Oral Neutrophil Levels: A Screening Test for Oral Inflammatory Load in Pregnancy in a Medical Setting

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Background: A multitude of studies suggest an association between periodontal disease and adverse birth outcomes, but the findings have been equivocal. Although the evidence is controversial, it is biologically plausible, and the key link may be inflammation. Because periodontitis is at times either active or inactive, trying to correlate the presence of pockets, for example, to adverse outcome of pregnancy might be preordained to failure or at least confusion. Alternatively, if inflammatory activity associated with periodontitis could be measured, it might be possible to correlate oral inflammatory load (OIL) to adverse pregnancy outcomes more precisely, but given the low incidence of adverse pregnancy outcomes, large populations must be studied. This underscores the need to use a means for assessment of OIL that is reliable, reproducible, and so simple to perform that it does not require dental expertise and can be used for large numbers of patients attending obstetrics units. The objective of this study was to demonstrate that OIL can be measured in a cohort of pregnant females presenting for obstetric care and secondarily to ensure that there was a realistic correlation to the presence of periodontitis.

Methods: Sixty-three pregnant females were recruited, and 15-second saline rinses were collected to measure OIL as represented by counts of oral neutrophil levels. Periodontal examinations were performed to determine the extent of the correlation between the presence of clinical markers of periodontitis, such as pockets and clinical attachment loss to the OIL.

Results: Using this small cohort of patients, a test for oral inflammatory disease could be administered successfully in a non-dental setting. In addition, there was a statistically significant increase (two-fold, $P<0.05$) in oral neutrophil counts found in patients with periodontitis compared with those without periodontitis.

Conclusion: The rinse assay can be used as a screening tool for oral inflammation, which was also related to the presence of periodontitis, in pregnant females attending a medical clinic. *J Periodontol 2015;86:72-81.*

KEY WORDS

Gingivitis; immunity, innate; inflammation; leukocyte count; periodontitis; pregnancy.

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Since 1996 when periodontitis was first reported to be a potential risk factor for preterm birth,[1] a multitude of studies have evaluated the association between periodontal disease and adverse pregnancy outcomes.[2-5] However, it has been difficult to establish an association between periodontitis and adverse pregnancy outcomes unequivocally because of the multifaceted nature of both periodontal diseases and adverse birth outcomes. It is believed that the focus in these linkage studies should be on oral inflammation rather than on pocket depths because pockets as measured in patients with periodontitis are indicative of past disease but not current disease activity. Hence, the concept that oral inflammation is the principal outcome parameter requiring measurement as a risk marker for non-oral diseases as opposed to focusing on specific oral diseases/diagnoses, including, but not limited to, gingivitis, periodontitis, or mucocutaneous diseases, is presented.[6] From a practical or clinical perspective, once elevated oral inflammation has been identified, it is then essential to make a diagnosis so that appropriate treatment can be rendered. To correlate oral inflammatory load (OIL) with adverse pregnancy outcomes in the hopes that treatment of periodontitis will reduce those risks, it must be shown that methods for assessment of OIL can be developed and used to show when the load is high and hence pathologically impactful. The use of this novel approach for measuring oral inflammation might be a more meaningful way to determine whether there is a correlation between oral inflammatory diseases including periodontitis and even gingivitis and adverse outcomes of pregnancy.

Polymorphonuclear neutrophils (PMNs) play a major role in the pathogenesis of periodontal diseases by releasing matrix metalloproteinases and other enzymes that upregulate inflammation.[7-9] Oral PMN (oPMN) levels measured using a rapid oral rinse assay paralleled the severity of periodontal disease showing that it is a valid, reproducible, and effective means for quantification of PMN levels that could be used to assess the presence and/or severity of periodontal disease.[6,10] This assay for oPMN is a simple non-invasive test that can be used by non-specialized staff to collect and quantify the degree of OIL. To reiterate, it is probable that a direct measurement of OIL has greater potential to demonstrate a putative relation between periodontal disease and preterm and/or low birth weight (PTLBW) outcome than by measurements of probing depths (PDs) and indices. This is not only important because PMNs can be used as biomarkers for risk but also because PMNs actually play a role in the pathophysiology of periodontitis (and potentially the development of PTLBW infants). Therefore, it is proposed that it would be useful to validate the quantification of oPMN using the assay system described above for use as a screening tool for OIL in pregnant females as a first step toward determining the extent, if any, of a correlation between these two factors.

The objective of the present study was to determine oPMN counts in a group of pregnant females using a brief oral rinse assay and to correlate this to the levels of periodontal disease as measured by conventional methods.

MATERIALS AND METHODS

This study was approved by the Scientific and Ethics Review Boards of Mount Sinai Hospital, Toronto, and the University of Toronto, Toronto, on February 4, 2011 before its commencement and was conducted in accordance with the Declaration of Helsinki of 1975, as revised in 2000.

