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A simple point of care test can indicate the need for periodontal therapy to reduce the risk for adverse pregnancy outcomes in mothers attending antenatal clinics

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ABSTRACT

Introduction: Although the association between periodontal disease (PD) and adverse pregnancy outcomes has gained recognition amongst antenatal healthcare workers, not much has changed in practice to address it. This prospective study tested the hypothesis that BANA (N-benzoyl-DL-arginine-2-naphthylamide), a diagnostic test for PD, may inform obstetricians and other antenatal healthcare practitioners, of the risk of adverse pregnancy outcomes in mothers attending antenatal clinics.

Methods: At first visit, the presence of suspected periodontopathogens was assessed by BANA testing of dental plaque from 443 mothers attending antenatal clinics in KwaZulu-Natal, South Africa and an association later sought with pregnancy outcomes. The accuracy of BANA to predict adverse pregnancy outcomes was evaluated by the calculation of likelihood ratios. The study complied with the Declaration of Helsinki.

Results: Significant differences were found between pregnancy outcomes of BANA-negative and BANA-positive mothers ($p < 0.0001$). BANA showed sensitivity and negative predictive values of 87% and 91%; 75% and 78%; 87% and 94% in detecting low birth weight, preterm delivery, and preterm low birth weight delivery respectively.

Conclusion: This study confirms that BANA may indicate the need for periodontal therapy to reduce the risk of adverse pregnancy outcomes and could form part of the routine antenatal examination.

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BANA; low birth weight; preterm birth; pregnancy-associated periodontal disease



Introduction

Adverse pregnancy outcomes such as preterm birth (PTB) and low birth weight (LBW) are major causes of maternal and neonatal morbidity and mortality. Increasing evidence points to an association between periodontal disease (PD) and adverse pregnancy outcomes and thus early detection will assist in treatment planning to reduce adverse pregnancy outcomes. It has been reported that 50%–70% of pregnant women develop PD between the second and eighth month of pregnancy (Barak *et al.* 2007, Leon *et al.* 2007). However, recent studies show that oral health assessment does not form part of the routine antenatal screening, resulting in many cases of maternal PD going undetected (Wilder *et al.* 2007, Thomas *et al.* 2008, Da Rocha *et al.* 2011, Suri *et al.* 2014). Although there appears to be an awareness of the association of PD with adverse pregnancy outcomes, very little is being done to address it and thus mothers with poor

oral health are often not detected nor granted the opportunity of being referred for dental therapy to reduce their risk of adverse pregnancy outcomes.


Treponema denticola, *Porphyromonas gingivalis* and *Tannerella forsythia*, collectively known as the red complex (Socransky *et al.* 1998), are bacterial species which have consistently been implicated in the aetiology of PD and are known to produce a trypsin-like enzyme capable of hydrolysing the substrate N-benzoyl-DL-arginine-2-naphthylamide, commonly referred to as BANA (Loesche *et al.* 1992). BANA has thus gained recognition as a diagnostic tool for PD through its ability to detect these species in dental plaque samples with the same accuracy as DNA probes (Loesche *et al.* 1992, Andrade *et al.* 2010).

Bayingana (2005) used BANA and PCR (polymerase chain reaction) to investigate the prevalence of the red complex in subgingival plaque samples in pregnant women and after associating these results with pregnancy outcomes, proposed

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Synopsis: A rapid diagnostic test for periodontal disease may alert health care practitioners of the risk for adverse pregnancy outcomes in mothers visiting antenatal clinics.

 Supplemental data for this article can be accessed [here](#).

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the wider use of BANA as a screening test for mothers at risk for delivering preterm or LBW infants. After controlling for other risk factors, Chan *et al.* (2010) also found an association between PTB and BANA-positive plaques, thus lending support to this thesis. However, to date, there has been very little other support for this association, despite these significant findings.

