periodontal disease, prompted an analysis of the feasibility of the spirochete counts as a screening method for subjects with destructive periodontal disease. A contingency table analysis revealed low odds ratios when the % of

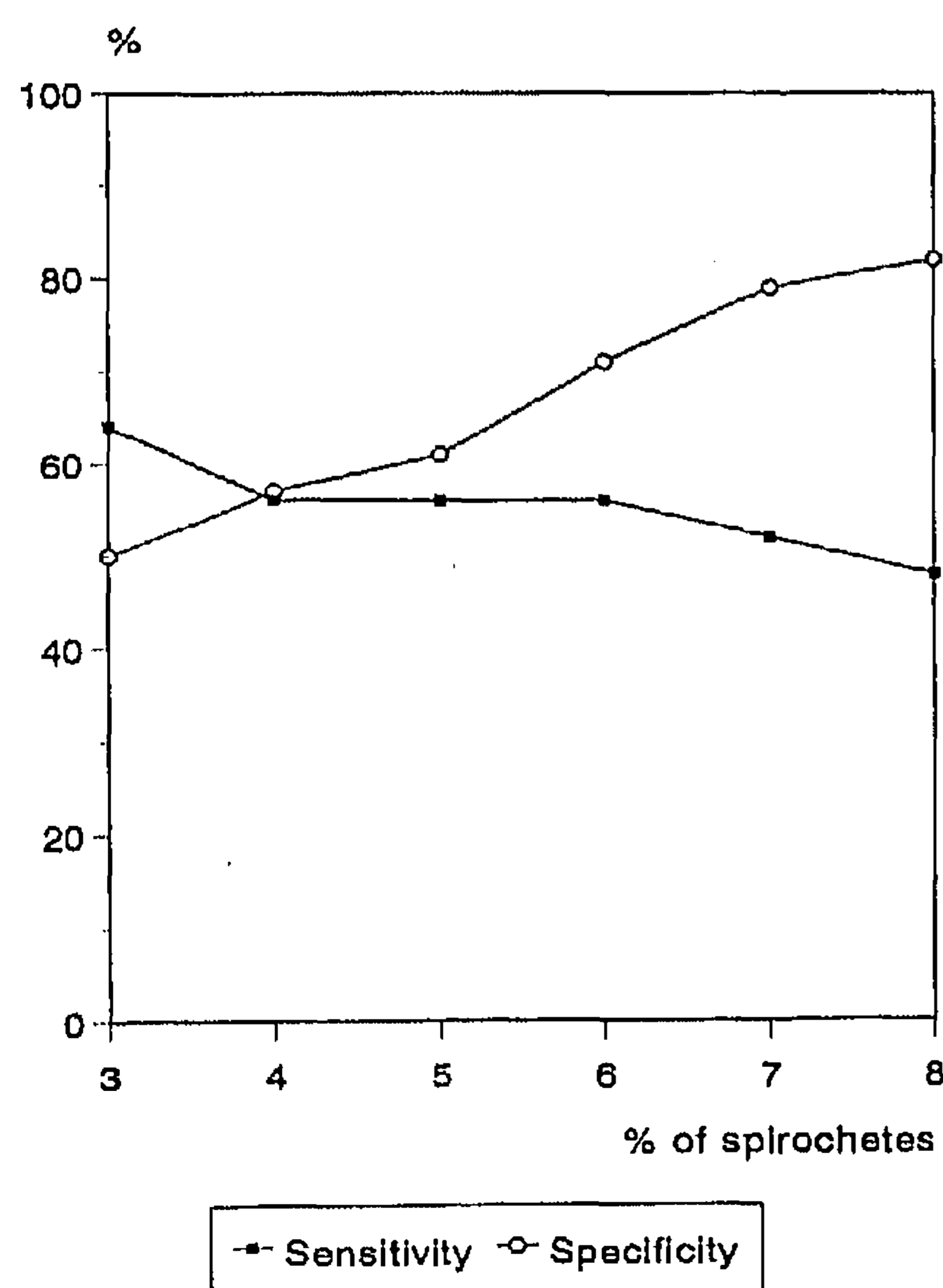


Fig. 1. Sensitivity and specificity values of the screenings criterion: % of spirochetes at non-destructive sites, as a screening method for subjects with periodontal disease.

spirochetes was used as screening criterion at different cut-off points. The sensitivity and specificity values indicate that this screening method is not accurate enough to justify its application.

