Original Research Article

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The Use of Benzoyl-Dl-Arginine-Naphthylamide (Bana) Test as a Screening Test for Mother at Risk for Delivery of Pre-Term and Low Birth Weight in A Rwandan Population

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Abstract

Oral infections can act as the site of origin for dissemination of periodonto-bacteria and their toxins as well as induce inflammatory mechanisms to distant body sites, thus linking periodontal diseases to pre-term delivery of low birth weight (PLBW) infants. Periodontal disease is an infection of the tissues surrounding and supporting the teeth. Researchers showed that between 18 and 50 % of all pre-term deliveries are associated with periodontal disease. 

Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia are among the subgingival microflora most frequently associated with periodontal disease. The presence of these bacteria can be identified by their ability to hydrolyse BANA. BANA is a rapid and effective diagnostic aid shown to correlate well with the clinical indices used to diagnose periodontal disease. The objective of this study was to investigate the association between the presence of member of the red complex (BANA positive species) in subgingival plaque and pre-term delivery of low birth weight in a Rwandan population. Three clinical indices (PI, GI and PD) were measured for each patient. Plaque sample were collected by inserting a sterile probe into the base of the pocket and this served for the measurement of the BANA enzyme test. The age of the population study was between 18 and 47 years with a mean of 30.8 (± 5.34). Among the 450 women examined, 57.1 % had a PI score of 2, 56.0 % had GI score of 2 and 89.6 % had a PD between 4-6 mm. At a level of 5%, a significant association of p-value=0.000 was found between PI vs. BANA, GI vs. BANA and PD vs. BANA. No significant association (p-value=0.073) was found between BANA and mothers who delivered preterm of low birth weight (PLBW). In conclusion, findings of this study showed that BANA cannot be used for now as screening test for mother at risk for preterm delivery, while waiting for a multicenter study which will help to verify the cause of these discrepancies of results. In the other hand we can recommend BANA to be used as a routine test for the detection of periodontal disease due it strong relationship with clinical indices used to diagnose periodontal disease.

Keywords

Rwandan Population, Weight, Delivery and BANA.

Introduction

Oral infections can act as the site of origin for dissemination of periodonto-bacteria and their toxins as well as induce inflammatory mechanisms to distant body sites, thus linking periodontal diseases to pre-term delivery of low birth weight (PLBW) infants (Han et al., 2004; Russel et al., 2006; Lin et al., 2007). Preterm birth is defined as birth before 37 weeks of gestation. Several organ systems in the normal human foetus mature between 34
and adequate maturity of the foetus is reached at the end of that period (Offenbacher et al., 1996; Davenport et al., 1998; Steer, 2005). Low birth weight infants are those who weigh less than 2500 gram. PLBW is increasing extensively and becoming an important problem in both developing and developed countries (McGaw, 2002; Vogel et al., 2005). The rate of low birth weight (LBW) in developing countries is more than double (16.5 %) that in developed countries (7 %) and in Subsaharan Africa, the rate is around 15 %. It is known that PLBW infants are exposed to serious health problems, including, neurodevelopmental disturbances, ear infections, respiratory infections, asthma and death (Shapiro et al., 1980). Ten % of neonatal mortality worldwide is caused by prematurity (Child Health Research Project Special Report, 1999). Preterm delivery is a significant cost factor in healthcare with a considerable cost of long-term care for children with disabilities resulting from it. A study in US showed a neonatal cost of $ 224, 400 for a newborn at 500-700 g while only $1,000 for a newborn at over 3,000 g (Gilbert et al., 2003).

Periodontal disease is an infection of the tissues surrounding and supporting the teeth. Female hormones during puberty, menses, pregnancy, contraceptive use and menopause have been suggested to play an important role in periodontal disease infection (Steinberg, 2000; Blagojevic et al., 2002; Krejci et al., 2002). The increase of estrogen and progesterone concentration in plasma stimulates bacterial growth and are associated with periodontal disease progression (Soory, 2000; Zachariasen, 1993).

Different factors have been linked with a higher risk of preterm delivery: Low socio-economic standards, educational level, single motherhood, age at the upper and lower end (> 35 or < 18 years of age), multiple pregnancies (twins, triplets etc…), smoking and alcoholism during pregnancy, maternal medical conditions such as high blood pressure, maternal diabetes, and heart disease (Parazzini et al., 2003; Rosenberg et al., 2005; Goldenberg et al., 2008). Researchers showed that between 18 and 50 % of all preterm deliveries are associated with periodontal disease and this appeared to increase more than 7 times after adjusting for smoking, vaginosis treatment history, marital status, previous preterm, race and age (Offenbacher et al., 1998a; McGaw, 2002).