This study aimed to inform obstetricians and other healthcare practitioners in antenatal clinics of the value of BANA as a point of care test (outside the dental environment) to detect potential periodontal pathogens and to reduce the risk of adverse pregnancy outcomes due to PD. It completes an earlier study which associated clinically diagnosed PD with pregnancy outcomes (Turton and Africa 2017), by using the BANA test to indicate the presence of suspected periodontal pathogens in these subjects.

Clinical significance

- Periodontal disease (PD) has been associated with adverse pregnancy outcomes, but not enough is being done to forge the collaboration between antenatal healthcare providers and oral healthcare professionals. The implementation of BANA (a diagnostic test for PD) as a routine point of care test at the start of an antenatal examination may deliver a result by the time her examination is complete. This will alert antenatal healthcare providers of any risk for adverse pregnancy outcomes and encourage them to refer mothers to a dental clinic for prompt treatment to reduce their risk.

Methods

Study population

The study included 443 randomly selected pregnant women aged ≥ 18 years, presenting at antenatal maternal obstetric units (MOU) in the province of KwaZulu-Natal in South Africa over a two-year period. Calculation of the estimates of the variance available from other studies revealed that 400 (± 10) samples would be an adequate sample size, given a 95% confidence interval. Non-probability sampling was employed to select the sites where pregnant women were enrolled using convenience sampling. Excluded from the study were smokers; patients with existing heart disease, hypertension, diabetes, asthma or chronic renal disease; mothers with induced labour, previous preterm delivery or multiple pregnancies; mothers currently using systemic corticosteroids or antibiotics, all of which may have influenced the outcomes of the study.

Ethical considerations

The study complied with the Declaration of Helsinki (WMA 2013) and was ethically approved by the research ethics

committee of the University of the Western Cape. The Provincial Department of Health and the Municipal health district managers granted permission for the research to be conducted at the various hospitals and clinics selected. Individuals submitted signed consent for participation in the study after the nature of the study was explained to them. Those who were unable to read or write had the contents of the consent forms explained to them and indicated their consent by means of an 'X' on the signature line.

Data collection

A cross sectional study structure was used with data collection in a standardized format using a structured questionnaire along with clinical data capture sheets. The questionnaire elicited demographic information about the participants and contained items designed to obtain information regarding factors which may predispose for PD such as medical history, maternal age, stage of pregnancy and personal hygiene practices (including oral hygiene). The gestational age of the participant, at the time of enrolment, was calculated from the date of the last menstrual period. This information was verified by cross referencing it with the participant's MOU file.

The presence and severity of PD was graded as absent, mild, moderate, or severe as described by Offenbacher *et al.* (2001).

For the BANA assay, an interdental subgingival plaque sample was collected with a periodontal probe between the first molar and second premolar of each quadrant of the mouth, or, if one of these teeth was missing, then between either the first and second molars, or between the premolars (Loesche *et al.* 1990).

The site from which the sample was collected was recorded on the BANA test card (Perioscan[®], Oral-B Laboratories Inc., Redwood City, CA), in the marked space. Briefly, the principle of the test is as follows. The BANA hydrolysis test is a plastic card with two separate reagent matrices (strips). The lower strip is impregnated with BANA reagent and the upper strip contains a chromogenic diazo reagent, Fast Black K B-naphthylamide. One of the hydrolytic products of the BANA reaction reacts with the Fast Black K producing a permanent blue colour (Loesche *et al.* 1990).

Following plaque collection, the tip of the periodontal probe containing the plaque was wiped onto the BANA-impregnated lower strip, located on the bottom of the BANA reagent card. The reaction was immediately activated by water applied to the upper strip containing the fast black dye. The lower strip was folded onto the upper strip and both strips were held in place with a metallic clip while incubated at 55°C for 15 min in the BANA incubator (Model KPT01, Knowell Periodontal Technologies, Inc., Toronto, Canada). The result of the BANA analysis was recorded as positive (blue spots at the sample site on the reagent card) or negative (no colour change) on the data capture sheet (Loesche *et al.* 1990).

Table 1. The association of BANA with periodontal disease status.