Study Population and Patient Selection

Sample size calculation determined that 63 females (mean age: 33.3 ± 3.8 years) were required for this study to detect a non-zero correlation of ≥0.4 between variation in oPMN counts in pregnant females and variation in clinical measures of periodontal status with an α of 0.05, using a two-sided test with 90% power (Fig. 1). Patients were recruited from the "low-risk" obstetrical clinic at the Mount Sinai Hospital, Toronto (February 24, 2011 to September 2, 2011) by the principle investigator (SH), and written informed consent was obtained.

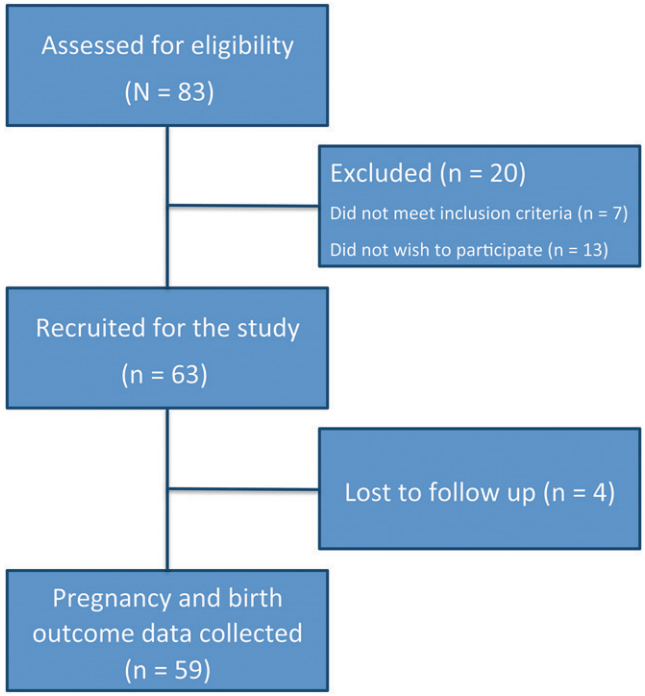

Figure 1.
Flow diagram following patient selection and follow-up.

The following were inclusion criteria: 1) pregnancy with gestational age >13 weeks but ≤26 weeks; 2) aged ≥16 years; 3) capable of giving informed consent; and 4) having ≥20 natural teeth. The following were exclusion criteria: 1) multiple gestations; 2) known fetal congenital anomaly or aneuploidy; 3) documented urinary tract and/or respiratory tract infections at the time of recruitment; 4) history of fetal invasive procedures (amniocentesis or chorionic villi sampling); 5) required antibiotic prophylaxis for dental procedures; 6) history of type 1 or type 2 diabetes; 7) immunocompromised state; 8) presence of cardiac or renal disease; and 9) presence of ≤20 teeth, rampant caries, or other gingival lesions not related to bacterial plaque.

A medical and dental history was taken from each patient using a structured questionnaire. Maternal demographic information, previous pregnancy history, and current gestational age were also recorded from the patient's medical records.

Assessment of oPMN Levels

The protocol used to collect and count oPMN was a modification of previous work published by Bender et al.[10] and Lakschevitz et al.[11] Participants rinsed their mouths once with 10 mL 0.9% saline for 15 seconds. They could not eat or drink for a minimum of 30 minutes before providing the second oral rinse (for testing) to avoid clearance of oPMNs before donations. Each sample was collected in a sterile vial[§] and stored at 4°C before transportation to the laboratory for processing. All samples were processed within 24 hours of collection.

The rinse was then centrifuged at 2,500 rpm for 5 minutes at 21°C.[‖] The cell pellet was resuspended in 1 mL double-distilled water. A total of 20 mg 2,2′-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS)[¶] was dissolved in 3.6 mL 1 mM phosphocitrate buffer to produce a 1× concentrated solution. A total of 45.6 μL 30% hydrogen peroxide was added to 3.952 mL double-distilled water to produce a hydrogen peroxide solution. For each 1 mL concentrated oral rinse sample, 100 μL of the ABTS solution followed by 100 μL 30% hydrogen peroxide solution was added to observe the color change. After the color reaction was complete, 250 μL of the sample was added to a 96-well plate in triplicate. The absorbance was measured at 420 nm for 10 cycles at 180 seconds/cycle using an automated microplate reader.[#] After all cycles, the absorbance values of the triplicate measurements were averaged to produce the final value. The average standard deviation and coefficient of variation of the absorbance of the 10 cycles for all the collected samples was 0.09% and 6.34%, respectively, indicating very low variation between the cycle absorbance readings. The assay was validated to confirm that the absorbance values were attributable to the number of neutrophils present in the rinse sample. Figure 2A demonstrates the strong and linear correlation between ABTS color intensity measured by absorbance and actual oPMN and isolated blood PMN counts. The color change measured by absorbance was eliminated when the neutrophils were removed by centrifugation before the assay was completed (10 patients; Fig. 2B). These data confirm that, although peroxidase might arise from non-PMN sources, actual measurement of color change/absorbance for the breakdown products of ABTS in this test reflects PMN counts, because peroxidase activity is undetected when PMNs are not present.