It is concluded that subjects with periodontal destruction carry higher %s of spirochetes at sites without periodontal destruction than subjects free of periodontal destruction. Despite the significant difference in % of spirochetes between cases and controls, the spirochete counts at sites without periodontal destruction did not provide an accurate marker for periodontal tissue destruction elsewhere in the mouth.

Acknowledgement

Thanks are due to Dr. Martin van 't Hof, University of Nijmegen, The Netherlands, for his statistical assistance and advice.

Zusammenfassung

Mikroskopische Auszählungen von Spirochäten bei nicht behandelten Personen mit und ohne parodontale Gewebedestruktion

Mit der vorliegenden Arbeit wurde beabsichtigt, den prozentualen Anteil der Spirochäten an nicht destruktiv veränderten Stellen von Personen mit destruktiven, parodontalen Erkrankungen (Fälle) mikroskopisch zu bestimmen, sowie bei nicht erkrankten Personen (Kontrollpersonen), bei denen keine professionell durchgeführten, prophylaktischen Maßnahmen eingesetzt worden waren. Fälle und Kontrollpersonen wurden aus einem Probandengut von 164, zwischen 30-44 Jahre alten, aus ländlichen und urbanen Regionen in Tanzania stammenden, Personen abgezweigt. Es wurden Fälle ($n=25$) ausgewählt, bei denen mindestens 3 Zähne mit einer Taschentiefe von >5 mm vorhanden waren. Bei den Kontrollpersonen ($n=28$) waren die Taschen höchstens 3 mm tief. Bei jeder Versuchsperson wurde am Mittelpunkt der lingualen Oberfläche einer der Oberkieferprämolaren mit gingivalem Blut nach dem Sondieren, jedoch ohne Zahnstein und vertiefte Taschen, ein subgingivaler Abstrich entnommen; außerdem ein subgingivaler Abstrich aus einer Zahnfleischtasche. Prozentual enthielten die Taschen die meisten Spirochäten, was frühere Befunde bestätigt. An nicht destruierten Stellen wurde ein deutlicher Unterschied des prozentualen Vorkommens zwischen Fällen und Kontrollpersonen konstatiert, was einen Einfluß des Wirts auf die subgingivale Mikroflora erkennen läßt. Die Gesamtzahl der kultivierbaren Spirochäten an nicht destruierten Stellen läßt sich

jedoch nicht als verlässlicher Maßstab zur Identifikation von Personen mit destruktiver parodontaler Erkrankung verwenden.

Résumé

Comptages microscopiques de spirochètes chez des patients non traités avec ou sans destruction parodontale

Le but de cette étude a été de déterminer par microscopie le % de spirochètes dans des sites sans destruction parodontale chez des sujets avec parodontite (cas) et chez d'autres sans (contrôles), n'ayant pas reçu de détartrage professionnel. Ces cas et contrôles ont été sélectionnés dans un échantillon de 164 individus âgés de 30 à 44 ans vivant tant en zone rurale qu'urbaine en Tanzanie. Les 25 cas sélectionnés montraient au moins 3 dents avec une profondeur de poche de plus de 5 mm. Les 28 contrôles n'avaient aucune poche plus profonde que 3 mm. Chez chaque sujet, un échantillon de plaque sous-gingivale a été prélevé au centre de la surface linguale d'une prémolaire supérieure avec saignement au sondage mais sans tartre ni poche. De plus un échantillon sous-gingival a été obtenu d'une poche. Les poches contenaient les plus hauts % de spirochètes, ce qui confirme les découvertes précédentes. Une différence significative dans le % des spirochètes entre les cas et les contrôles a été trouvée au niveau des sites non-destructeurs impliquant un effet de l'hôte sur la microflore sous-gingivale. Cependant le comptage de spirochètes au niveau des sites non-destructeurs n'a pas apporté une mesure fiable permettant d'identifier les sujets avec maladie parodontale destructrice.

References

- Africa, C. W., Parker, J. R. & Reddy, J. (1985) Bacteriological studies of subgingival plaque in a periodontitis-resistant population. *Journal of Periodontal Research* **20**, 1-7.
- Armitage, G. C., Dickinson, W. R., Jendersack, R. S., Levine, S. M. & Chambers, D. W. (1982) Relationship between the percentage of subgingival spirochetes and the severity of periodontal disease. *Journal of Periodontology* **53**, 550-556.
- Dahlén, G., Manji, F., Baelum, V. & Fejerskov, O. (1992) Putative periodontal pathogens in diseased and non-diseased persons exhibiting poor oral hygiene. *Journal of Clinical Periodontology* **19**, 35-42.
- Evian, C. I., Rosenberg, E. S. & Listgarten, M. A. (1982) Bacterial variability within diseased periodontal sites. *Journal of Periodontology* **53**, 595-598.
- Lindhe, J., Liljenberg, B. & Listgarten, M. (1980) Some microbiological and histopathological features of periodontal disease in man. *Journal of Periodontology* **51**, 264-269.