BANA (<i>n</i> = 443)	Periodontal disease			
	Absent	Mild	Moderate	Severe
Negative (<i>n</i> = 161)	117	38	5	1
Positive (<i>n</i> = 282)	6	155	76	45

Chi-squared test revealed that as periodontal disease severity increased, the number of BANA samples decreased significantly ($\chi^2 = 260.3$, $df = 3$, $p < 0.0001$).

Pregnancy outcomes

Post-delivery, gestational age, infant weight and mode of delivery were recorded. Infants with birth weight (BW) < 2500 g were classified as LBW and those delivered before 37-week gestation were classified as PTB. Infants born both preterm (<37 weeks) and with LBW (<2500 g) were classified in the category preterm LBW (PTLBW).

Data analysis

The STARD checklist and flow chart for studies of diagnostic accuracy (Bossuyt *et al.* 2015) were employed to ensure accurate reporting of the data. Descriptive statistics were derived from demographic and socio-economic data. The diagnostic accuracy of BANA in predicting PTB and LBW was determined by measurement of the area under the curve (AUC) in a receiver operating characteristic (ROC) curve analysis and by calculating the sensitivity, specificity, predictive values and likelihood ratios.

MedCalc Statistical Software version 17.1 (MedCalc Software bvba, Ostend, Belgium) was used to perform the statistical analyses. After examining for normal distribution of the data by means of the Chi-squared test, non-parametric tests (Spearman rank correlation, Mann-Whitney test and Kruskal-Wallis test) were applied. Statistical significance was defined as $p < 0.05$.

Results

The participants ranged in age between 18 and 42 years with a mean (SD) age of 24.13 (± 5.30) years. Pregnancy stage varied, with 62.3% in their second trimester of pregnancy, 33.4% in the third trimester and 4.2% in the first trimester.

BANA

A description of the clinical presentation of PD status of these mothers is published elsewhere (Turton and Africa 2017), and will not be repeated here. In line with the PD grading, 282 (64%) of the mothers produced a positive BANA test result, while BANA-negative mothers constituted 161 of the 443 subjects recruited to this study (36%).

BANA was significantly associated with the presence of PD in these mothers as revealed by Chi-squared analysis ($\chi^2 = 260.3$, $df = 3$, $p < 0.0001$, Table 1). As the severity of PD increased from mild to severe, the number of BANA-negative plaques decreased (Table 1). On the other hand, the number of BANA-positive plaques was low in the absence of PD and increased in patients with PD (Table 1).

Pregnancy outcomes

Birth outcomes in terms of gestational age ($r = 0.230$), BW ($r = 0.491$) and BW for gestational age ($r = 0.409$) showed a significant negative correlation ($p < 0.0001$) with PD status.

The mean gestational age (GA) was 37.32 weeks (SD = 2.5, Min = 22, Max = 40). The Kruskal-Wallis test showed that GA and BW differed significantly ($p < 0.0001$) with PD status (i.e. from absent to severe) and between BANA-positive and BANA-negative samples (Figure 1(a,b), respectively). Since not all BANA-negative samples indicated an absence of PD, and since not all BANA-positive samples indicated PD (Table 1), we elected to group samples into two groups, namely BANA-positive/PD-positive, and BANA-negative/PD-negative, in order to compare their pregnancy outcomes. A significant difference between the two groups was observed for both gestational age and BW (Figure 1(c)).

Seventy-five per cent (107/142) of the mothers who delivered PTB (i.e. <37-week gestation) were BANA-positive, while 78.3% (126/161) of the mothers who were BANA-negative delivered full term (FTB, Table 2). Chi-squared analysis revealed significant differences between PTB and FTB for BANA ($p < 0.0001$).

One hundred and nine mothers delivered LBW infants of whom 95 (87.2%) were BANA-positive (Table 2). Of the mothers who tested BANA-negative, 91% (147/161) delivered infants of normal birth weight (NBW). BANA comparisons for LBW and NBW differed significantly ($p < 0.0001$). This significance remained when the BW and gestational age were combined and classified as preterm LBW (PTLBW) and full term NBW (FTNBW). Of the 71 infants classified as PTLBW, 62 (87%) were BANA-positive while 94% of those born of mothers with a negative BANA were born FTNBW (Table 2).