A series of standard oPMN solutions with known oPMN numbers were prepared. The absorbance was then measured as above. The standard line equation was then obtained by plotting the absorbance of the standard solutions versus the known concentrations, thus allowing calculation of the oPMN counts using the absorbance value obtained for any one sample. The standard curve and resulting equation was generated on four separate occasions with triplicate samples at each value to generate an accurate and reproducible equation. The equation mean generated varied only by ±10% over time.

The Periodontal Examination

After the oral rinse, SH performed a complete periodontal examination on each patient. Bleeding on probing (BOP), PD, recession, and clinical attachment level (CAL)[12] at six sites of each tooth, as well as mobility, plaque index (PI), calculus index,[13] modified gingival index (GI),[14] and bleeding index (BI)[14] using a standardized method for data collection were recorded. Examiner reliability was assessed by calculating intraexaminer κ scores between measurements of PD and clinical attachment loss on a volunteer patient lying flat on a hospital bed on two separate occasions (2 weeks apart to duplicate the study conditions). The intraexaminer correlation or κ scores exceeded 0.95 for both measurements, a nearly perfect score demonstrating a high degree of examiner reliability and reproducibility.[15]

Mild periodontitis was defined as at least two interproximal sites with CAL ≥3 mm or at least two interproximal sites with PD ≥4 mm (not on the same tooth) or one site with PD ≥5 mm. Moderate periodontitis was defined as at least two interproximal sites with CAL ≥4 mm or at least two interproximal sites with PD ≥5 mm (not on the same tooth). Severe periodontitis was defined as at least two interproximal sites with CAL ≥6 mm and at least one interproximal

§ Falcon tube, BD Biosciences, San Jose, CA.
‖ Hettich Rotina 35R, Rare Scientific, Edmonton, AB.
¶ Sigma-Aldrich, Oakville, ON.
# FLUOstar OPTIMA, BMG LABTECH, Offenburg, Germany.

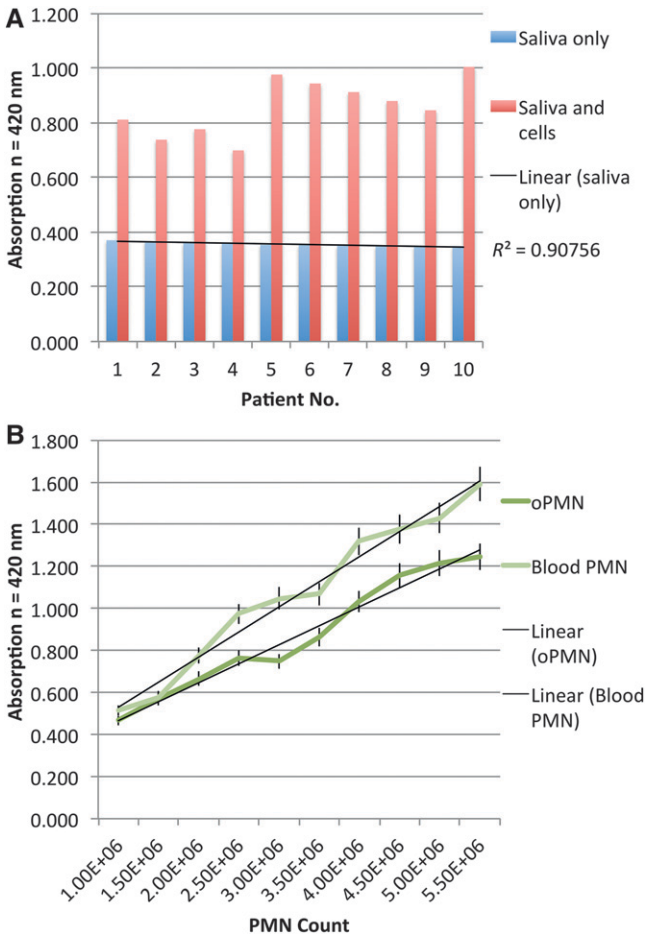

Figure 2.
A) *Absorption in samples attributable to cellular constituents: comparison between rinse samples and samples with cells removed by centrifugation. Bars represent absorption at 420 nm for saliva-only samples and saliva with cells for 10 different patients. To generate samples without cells, half the rinse sample was spun down at 2,500 rpm for 5 minutes, and the supernatant alone was processed for ABTS sampling.* ***B)*** *Relationship between ABTS absorption at 420 nm after 180 seconds and PMN counts.*

site with PD ≥5 mm (not on the same tooth). These measurements excluded third molars. Gingivitis was diagnosed by the presence of inflammation and BOP in the patients who did not present with periodontitis as defined above.

