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Periodontal Bacterial Load: A Proposed New Epidemiological Method for Periodontal Disease Assessment

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Abstract

Aim: The purpose of this study was to identify a periodontal clinical measure that correlates with red complex bacteria usually associated with periodontal disease.

Methods and Materials: Periodontal clinical parameters were recorded in 116 postpartum women at six sites per tooth for all teeth excluding third molars. Two subgingival plaque samples per subject were collected and analyzed for 39 bacterial species using the Checkerboard DNA-DNA hybridization technique. Periodontal Bacterial Load (PBL) was calculated as the sum of all pocket depth measurements of 4 mm at sites with a Clinical Attachment Level (CAL) of 4 mm. The association of clinical and bacterial scores was analyzed using the Spearman correlation coefficient and the Kruskal-Wallis test.

Results: The PBL was correlated with microorganisms from the red complex that included *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*, individually or grouped ($p<0.05$). The PBL was not associated with periodontally beneficial species from the yellow, green, purple, and blue complexes ($p>0.05$). The proportions and mean counts of the red complex were increased according to the quartile groups of distribution of the PBL.

Conclusions: PBL appears to be a reliable measure of periodontal status in postpartum women.

Clinical Significance: PBL avoids bias in the assessment of periodontal status in studies of periodontal disease.

Keywords: Periodontitis, periodontal disease, epidemiology, microbiology

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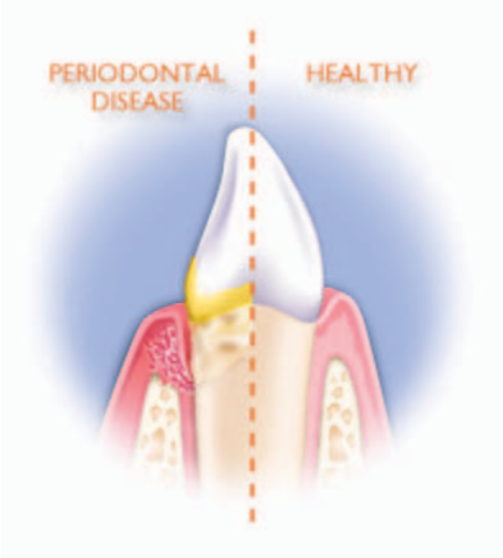
Introduction

The most common periodontal indices used in epidemiologic studies, such as the Periodontal Index,[1] Periodontal Disease Index,[2] Community Periodontal Index of Treatment Need (CPITN), the Community Periodontal Index (CPI),[3] and the Extent and Severity Index[4] as well as partial mouth recording protocols[5] all have limitations. Therefore, recent studies in periodontal medicine have used full-mouth measurements of periodontal parameters to measure periodontal disease.[6,7] Such assessments have led to the use of a wide range of cutoff points for the frequency of deep pockets, sites with loss of attachment, and/or bleeding on probing to assess associations between periodontal status and systemic conditions.[8–10] For example, those evaluating the relationship between periodontitis and preterm low birth weight (PTLBW) used 13 different measures and cutoff points to characterize periodontal disease. That indicates a lack of standardized definitions of periodontal disease.[11]

Assessment of periodontal status based exclusively on the clinical attachment level (CAL) could represent a problem since this parameter does not take into account the extent of current status of inflammation of the periodontium and might reflect only the past history of disease. Use of only the CAL parameter to characterize disease can result in classification bias. For example, in many cases there is no periodontal disease activity at sites with loss of attachment but without deepening of pockets.[12] Such a methodological problem has generated conflicting results, especially in studies dealing with younger people like those that have examined the relationship between periodontal disease and PTLBW.[7,8,9] Furthermore, even those studies that took into consideration different thresholds of periodontal pocket depth (PPD) and/or CAL measures as the diagnostic criteria for periodontal disease usually do not correlate those periodontal parameters with the bacteria related to periodontal disease.

Reliable clinical indices to determine periodontal disease status should be associated with specific



periodontal pathogens such as microorganisms of the red complex: *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*.[13] These species are associated with periodontal tissue breakdown and active lesions of destructive periodontal disease.[14,15]

Conversely, a reliable periodontal measure should not be related to bacterial beneficial species, such as those from the yellow (*Streptococcus spp*), purple (*Veilonella parvula* and *Actinomyces odontolyticus*) and blue (*Actinomyces spp*) complexes, which are usually in higher proportions at healthy periodontal sites.[13,16]

The lack of standardization on definitions of periodontal disease in studies that investigated the possible relationship between periodontal disease and PTLBW and the use of measures without reliability analysis through microbiological assessment prompted the present study, which aimed to develop a periodontal clinical measure that correlates with red complex bacteria that are associated with periodontal disease in postpartum women, what we have termed the periodontal bacterial load (PBL).