Evaluation of BANA as a screening test for pregnancy outcomes

The evaluation of BANA as a screening test for poor pregnancy outcomes revealed a positive likelihood ratio (LR+) of >1 for PD, preterm (PTB), LBW and preterm LBW (PTLBW). BANA showed a sensitivity of 86% for predicting PD (CI = 82%–89%), 75% for preterm (CI = 67%–82%), 87% (CI = 79%–92%) for LBW and 87% (CI = 77%–94%) for PTLBW (Table 3). Although the positive predictive value (PPV) was only high for PD, a good negative predictive value (NPV) and negative likelihood ratio (LR-) of <1 was demonstrated for PD, PTB, LBW and PTLBW (Table 3).

Figure 2 shows the ROC-curve comparison of BANA with each of the reference standards used, namely, PD, BW, GA and BW for gestational age (BWGA). The standard methods used for establishing these outcomes are outlined in the methodology section above. AUC measurement of the ROC-curve analysis revealed that BANA accurately predicted PD (AUC = 0.895, $p < 0.0001$) and was a better predictor of BW (AUC = 0.697; $p < 0.0001$) and BWGA (AUC = 0.669; $p < 0.0001$) than GA (AUC = 0.621; $p = 0.002$).

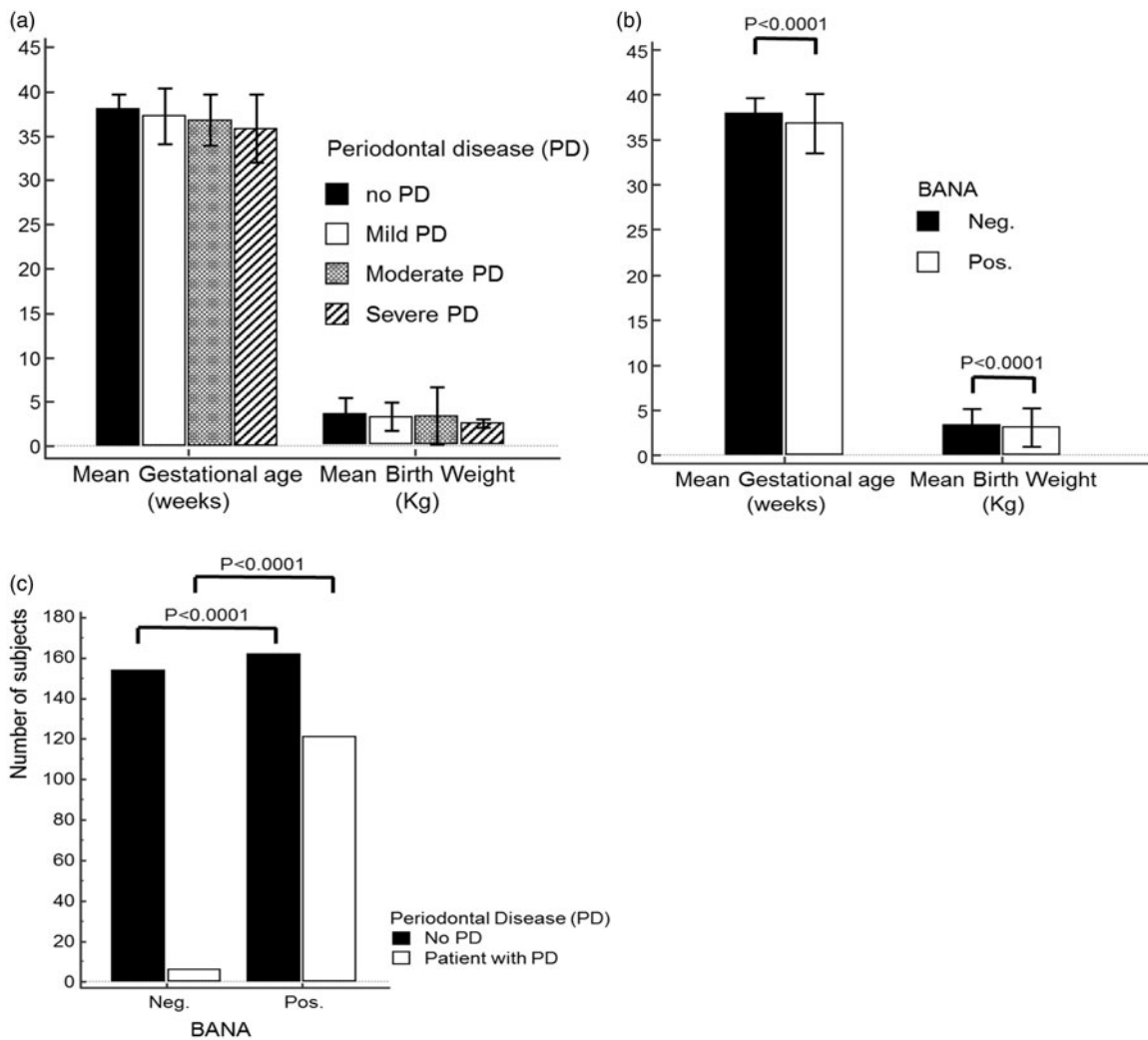


Figure 1. Association of pregnancy outcomes with periodontal disease and BANA. (a) There is a highly significant ($p < 0.0001$) correlation between gestational age/birth weight and periodontal disease status with gestational age and birth weight declining significantly with increased periodontal disease severity. (b) Kruskal–Wallis test shows highly significant ($p < 0.0001$) differences in gestational age and birth weight in infants born of BANA-positive and BANA-negative mothers. (c) Number of BANA-positive and BANA-negative subjects with and without periodontal disease.