All patients received individualized oral hygiene instructions (i.e., brushing and flossing). Patients diagnosed clinically with periodontal disease were advised to visit a dentist for treatment.

Maternal and Pregnancy Demographic Data

Information regarding the course of the pregnancy and the outcome was collected from the antenatal chart and delivery records of the participating patients at the end of the pregnancy. Preterm birth was defined as birth at <37 weeks of gestation. Low birth weight (LBW) was defined as birth weight <2,500 g at delivery.

Statistical Analysis

Descriptive statistics were calculated for all the demographic characteristics, outcomes, and diagnostic factors. Because of the positively skewed nature of oPMN counts, a natural log transformation was used to normalize the distribution before inferential analyses.

Spearman rank correlation was used to examine the association between the natural log-transformed PMN counts and the BI, average PDs, age, gestational age at recruitment, and the number of teeth.

Tukey honestly significant difference (HSD) test was used in conjunction with an analysis of variance (ANOVA) to examine the association among the natural log-transformed oPMN counts, the modified GI and calculus index, and periodontal status classification (i.e., health, gingivitis, periodontitis). An independent-sample *t* test was used to examine the association between the natural log-transformed oPMN counts and the PI and dental visit frequency (over the past 6 months).

All statistical analyses were conducted in a statistical software program,** and a Type I error rate of ≥0.05 was used to indicate statistical significance.

RESULTS

Sixty-three individuals met the eligibility criteria and were recruited, with a mean ± SD age of 33.3 ± 3.8 years and a gestational age of 19.3 ± 4.2 weeks. This met the sample size requirement (Fig. 1). The mean ± SD number of teeth present was 28.8 ± 1.8. The patient population was a predominantly white (69.8%), well-educated group, with all the patients either attending or having completed postsecondary education. All the patients reported taking some form of prenatal supplements. None of the patients reported taking antibiotics recently (within 2 weeks) at the time of recruitment. The two patients that reported smoking claimed to be smoking <5 cigarettes per day during the pregnancy. One patient reported drinking 1 unit of alcohol every 1 to 2 week(s). Nearly two thirds of the patients had visited a dentist within the past 6 months for examination, scaling, and prophylaxis.

Approximately 43% of the patients had gingivitis, whereas 40% had some form of periodontitis as per the definitions of the study protocol. However, none of the patients presented with severe periodontitis. The mean oPMN count was 4.66×10^6 cells. The counts of oPMN and clinical parameters are shown in Table 1.

The relationship between the level of oPMN and severity of periodontal disease was analyzed.

** SPSS v.20.0.0 for Microsoft Windows Vista, IBM, Armonk, NY.

Table 1.

Mean ± SD Values of the oPMN Counts (x 10^6 cells per rinse), Average PDs (mm), and BI of the Study Population at Recruitment

Factor	n	Mean ± SD	Median	Minimum	Maximum
Periodontal status					
Healthy	11	2.86 ± 0.78*			
Gingivitis	27	4.31 ± 1.60*			
Periodontitis	25	5.83 ± 2.22*			
Mild periodontitis	20	5.12 ± 1.86*			
Moderate periodontitis	5	8.63 ± 0.91*			
Outcome					
PMN counts (×10^6 cells per rinse)		4.66 ± 2.06	4.36	2.22	9.53
Average PD (mm)		2.43 ± 0.12	2.42	2.17	2.78
BI*		4.44 ± 3.89	3.57	0.00	14.88

* The BI is calculated as the number of sites with bleeding divided by the total number of sites probed × 100. Mean oPMN counts (×10^6 cells per rinse) of the study population according to their periodontal status (n = 63; ANOVA, P <0.05; Tukey HSD test showed a significant difference between all the groups).

A significant positive correlation between PMN counts and the BI was shown (Fig. 3A; Spearman test, r_s = 0.81, P <0.001). A significant positive correlation between the PMN counts and the average PDs (Fig. 3B; Spearman test, r_s = 0.58, P <0.001) was also found. There were no significant correlations found between PMN counts and age, number of teeth present, or the gestational age at recruitment.