Methods and Materials

Experimental Design

The study was approved by the Committee of Ethics and Research of the Oswaldo Cruz Foundation.

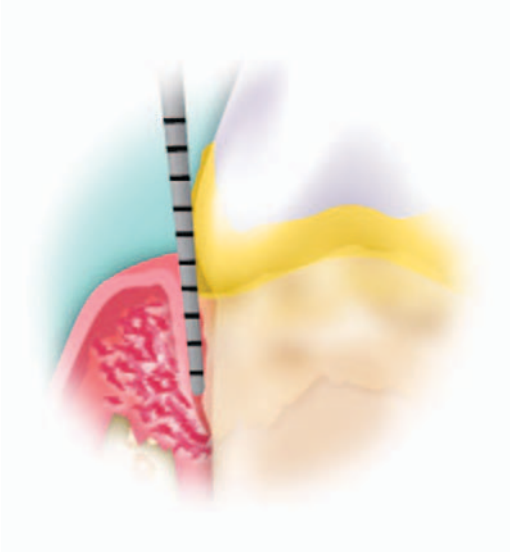
Periodontal measurements were recorded in 116

postpartum women aged 30 years and over who received care at public maternity hospitals in Rio de Janeiro, Brazil. The women were enrolled in a large case control study to test the relationship between periodontal disease and PTLBW.[17] Subjects had clinical and microbiological assessments performed during a single one-hour appointment.

The inclusion criteria were that women were at least 30 years old without any systemic conditions, or currently taking medicines related to periodontal changes. Other inclusion criteria were the presence of 15 or more teeth, no periodontal treatment within the last six months, and those who had not taken antibiotics within the last week. Exclusion criteria were the presence of HIV infection, chronic hypertension, chronic diabetes, and those requiring prophylactic antibiotics for periodontal examination.

Clinical Assessment

Periodontal clinical measures including visible dental plaque[18] and bleeding on probing index[19] were measured at four sites (mesiobuccal, buccal, distobuccal, and lingual) per tooth. CAL and PPD measures were assessed at six sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual, and mesiolingual) for all teeth excluding third molars.[20] The PPD and CAL measures were recorded using the North Carolina periodontal probe (Hu-Friedy®, Chicago, IL, USA). This evaluation was done by six calibrated examiners. The results of a kappa test for PPD were ≥0.78 for intra-examiner and ≥0.77 for interexaminer reliability.



Microbiological Assessment

Sample Collection

Subgingival plaque samples were collected from the two deepest periodontal sites from different teeth for each woman. When the subject had no periodontal pockets, the selected sites were randomized in different quadrants. After the clinical parameters had been recorded, the supragingival plaque was removed and the samples were taken using individual sterile Gracey curettes and immediately placed in separate Eppendorf tubes containing 0.15 ml TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.6). Next, 0.10 ml of 0.5 M NaOH was added to each tube and the samples were dispersed using a vortex mixer.

Checkerboard DNA-DNA Hybridization

Counts of 39 bacterial species were determined in each sample using the Checkerboard DNA-DNA hybridization technique.[21] The assay sensitivity was adjusted to permit detection of 104 cells of a given species by adjusting the concentration of each DNA probe.

Description of the Periodontal Bacterial Load Measure

The proposed new measure is named the periodontal bacterial load (PBL) because it assesses periodontal disease in all teeth and all sites, using periodontal bacteria as a gold standard. The PBL is a continuous measure expressed as the sum of all PPD≥4 mm in sites with CAL≥4 mm. The PBL can be used as a continuous or a categorical measure. To use PBL as a categorical measure, the sample population should be divided into subgroups according to quartiles of distribution of PBL in the total population. The PBL groups according to quartiles of distribution were as follows:

- Level 1: 0–7 mm
- Level 2: 8–45 mm
- Level 3: 46–100 mm
- Level 4: ≥101 mm

Assessing the Reliability of the PBL

A proxy measure for periodontal disease should represent the periodontal pathogen load. Therefore, the PBL should be related only with periodontal pathogens in a separated and aggregated analysis. Furthermore, the PBL should not be associated with beneficial or host compatible species.