- Listgarten, M. A. & Helldén, L. (1978) Relative distribution of bacteria at clinically healthy and periodontally diseased sites in humans. *Journal of Clinical Periodontology* **5**, 115-132.
- Listgarten, M. A. & Levin, S. (1981) Positive correlation between the proportions of subgingival spirochetes and motile bacteria and susceptibility of human subjects to periodontal deterioration. *Journal of Clinical Periodontology* **8**, 122-138.
- Listgarten, M. A. (1984) Subgingival microbial differences between periodontally healthy sites and diseased sites prior to and after treatment. *International Journal of Periodontology and Restorative Dentistry* **4**, 27-33.
- Loesche, J. (1988) The role of spirochetes in periodontal disease. *Advances in Dental Research* **2**, 275-283.
- MacFarlane, T. W., McCourtie, J. & Watkins, T. R. (1986) A new method for assessing the % of spirochetes in subgingival plaque. *Journal of Clinical Periodontology* **13**, 651-653.
- Mikx, F. H. M., Matee, M. I. N. & Schaeken, M. J. M. (1986) The prevalence of spirochetes in the subgingival microbiota of Tanzanian and Dutch children. *Journal of Clinical Periodontology* **13**, 289-293.
- Mousquès, T., Listgarten, M. A. & Phillips, R. W. (1980) Effect of scaling and root-planing on the composition of the human subgingival microbial flora. *Journal of Periodontal Research* **15**, 144-151.
- Omar, A. A. & Newman, H. N. (1986) False results associated with darkground microscopy of subgingival plaque. *Journal of Clinical Periodontology* **13**, 814-824.
- Savitt, E. D. & Soeransky, S. S. (1984) Distribution of certain subgingival microbial species in selected periodontal conditions. *Journal of Periodontol Research* **19**, 111-123.
- Schlesselman, J. J. (1982) *Case - control studies. Design, conduct, analysis.* Oxford University Press, UK.
- Simonson, L. G., McMahon, K. T., Childers, D. W. & Morton, H. E. (1992) Bacterial synergy of *Treponema denticola* and *Porphyromonas gingivalis* in a multinational population. *Oral Microbiology and Immunology* **7**, 111-112.
- Singletary, M. M., Crawford, J. J. & Simpson, D. M. (1982) Darkfield microscopic monitoring of subgingival bacteria during periodontal therapy. *Journal of Periodontology* **53**, 671-681.
- Slots, J. & Geneo, R. J. (1984) Black pigmented *Bacteroides* species, *Capnocytophaga* species and *Actinobacillus actinomyces* in human periodontal disease: Virulence factors in colonization, survival and tissue destruction. *Journal of Dental Research* **63**, 412-421.
- Soeransky, S. S. & Haffajee, A. D. (1992) The bacterial etiology of destructive periodontal disease: Current concepts. *Journal of Periodontology* **63**, 322-331.
- Ter Steeg, P. E., van der Hoeven, J. S., de

- Jong, M. H., van Munster, P. J. J. & Jansen, M. J. H. (1988) Modelling the gingival pocket by enrichment of subgingival microflora in human serum in chemostats. *Microbial Ecology in Health and Disease* **1**, 73-84.
- Van Palenstein Helderma, W. H. (1981) Microbial etiology of periodontal disease. *Journal of Clinical Periodontology* **8**, 261-280.
- Van der Velden, U. (1979) Probing force and the relationship of the probe tip to the periodontal tissues. *Journal of Clinical Periodontology* **6**, 106-114.
- Wolff, L. F. Pihlstrom, B. L., Liljemark, W. F., Schaffer, E. M. & Bandt, C. L. (1985) Distinct categories of microbial forms associated with periodontal disease. *Journal of Periodontal Research* **20**, 497-502.

Address:

F. H. M. Mikx
Tandheelkunde KUN
Postbus 9101
NL 6500 HB
The Netherlands
Fax +31 80 541314