When PMN counts were compared with the modified GI, patients with higher visually detected inflammation exhibited higher oPMN counts (ANOVA, P <0.001; Tukey HSD test showed a significant difference between the groups). No patients presented with a modified GI of 4 or "severe inflammation." Patients who presented with plaque had higher oPMN counts compared with those who did not (t test, P <0.05). However, no patients presented with a PI >1. No patients had pathologically mobile teeth. Patients with moderate amounts of supragingival and subgingival calculus or subgingival calculus only exhibited significantly higher oPMN counts compared with the others (ANOVA, P <0.001). No participants had supragingival and subgingival calculus or a calculus index score of 3. There was no significant relationship found between oPMN counts and the regularity of dental office visits (t test, P = 0.48). Participants with gingivitis exhibited a 1.5-fold greater level of PMNs, whereas the patients with periodontitis exhibited a two-fold greater level of oPMNs compared with the periodontally healthy patients (ANOVA, P <0.05; Tukey HSD test showed a significant difference between all the groups). Furthermore, the oPMN counts of the patients with moderate periodontitis were three-fold greater than those who were periodontally healthy. The mean oPMN counts of the patients by their periodontal status are reported in Table 2.

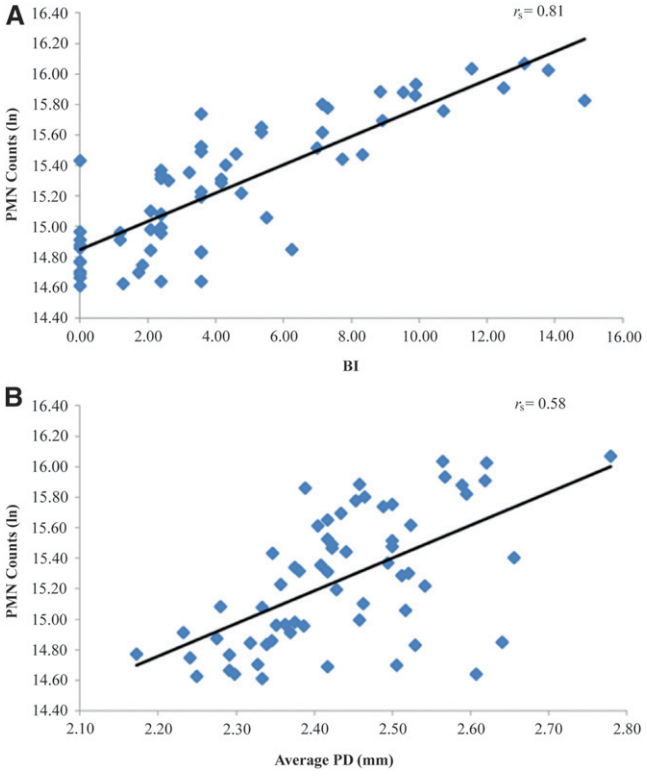

Figure 3.
A) *Relationship between natural log of PMN counts and the BI of the study population showing a significant positive correlation (r_s = 0.81, P <0.001.* ***B)*** *Relationship between natural log of PMN counts and the average PDs of the study population showing a significant positive correlation (r_s = 0.58, P <0.001).*

Table 2.

Pregnancy Characteristics of the Study Population at Follow-Up (n = 59) and Incidence of Overall Adverse Pregnancy Outcomes Among the Study Population Categorized by Certain Risk Factors, Periodontal Status, and Other Indicators of the Status of Periodontal Disease

Factor	n	Percentage
Previous pregnancy history		
History of any pregnancy	38	64.4
History of spontaneous abortions	14	23.7
History of induced abortions	4	6.8
History of preterm birth	2	3.4
Fertility treatment	4	6.8
Gestational diabetes development	2	3.4
Premature rupture of membranes		
Term	3	5.1
Preterm	2	3.4
Delivery		
Vaginal	40	67.8
Cesarean	19	32.2
Elective	13	22.0
In labor	6	10.2
Labor		
Spontaneous	33	55.9
Induced	13	22.0
No labor and not induced	13	22.0
Reason for induction		
After the due date	4	6.8
Term premature rupture of membranes	3	5.1
Preterm premature rupture of membranes	1	1.7
Intrauterine growth restriction	1	1.7
Other*	4	6.8
Adverse birth outcomes		
Preterm birth and/or LBW infant	6	10.2
Risk factors		
Age ≥35 years	3	5.1
Race		
White	5	8.5
Black	0	0
Asian	0	0
East Indian	1	1.7
Other	0	0
History of spontaneous abortions†	2	3.4
History of induced abortions†	0	0

To determine the usefulness of the oPMN counts as a tool for detection of periodontal disease in pregnant females, a BI of 5.5 or BOP at ≥10 sites was chosen as the cutoff point for the diagnostic test.[6,10] This is a clinically relevant number of sites, because this can be considered to represent localized inflammation. Similarly, the cutoff point of four interproximal sites with a PD ≥4 mm was chosen as a clinically relevant number of PDs because detection of localized periodontitis would be clinically useful. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and receiver operator curve (ROC) were determined for the BI cutoff point (Figs. 4A and 4B) and PD cutoff point (Figs. 4C and 4D) at various oPMN counts. For the BI cutoff point, a PMN count of ≈4.5 million cells yielded a sensitivity of 0.94 and a specificity of 0.76. The PPV and NPV were calculated to be 0.60 and 0.97, respectively. Similarly, for the PD cutoff point, an oPMN count of ≈4 million cells was found (sensitivity of 0.80 and specificity of 0.63). The PPV and NPV were 0.59 and 0.83, respectively.