Statistical Analysis

First, the PBL measure was examined as a continuous variable. The associations of PBL measures, mean PPD, frequency of PPD≥4 mm, mean CAL, and frequency of CAL≥3 mm with counts of microbial complexes and counts of periodontal pathogens were examined using Spearman correlation coefficients. Second, the PBL measures were divided into four groups according to the PBL's quartiles of distribution.

In order to compare counts of each bacterial complex and individual periodontal pathogens, the data were expressed as counts times 106, averaged within a subject and then across subjects. Significance of differences between groups in mean counts of bacterial complexes and periodontal pathogens were analyzed using the Kruskal-Wallis test. The total DNA probe count also was computed at each sampled site within each subject and the proportion of each bacterial complex was determined. The Kruskal-Wallis test also was used to compare means of proportions of different microbial complexes[13] in subgingival biofilm samples among the four quartile groups of distribution of the PBL. The significance level established for all analyses was 5%.

Results

The mean age of the tested population was 34.1±3.6 years. The clinical features of the subjects are presented in Table 1.

Association between the PBL and Bacterial Species Related to Periodontal Status

Table 2 presents the correlation between microbial complexes and different categories of PPD, CAL, and PBL. There was an association between the red complex and three of the measurements evaluated, PBL, frequency of PPD≥4 mm, and frequency of PPD and CAL≥4 mm ($p \leq 0.01$).

Full-mouth mean PPD was associated with the purple and yellow microbial complexes ($p<0.05$). Negative correlations between frequency of PPD≥5 mm and blue, yellow, and green complexes, and the group formed by other species and new DNA probes, also were observed ($p<0.05$). Full-mouth mean CAL and frequency of CAL≥3 mm were positively correlated with purple, yellow, and orange microbial complexes.

Table 1. Clinical characteristics of subject group (n=116).

	Mean (± SD)	Range
Number of teeth	23.3±3.8	15-28
Mean PPD (mm)	2.4±1.0	1-5
Mean CAL (mm)	2.5±0.7	1-6
Percentage of sites with		
VPI	52±43	0-100
BOP	20±28	0-100
PPD <4 mm	88.1±13.5	8-100
PPD 4–6 mm	11.8±13.5	0-89
PPD >6 mm	0.10±0.6	0-11
CAL <4 mm	85.0±16.9	0-100
CAL 4–6 mm	14.6±16.3	0-100
CAL >6 mm	0.3±1.5	0-24

Legend: VPI: Visible Plaque Index; **BOP:** bleeding on probing; **PPD:** periodontal pocket depth; **CAL**: clinical attachment level

Table 2. Correlation matrix of PBL and clinical parameters of periodontal disease with mean counts of different microbial complexes.

Clinical Parameters	Microbial Complexes (Total Counts)						
	Blue Complex	Purple Complex	Yellow Complex	Green Complex	Orange Complex	Red Complex	Other Species and New DNA Probes
PBL	-0.094	0.035	-0.009	-0.038	-0.038	0.289*	-0.019
Mean of PPD	0.048	0.233*	0.240*	0.074	0.178	0.161	0.072
%PPD ≥4 mm	-0.064	0.072	0.027	-0.016	0.175	0.308*	0.010
%PPD ≥5 mm	-0.214*	-0.178	-0.333*	-0.240*	-0.047	0.147	-0.256
Mean of CAL	0.070	0.218*	0.190*	0.1605	0.190*	0.166	0.076
%CAL ≥3 mm	0.145	0.291*	0.284*	0.164	0.239*	0.181	0.147
%PPD ≥4 mm and CAL ≥4mm	-0.113	0.006	-0.008	0.013	0.165	0.252*	-0.002

Legend: PPD: periodontal pocket depth; **CAL:** clinical attachment level
*Spearman coefficient, $p<0.05$

Table 3. Correlation matrix of PBL and clinical parameters of periodontal disease with mean counts of selected periodontal pathogens.