Most periodontal diseases are associated with the presence or overgrowth of anaerobic bacteria either alone or in association. Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia are among the subgingival microflora most frequently associated with periodontal disease (Socransky et al., 2009). Frequent association of these three species with periodontal disease resulted in this group of anaerobic Gram negative bacteria being referred to the “red complex”. Members of the red complex possess a trypsin-like enzyme capable of hydrolysing the synthetic peptide N-Benzoyl-DL-Arginine-Naphthylamide (BANA). The presence of these bacteria can be identified by their ability to hydrolyse BANA. BANA is a rapid and effective diagnostic tool shown to correlate well with the clinical indices used to diagnose periodontal disease (Loesche et al., 1992). In our previous study done in South Africa but with a very small number of participants with pre-term delivery infants (Africa et al., 2009), we found that BANA was associated with indices used to diagnose periodontal disease and could be associated with pre-term delivery of low birth weight infants. The objective of this study was to investigate the association between the presence of member of the red complex (BANA positive species) in subgingival plaque and pre-term delivery of low birth weight in a Rwandan population.
Materials and Methods

This study was conducted at Butare University Teaching Hospital (CHUB). Four hundred and fifty women attending the department of obstetrics-gynecology of CHUB were enrolled in this study. Participants were informed the purpose of the study and written informed consents were obtained. Clinical assessment includes patient medical history, dental clinical indices, oral lesion, factors predisposing for periodontal disease, previous preterm delivery, and oral hygiene habits were recorded. All oral clinical examinations and sample collections were done by a dentist. The amount of plaque index (PI) was assessed using the criteria of Silness and Loe (1988) and scored from 0 to 3. Gingival inflammation (GI) was scored from 0 to 3 according to the stage of gingival inflammation (Loe et al., 1963). Probing depth (PD) was measured from the gingival margin to the base of the pocket using a periodontal probe. The average of each clinical index for each patient was determined by dividing by 4 the sum of each clinical index measured in the 4 following teeth: Maxillary right first molar, maxillary left first molar, mandibular left first molar, and mandibular right first molar.

Plaque sample were collected from the above four teeth by inserting a sterile probe into the base of the pocket and this served for the measurement of the BANA enzyme test. The BANA test was done according to the manufacturer’s instructions (Perioscan, Oral B). BANA hydrolysis test is a plastic card with two separates reagents matrices (strips). The lower strip has BANA reagent and the upper strip contains a chromogenic diazo reagent, fast black K. B-naphtylamide, one of the hydrolytic products of the BANA reaction reacts with the fast black K, producing a permanent blue colour. Sites informations were recorded on the BANA test card in the marked space.

Plaque sample were applied on the lower reagent strip of the BANA test card. The upper strip of the BANA card containing the fast black dye was activated by moistening with distilled water and BANA card was folded at the perforation so that the lower and upper matrices come in contact with each other and were placed in the BANA incubator for 15 minutes at 55°C. The BANA test results were read on the upper strip and the results were recorded on the patient’s chart as negative (no blue colour), or positive (blue colour). A positive result suggests that the plaque contained at least $10^4$ to $10^5$ BANA positive organisms or member of the red complex (Loesche et al., 1990).

All questionnaires, oral examination and laboratory data were entered in Excel. Statistical analysis was performed using Excel. Frequencies, means and standard deviations were calculated using descriptive statistics. The significance of associations was determined using chi-squared and Fisher’s exact test. A p value of < 0.05 was considered statistically significant.

Results and Discussion

The age of the population study was between 18 and 47 years with a mean of 30.8 (± 5.34). The age group of 30-35 years was the most frequent with 42 % followed by the age group of 24-29 years with 29.3 % (Table 1).

Only PI, GI and PD will be discussed because most of sites showed no LA. Among the 450 women examined, 57.1 % had a PI score of 2, 33.3 % had a score of 3 and the mean (±SD) PI was 2.24 (±0.617). The mean (±SD) GI was 2.24 (±0.624), 56.0 % had a score GI of 2 and 34.2 % of them had a score GI of 3. The mean (±SD) PD was 4.20 (±0.735) and 89.6 % had a score PD between 4-6 mm (Table 2).

At a level of 5%, a significant association of p-value=0.000 was found between PI vs.
BANA, GI vs. BANA and PD vs. BANA. The more the score of PI, GI and PD increased, the more the number of patients with BANA positive increased. The other way around observation was found in mothers with BANA negative (Tables 3, 4, 5). A significant relationship ($p = 0.019$) was found between BANA and Birth weight. Mothers who delivered babies with <2500g had a tendency to have more BANA positive than mother who delivered babies with > 2500g (Table 6a).

No significant relationship ($p = 0.196$) was found between BANA and Birth weight when all the mothers who delivered infants with > 2500g were pooled together (Table 6b). However no significant relationship ($p$-value=0.090) was found between BANA and gestation Term (Table 7).

At a level of 5%, no significant association ($p$-value=0.073) was found between BANA and mothers who delivered preterm of low birth weight (PLBW) (Table 8).

The objective of this study was to investigate if there is an association between BANA and pre-term delivery of low birth weight and thus to be used for pregnant women with periodontal disease and therefore at risk for adverse pregnancy outcomes. Among the 450 women examined, 57.1% had a PI score of 2 and 33.3% had a score of 3?