Four of the 63 patients (6.3%) moved away from the city of Toronto and did not deliver at the Mount Sinai Hospital, Toronto; consequently, the course of the their pregnancies and birth outcomes could not be collected.

For details of the pregnancies, see Table 2. Of note, only six (10.2%) patients either delivered preterm and/or had an LBW infant. The incidence of overall adverse birth outcomes classified by certain risk factors (age, race, pregnancy characteristics, medical history, and smoking status), periodontal status (healthy, gingivitis, periodontitis), and indicators of the status of periodontal disease (modified GI, PI, calculus index) is presented in Table 2. The majority of patients who experienced an adverse birth outcome were white. The expected number of preterm deliveries (based on population statistics)—five pregnancies—was observed, but only one of these patients was diagnosed with periodontal disease at recruitment. At recruitment, the mean oPMN count for those who delivered preterm was 4.03×10^6 cells, and for patients delivering LBW infants, the mean oPMN count was 3.91×10^6 cells. There were no statistically significant differences between the PMN counts, average PDs, and BI of the preterm delivery patients and those who delivered at term (*t* test, $P = 0.42$, $P = 0.51$, and $P = 0.83$, respectively). There were no statistically significant differences in oPMN, average PDs, and BI in patients who delivered an LBW infant versus those who delivered normal-weight infants (*t* test, $P = 0.35$, $P = 0.51$, and $P = 0.72$, respectively).

DISCUSSION

The primary objective of this study is to correlate the levels of periodontal disease as measured by

Table 2. (continued)

Pregnancy Characteristics of the Study Population at Follow-Up (n = 59) and Incidence of Overall Adverse Pregnancy Outcomes Among the Study Population Categorized by Certain Risk Factors, Periodontal Status, and Other Indicators of the Status of Periodontal Disease

Factor	n	Percentage
History of preterm birth†	0	0
Fertility treatment†	1	1.7
Gestational diabetes†	1	1.7
Smoker†	0	0
Periodontal status classification		
Healthy	5	8.5
Gingivitis	0	0
Periodontitis	1	1.7
Periodontal disease status indicators		
Modified GI‡		
0	1	1.7
1	4	6.8
2	0	0
3	1	1.7
PI§		
0	3	5.1
1	3	5.1
Calculus index‖		
0	4	6.8
1	1	1.7
2	1	1.7

* Other reasons included gestational diabetes, previous trauma with delivery, oligohydraminos, and fetal anomaly.
† These risk factors are not mutually exclusive.
‡ Modified GI is classified as follows:[15] 0, absence of inflammation; 1, mild inflammation, with slight change in color, little change in texture of any portion but not the entire marginal or papillary gingival unit; 2, mild inflammation, same criteria as above but involving the entire marginal or papillary gingival unit; and 3, moderate inflammation, with glazing, redness, edema, and/or hypertrophy of the marginal or papillary gingival unit.
§ PI is classified as follows:[14] 0, no plaque present; and 1, plaque present on some but not all of the interproximal and gingival surfaces of the tooth.
‖ Calculus index is classified as follows:[14] 0, absence of calculus; 1, supragingival calculus extending only slightly below the free gingival margin (not more than 1 mm); and 2, moderate amount of supragingival and subgingival calculus or subgingival calculus only.

conventional methods with oPMN counts from an oral rinse assay in a group of low-risk pregnant females. The present findings clearly demonstrate that the levels of periodontal disease, as measured by conventional methods (visual assessment of inflammation, presence of plaque and calculus, PDs, and BOP), correlate significantly with oPMN counts in this study population. Furthermore, the relatively high PPV, NPV, sensitivity, and specificity values for BOP at ≥10 sites and PDs ≥4 mm when counts of oPMN were between 4 and 4.5 million cells indicates that this oral rinse assay is a very good predictor of the presence of significant OIL and deep pockets. These findings are consistent with the previously reported findings in otherwise healthy non-pregnant females being treated for moderate to severe periodontal diseases.[10] Importantly, however, the quantification of oPMN was simplified greatly by collecting a single (as opposed to two) 15-second rinse sample and by measuring the optical density of the sample instead of using the much more labor-intensive cell counting methods described previously. The relative ease and rapidity with which oPMN can be collected and quantified, as well as the low cost per patient for the reagents used to quantify oPMNs highlight the simplicity and cost effectiveness of this assay. This diagnostic test for OIL shows that it is a valid and reliable method of identifying increased OIL that frequently accompanies periodontal diseases in pregnant females.