Clinical Parameters	Periodontal Pathogens (Total counts)		
	P. gingivalis	*T. denticola*	*T. forsythia*
PBL	0.242*	0.237*	0.294*
Mean of PPD	0.100	0.059	0.178
%PPD ≥4 mm	0.255*	0.250*	0.312*
%PPD ≥5 mm	0.085	0.106	0.159
Mean of CAL	0.113	0.075	0.169
% CAL ≥3 mm	0.092	0.107	0.183*
%PPD ≥4 mm and CAL ≥ 4mm	0.238*	0.161	0.259*

Legend: PPD: periodontal pocket depth; **CAL:** clinical attachment level
*Spearman coefficient, $p<0.05$

The correlations between the individual members of the red complex, *P. gingivalis*, *T. denticola*, *T. forsythia*, and periodontal disease measures are presented in Table 3. The PBL and frequency of PPD≥4 mm were significantly associated with all species of the red complex. The frequencies of CAL≥3 mm and PPD and CAL≥4 mm were positively correlated with *T. forsythia*. PPD and CAL≥4 mm also were associated with *P. gingivalis*.

Comparisons of mean counts and mean proportions of each microbial complex in

subgingival plaque samples among the four quartile groups of distribution of the PBL are shown in Figures 1 and 2. The PBL groups according to quartiles of distribution were as follows:

- Level 1: 0–7 mm
- Level 2: 8–45 mm
- Level 3: 46–100 mm
- Level 4: ≥101 mm

The differences in mean counts of the orange and red complexes were significant among the different groups of distribution of the PBL. As the PBL measure of periodontal disease increased (from levels 1 to 4), the mean counts of red complex species also increased. Mean proportions of the red complex in the four PBL groups were significantly different ($p<0.01$). The proportion of this microbial group of pathogens progressively increased from levels 1 to 4 of the PBL. The PBL levels 3 and 4 showed higher proportions of orange complex and lower proportions of blue complex, compared with PBL levels 1 and 2. However, these differences were not statistically significant.

Discussion

There is a lack of an assessment standard to determine periodontal disease status in epidemiological studies. This creates a problem in evaluation of data from different research protocols, especially those studies of the relationship between periodontal disease and PTLBW. Therefore, the present study tested the reliability of a new measure, the PBL, to determine the periodontal disease status in postpartum women in epidemiological studies. The PBL measure is the sum of all PPD values equal to or greater than 4 mm in sites with a CAL≥4 mm. The logic of this approach is that by including all potential sites with loss of attachment in association with pocket depth in the assessment, the PBL value should represent the extent of the periodontal infection related to all the individual's natural teeth.

The reliability of the method was demonstrated by a strong association between PBL values and periodontal pathogens from the red complex—*P. gingivalis*, *T. denticola*, and *T. forsythia*—using

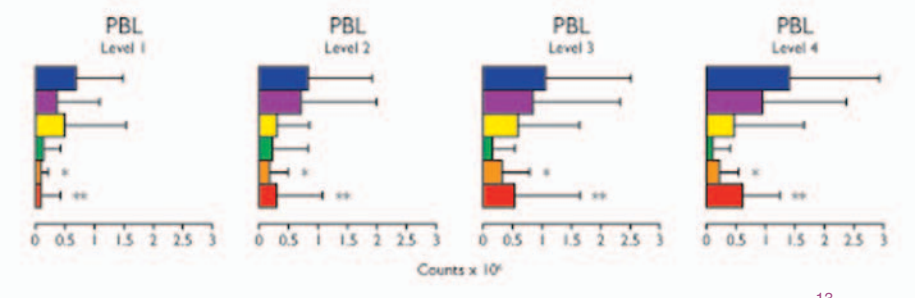


Figure 1. The bars represent the mean counts of each microbial complex.[13] The different colors correspond to the colors of the complexes. Significance of differences in mean proportions between group mean values for each complex was tested using the Kruskal-Wallis test.
Note: *p<0.05 **p<0.01 PBL (sum of all pockets ≥4 mm in sites with CAL≥4 mm) level 1: 0–7 mm; level 2: 8–45 mm; level 3: 46–100 mm; level 4: ≥101 mm