Table 2. Comparison of normal and adverse pregnancy outcomes with BANA.

BANA	Gestational age		Birth weight		Birth weight for gestational age	
	Preterm (<37 weeks)	Full term (≥ 37 weeks)	Low birth weight (<2500 g)	Normal birth weight (≥ 2500 g)	Preterm low birth weight	Full-term normal birth weight
BANA+ $n = 279$	107 (24.3)	172 (39)	95 (21.5)	184 (42)	62 (14)	217 (49.3)
BANA- $n = 161$	35 (7.9)	126 (8.6)	14 (3.2)	147 (33.3)	9 (2)	152 (34.5)
Total $N = 440$	142 (32.2)	298 (67.7)	109 (24.7)	331 (75.3)	71 (16)	369 (83.8)

Missing = 3.

Table 3. BANA test evaluation.

BANA test	Periodontal disease (PD)	Preterm (PTB)	Low birth weight (LBW)	Preterm low birth weight (PTLBW)
Sensitivity	86%	75%	87%	87%
Specificity	95%	42%	44%	41%
LR+	17.6	1.3	1.5	1.5
LR-	0.1	0.6	0.3	0.3
PPV	98%	38%	34%	22%
NPV	73%	78%	91%	94%

Confidence intervals (CI) = 95%. LR+ = positive likelihood ratio; LR- = negative likelihood ratio; PPV = positive predictive value; NPV = negative predictive value.

Discussion

PD is a common complication of pregnancy, and until recently has not received the attention it deserves as a risk indicator for poor pregnancy outcomes. Microbiological techniques used to diagnose PD include microscopy, culture and more recently, molecular techniques, all of which are expensive and time-consuming and cannot be conducted in a routine clinical setting.