The exact mechanism by which hormonal changes in pregnancy increase the susceptibility of gingival tissues to inflammation is not known. Most explanations for pregnancy-related gingival changes have suggested increased vascularity and vascular flow, directly or indirectly, as the main factor because both estrogen and progesterone have effects on the vascular system during pregnancy. The increase in progesterone has been shown to cause increased vascular permeability, gingival edema, crevicular fluid flow levels, and prostaglandin production, which may then lead to gingival inflammation.[16] Other proposed mechanisms include changes in the immune system or changes in connective tissue metabolism. Although the number of peripheral PMNs increases during pregnancy, their function appears to be altered,[17] which may then render gingival tissues less resistant to challenges caused by bacteria. In addition, progesterone has been shown to decrease interleukin-6 production by human gingival fibroblasts, which in turn may lower resistance to infectious challenges.[18]

It is noteworthy that the average oPMN count for this group of participants (with moderate periodontitis) was ≈9 million cells, in contrast to levels of ≈6 million cells for a group of non-pregnant patients studied previously.[10] Despite finding that the oPMN counts in the population studied here were comparatively higher, it becomes difficult to make any definitive statements because of the differences in the criteria used to define moderate periodontitis between the two studies (Bender et al.[10] had defined moderate periodontitis as the presence of ≤10 pockets >5 mm). A previous study by the present authors also did not define mild periodontitis; therefore, no comparisons could be made for the

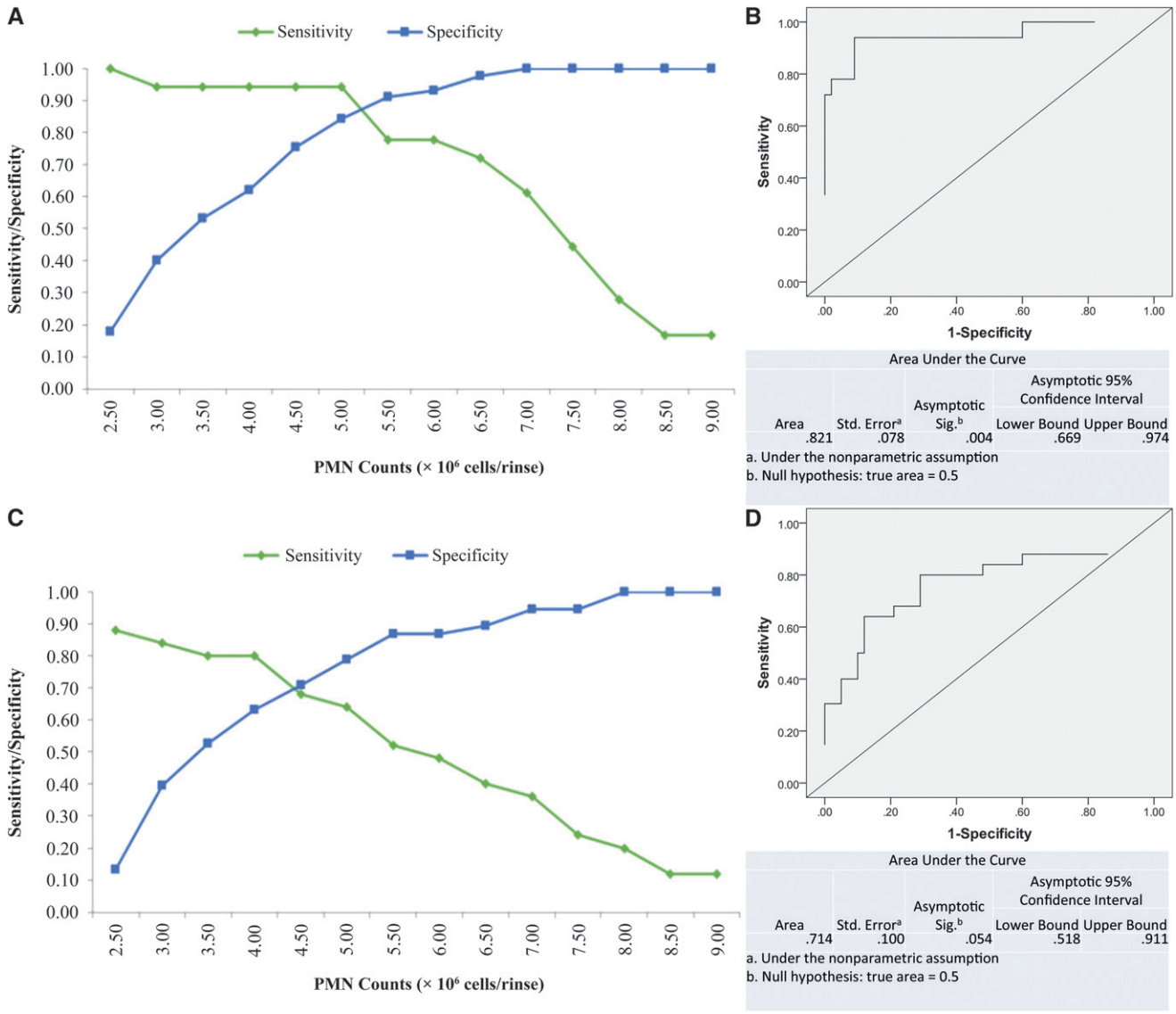

Figure 4.