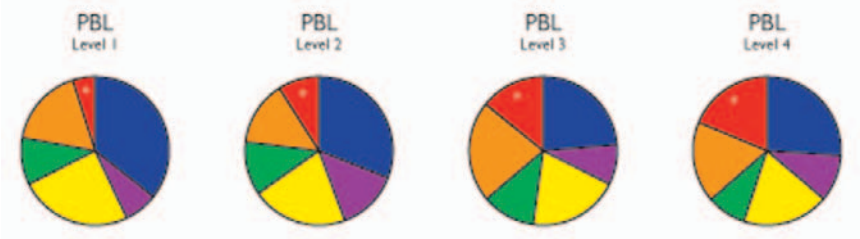
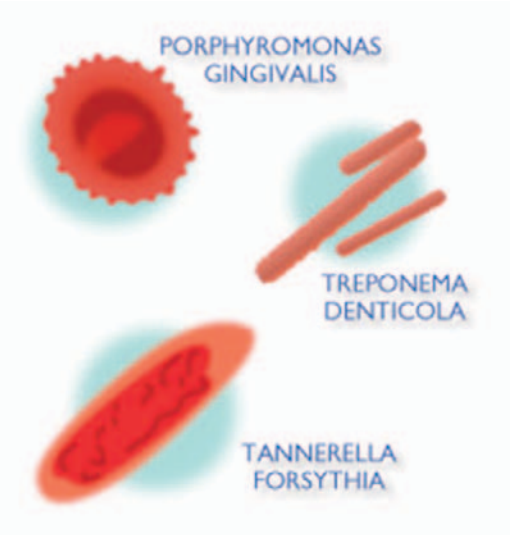


Figure 2. The different PBL levels are described in Figure 1. The colors in the pie charts correspond to the colors of microbial complexes.[13] Significance of differences in mean proportions between group mean values for each complex were determined using the Kruskal-Wallis test. (* $p<0.01$)



isolated and aggregated analyses. As the sample was postpartum women randomly selected from public maternity care facilities, the level of periodontal disease was similar to the general Brazilian population. Despite using people with low to moderate periodontal disease, the levels of the pathogens, individually or grouped, were significantly associated with PBL measurements. Moreover, the red complex was the only group of bacteria with statistically significant differences in proportions among the four quartile groups of distribution of the PBL. It was interesting to observe that mean proportions of this complex consistently increased with increases in the degree of periodontal disease determined by the PBL levels from 1 to 4. In terms of total counts of the microbial complexes, there was a positive relationship between PBL levels and the red and orange complexes. This is an important observation since the red complex harbors the three most important periodontal pathogens and several species considered to be possible pathogens belong to the orange complex. The lack of an association between host-compatible species from the yellow, green, purple, and blue complexes and PBL values was also a relevant finding, and suggests that the PBL is a reliable measure to assess periodontal disease.

Misclassification of the periodontal status is commonly observed in studies of periodontal medicine. For example, up to 13 different methods have been used in studies of periodontal disease and PTLBW.[11] Some studies used cutoffs for periodontitis diagnosis varying in the number of teeth/sites involved, the clinical measurements used, and the values used as thresholds. The risk of misclassification increases when the assessment of periodontal disease uses only CAL measurements, which is considered an appropriate measure of periodontal status in terms of cumulative periodontal destruction over time. However, this parameter alone might reflect only gingival recession without periodontal infection. Some studies using the CAL to define periodontal infection did not find an association between red complex or other periodontal pathogens and the criteria employed, such as ≥5 sites with CAL≥3 mm[22] ; ≥60% sites with CAL≥3 mm.[9]

It is important to note that in the present study the parameter "%PPD≥4 mm" also had a positive correlation with the total counts of red complex pathogens, a similar pattern observed for the PBL. However, the shortcoming in using the "%PPD≥4 mm" for periodontal status assessment is that patients with the same frequency of PPD≥4 mm can present with different levels of periodontal disease depending on the PPD of these sites. With the PBL measure it is possible to distinguish the severity of periodontal disease in patients with the same frequency of PPD≥4 mm. So the frequency of PPD≥4 mm refers to the extent of the disease and does not include information about severity. On the other hand, the PBL refers to both the extent and severity of periodontal disease.

Conclusions

PBL appears to be a reliable epidemiologic measure of periodontal disease status in postpartum women. Further studies testing this measure on different populations and evaluating greater numbers of plaque samples and subjects need to be conducted to test the reliability of the PBL.

Clinical Significance

In epidemiological studies, periodontal disease is conventionally assessed using periodontal indices or clinical parameters. Those measures have shortcomings for assessing the periodontal status. This study tested a new approach, the PBL, which is a continuous variable measuring the sum of periodontal pocket depth ≥4 mm in sites with the CAL≥4 mm. PBL was shown to be a valid measure of periodontal condition due to its strong association with periodontal pathogens and avoids bias in the assessment of periodontal status in studies of periodontal disease.

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