The accuracy of BANA to detect PD has already been well established (Loesche *et al.* 1992), showing good sensitivity

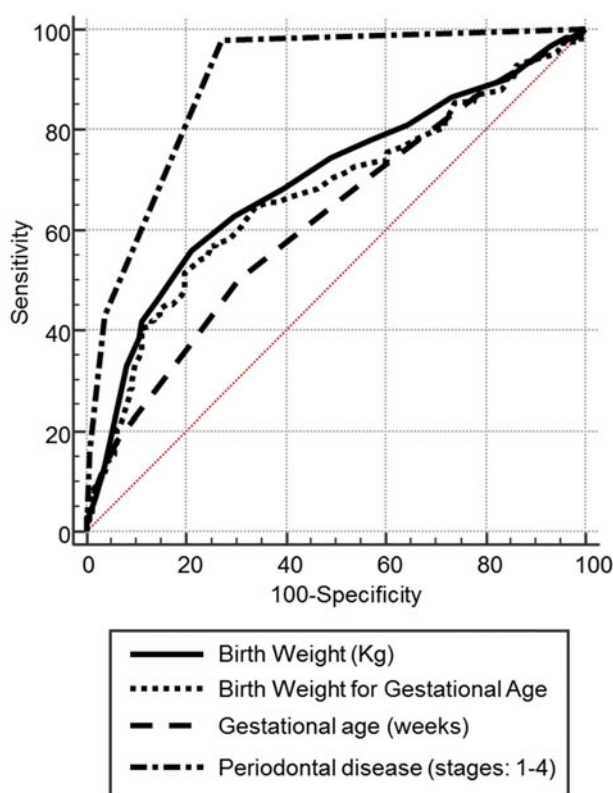


Figure 2. Comparison of ROC curves to determine the accuracy of BANA in predicting periodontal disease and pregnancy outcomes. Details of each of the reference standards compared with BANA, namely, birth weight, gestational age and birth weight for gestational age, are outlined in the methodology section of this paper. BANA predicted periodontal disease most accurately (AUC = 0.895; $p < 0.0001$), followed by birth weight (AUC = 0.697, $p < 0.0001$) and birth weight for gestational age (AUC = 0.669; $p < 0.0001$) with gestational age showing the lowest AUC (AUC = 0.621, $p = 0.002$).

and specificity when compared with DNA probes and polyclonal immunological reagents (92% and 70%, respectively), and 95% sensitivity when compared with chequerboard DNA–DNA hybridization using highly specific whole genomic DNA probes to *P. gingivalis*, *T. forsythia* and *T. denticola*. It is thus recognized in epidemiologic studies as a diagnostic tool for PD (Loesche *et al.* 1992) and associated infections (Bretz *et al.* 1993; Grisi *et al.* 2001; Loesche *et al.* 1998).

A strength of this study is that the mean BW of infants born of BANA-negative and BANA-positive mothers differed significantly, as did the gestational age and BWGA. BANA showed a sensitivity of 87% and a NPV of 91% in detecting LBW, a sensitivity of 75% and NPV of 78% in detecting PTB, and a sensitivity of 87% with a NPV of 94% for PTLBW, thereby demonstrating the usefulness of the BANA test as a screening test for mothers at risk for preterm delivery of LBW infants. Although we were unable to record the pregnancy outcomes of three of the patients originally recruited to the study, we did not consider this a limitation since we had made allowance for attrition by exceeding the number of subjects originally decided upon. We speculate that the finding of BANA-positive results in the absence of PD in this study may be due to bacteria other than the red complex, such as *Rothia* and *Capnocytophaga* species, which are also known to be BANA-positive, but unlike the red complex,

have not consistently been associated with PD (Loesche *et al.* 1990).

There is a controversy around the benefit of dental treatment during pregnancy with some studies reporting a reduction in adverse pregnancy outcomes (Slots and Research, Science and Therapy Committee 2004, Lopez *et al.* 2005), and others showing no benefit (Michalowicz *et al.* 2006, Newnham *et al.* 2009, Offenbacher *et al.*, 2009, Macones *et al.* 2010, Jeffcoat *et al.* 2011). However, the argument in favour of treatment far outweighs the argument against it. It has been confirmed by the American Academy of Periodontology (Rubenfire *et al.* 2007) that treatment for PD during the second trimester of pregnancy is not only beneficial but also safe and effective.