A) *Sensitivity and specificity values of the oral rinse assay for increasing PMN counts using BI ≥5.5 as the cutoff point. A PMN count of 4.5 million cells yielded a sensitivity of 0.94 and a specificity of 0.76.* ***B)*** *ROC curves for PMN counts and the BI of the study population.* ***C)*** *Sensitivity and specificity values of the oral rinse assay for increasing PMN counts using PD ≥4 mm in at least four sites on different teeth as the cutoff point. A PMN count of 4 million cells yielded a sensitivity of 0.80 and specificity of 0.63.* ***D)*** *ROC curves for PMN counts and the average PDs of the study population. Std. Error = standard error; Sig. = significance.*

average oPMN counts of the group of pregnant females who presented with mild periodontitis in this study and the findings obtained previously. However, it should be emphasized that the average oPMN counts for the patients with healthy periodontal status were similar in both investigations.

The above notwithstanding, it is conceivable that this simple assay for oPMNs for assessment of OIL in female patients trying to become pregnant (high risk or otherwise) or who are already pregnant and can be administered by relatively non-specialized staff, could provide meaningful data that might finally demonstrate more clearly any linkages between oral inflammation and adverse pregnancy outcomes, particularly because it can be used easily to screen large populations and, most importantly, in non-traditional settings, including medical offices. At this juncture, although it is recognized that to answer the question relating to whether there is a correlation between oral inflammation and adverse pregnancy outcomes, it had to be shown initially that administration of this test was feasible in a medical setting. This will improve overall applicability and acceptability for use in large multicenter studies.

There were several other publications in which this screening test for periodontal disease/OIL has been validated (Bender et al.,[10] Landzberg et al.,[6,19] and Moosani et al.[20]). More importantly, hopefully, a case has been made for this paradigm shift that, in reality, it is oral inflammation that is being measured as defined by OIL. It is recognized and appreciated that oral inflammation might arise from any source ranging from gingivitis to periodontitis, as well as, for example, mucocutaneous diseases. It was shown that high levels of oPMN do correlate with severe periodontitis, but perhaps this is beside the point when it comes to the putative relationship between oral and non-oral conditions (including adverse pregnancy outcomes). It is proposed that a specific diagnosed disease, such as periodontitis, is not actually what should be evaluated when attempting to correlate oral and non-oral diseases. Rather, it is posited that, for "oral and systemic diseases," the focus should be on oral inflammation, regardless of the oral disease that might be the source of the inflammation. Of course, once oral inflammation has been identified, a diagnosis must be made so that appropriate treatment can be rendered. In consideration of the above, it is hypothesized that the test is not necessarily one that is specific for periodontitis but, rather, for oral inflammation, the source of which, at least in this study, appears to be correlated to the presence of periodontitis.

Perhaps inflammation is the key risk indicator or even link between periodontitis and other non-oral conditions ranging from cardiovascular diseases to adverse pregnancy outcomes. Admittedly, the bacterial elements of periodontal diseases (or other diseases in the oral cavity) could also play some role as risk indicators or risk factors insofar as non-oral diseases and conditions are concerned. However, there are several diseases of inflammation in which microbiologic factors are absent, including rheumatoid arthritis and even osteoarthritis.[21-23] These also increase the risk for other non-oral conditions, such as cardiovascular diseases, possibly through the effects of inflammation-associated increases in degradative enzymes that destroy an anti-inflammatory protein, fetuin, and are decidedly not microbial in origin.[24,25]

CONCLUSIONS

Finally, although in this study there is no evidence found to support the notion that oral inflammation increased the risk for adverse pregnancy outcomes, it might still be possible that increased inflammatory load could be more important when present before pregnancy. Moreover, the degree of oral inflammation demonstrated in this investigation is relatively low. Including a surrogate marker for systemic inflammation (e.g., C-reactive protein) would be an excellent addition to future studies to determine the true link between OIL and systemic inflammation. This type of investigation should also be focused on patients in lower socioeconomic strata and who may be more likely to have more severe forms of periodontitis or higher levels of OIL. As noted above, the relative ease of administration of the oPMN assay described here means that assessments of OIL can be simplified and hence used more assiduously in these types of studies. This could lead to clearer findings with regard to the still controversial issues surrounding the question of the relationships between adverse outcomes of pregnancy and the presence of oral inflammation. Solving this problem could conceivably lead to important improvements in perinatal health.

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