Unfortunately, not many mothers seek dental treatment during pregnancy and antenatal clinics pay little if any attention to the oral health status of the mother, with the result that this complication often goes undetected. Surveys conducted in USA, Brazil and India on the knowledge and practice behaviours of obstetricians reveal an increasing awareness amongst obstetricians of an association of PD with adverse pregnancy outcomes while simultaneously suggesting that a change in attitude is needed to adequately address the risk (Da Rocha *et al.* 2011, Suri, Rao and Aggarwal 2014, Wilder *et al.* 2007).

In the survey conducted in North Carolina, USA (Wilder *et al.* 2007), 84% of obstetricians recognized PD as a risk factor for adverse pregnancy outcomes; only 22% admitted examining patients' mouths at first visit, while the remainder reported either doing an oral examination periodically (9%), or when a problem was reported by a patient (48%). Forty-nine per cent reported that they rarely if ever, recommended a dental examination. A later survey of the same region by Thomas *et al.* (2008), involving nurse practitioners, physician assistants and certified midwives, looked more encouraging and revealed that 63% reported looking in the patient's mouth to screen for oral problems at the initial visit, 20% felt that their knowledge of PD was current, and all agreed that their discipline should receive instruction regarding PD. Ninety-five per cent agreed that a collaborative effort between the healthcare provider and the oral healthcare professionals was needed and would reduce the patient's risk of having an adverse pregnancy outcome.

In Brazil, 80% of obstetricians surveyed, believed that PD was a risk factor for poor pregnancy outcomes (Da Rocha *et al.* 2011) while only 30%–52% (influenced by increasing years of practical experience) referred their patients for dental examinations during pregnancy. Similar results were reported from India (Suri, Rao and Aggarwal 2014) where 70% of obstetricians recognized the effects of poor dental health on pregnancy outcomes, but only 40% advised their patients on the need for routine dental examination during pregnancy.

It is evident from the above that even though the importance of oral health during pregnancy is acknowledged, not enough is being done to forge the collaboration between the antenatal healthcare provider and oral healthcare professionals. One of the reasons given for this lack of action is the limited time for consultation, leaving no time to perform an oral examination (Wilder *et al.* 2007). This can be overcome

by incorporating a rapid point of care test such as BANA into the routine antenatal examinations. This could alert antenatal healthcare providers of the risk of adverse pregnancy outcomes and encourage them to refer mothers to a dental clinic for prompt treatment to reduce their risk and improve their own health and general wellbeing as well as the wellbeing of their infants.

BANA is a simple, rapid and inexpensive point of care test, if set up prior to the start of the patient's examination, which can deliver a result by the time her examination is complete. Although a periodontal probe was used in this study to collect plaque from between the teeth, a sterile wooden toothpick will work as efficiently, thus lending this technique to use outside of the dental clinic. In addition, the size of the BANA incubator is relatively small (16.5 × 13 cm) and can easily be accommodated in a clinical setting.

Limitations of the study include the difficulty in making comparisons with other studies due to differences in (amongst others) sampling techniques, diagnostic criteria, pregnancy stage at recruitment, ethnicity and geographical location. Within the limitations of the study, the findings support the hypothesis that a positive BANA test during pregnancy may have a significant association with negative pregnancy outcomes such as miscarriage, PTB and LBW. Its implementation as a chairside screening test during antenatal visits should be considered.

Conclusion

This study is one of very few studies which examined for potential periodontal pathogens in mothers attending antenatal clinics, using a simple and rapid point of care test (BANA) which reliably identified mothers at risk for preterm delivery of LBW infants. Since the objective of the current study was to inform healthcare practitioners in antenatal clinics of the predictive value of BANA as a point of care test for detecting maternal PD outside of the dental environment with the purpose of referring them to a dentist for treatment, a prospective randomized treatment study is needed to prove that prompt treatment of PD following BANA testing of mothers may significantly reduce the preterm delivery of LBW infants.

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Disclosure statement

The authors declare that they have no competing interests.

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