The mean ($\pm$SD) PI was 2.24 ($\pm$0.617). These scores are too high compared to our previous study done in South Africa (Cape Town), where 68.5% had a PI=0, with a mean (SD) of 0.4 ($\pm$0.47). Tilakaratne et al., reported a mean PI of 0.69 at the third trimester of pregnancy (15). The high PI in this study may be due to the bad oral hygiene of the population study.

The mean ($\pm$SD) GI was 2.24 ($\pm$0.624), 56.0% had a score GI of 2 and 34.2% of them had a score GI of 3. These scores are high comparing with our previous study (26) where the mean ($\pm$SD) GI was 1.10 ($\pm$0.49) in the third trimester. This high score of GI may be the consequence of the bad oral hygiene of the study population.

It was reported also that pregnancy progesterone and estrogen levels increase 10 to 30 times more in the thirds trimester than during menstruation cycle, which may cause dilatation of gingival capillaries, gingival permeability, exudates and therefore gingival inflammation (Sooriyamoorthy et al., 1989). The mean ($\pm$SD) PD was 4.20 ($\pm$0.735) mm.

### Table 1 Distribution of age ($\pm$SD)

| Characteristics | Frequency |
|-----------------|-----------|
| AGE             |           |
| Mean : 30.8 (±5.34) |   |
| 18-23years      | 42 (9.3)  |
| 24-29years      | 132 (29.3)|
| 30-35years      | 189 (42.0)|
| 36-41years      | 76 (16.9)|
| 42-47years      | 11(2.4)   |

Note: SD=Standard deviation
Table 2: Means (±SD) of clinical indices

| Clinical Indices     | Frequency |
|----------------------|-----------|
| Plaque Index         |           |
| 0                    | 1 (0.2%)  |
| 1                    | 42 (9.3%) |
| 2                    | 257 (57.1%) |
| 3                    | 150 (33.3%) |
| Mean (±SD)           | 2.24 (±0.617) |
| Gingival Index       |           |
| 0                    | 1 (0.2%)  |
| 1                    | 43 (9.6%) |
| 2                    | 252 (56.0%) |
| 3                    | 154 (34.2%) |
| Mean (±SD)           | 2.24 (±0.624) |
| Pocket Depth         |           |
| 1-3 mm               | 47 (10.4%) |
| 4-6 mm               | 403 (89.6%) |
| Mean (±SD)           | 4.20 (±0.735) |

Table 3: Association between BANA and plaque index

| BANA (n) | Plaque Index | 0 | 1   | 2   | 3   | Total |
|----------|--------------|---|-----|-----|-----|-------|
| Negative (%)| 1 (0.5%) | 29 (14.4%) | 139 (68.8%) | 33 (16.3%) | 248 (100%) |
| Positive (%) | 0 (0.0%) | 13 (5.2%) | 118 (47.6%) | 117 (47.2%) | 202 (100%) |
| Total (%)     | 1 (0.2%) | 42 (9.3%) | 257 (57.1%) | 150 (33.3%) | 450 (100%) |

At a level of 5%, a significant association (p-value=0.000) was found between plaque index and BANA.

Table 4: Association between BANA and gingival index

| BANA (n) | Gingival Index | 0 | 1   | 2   | 3   | Total |
|----------|----------------|---|-----|-----|-----|-------|
| Negative (%)| 1 (0.5%) | 30 (14.9%) | 136 (67.3%) | 35 (17.3%) | 202 (100%) |
| Positive (%) | 0 (0.0%) | 13 (5.2%) | 116 (46.8%) | 119 (48.0%) | 248 (100%) |
| Total (%)     | 1 (0.2%) | 43 (9.6%) | 252 (56.0%) | 154 (34.2%) | 450 (100%) |

At a level of 5%, a significant association (p-value=0.000) was found between gingival index and BANA.

Table 5: Association between BANA and pocket depth

| BANA (n) | Pocket Depth in mm | 1-3mm | 4-6mm | Total |
|----------|---------------------|-------|-------|-------|
| Negative (%)| 33 (16.3%) | 169 (83.7%) | 202 (100%) |
| Positive (%) | 14 (5.6%) | 234 (94.4%) | 248 (100%) |
| Total (%)     | 47 (10.3%) | 403 (89.6%) | 450 (100%) |

At a level of 5%, a significant association (p-value=0.000) was found between pocket depth and BANA.
Table 6a Relationship between BANA and birth weight

| BANA (n) | Infant Weight in Grams |          |          |          |          | Total |
|---------|-------------------------|----------|----------|----------|----------|-------|
|         | <2500g                  | 2500-3000g | 3001-3500g | 3501-4000g | >4000g  |       |
| Negative (%) | 21 (11.8%) | 70 (39.3%) | 67 (37.6%) | 11 (6.2%)  | 9 (5.1%) | 178   |
| Positive (%) | 36 (16.7%) | 100 (46.3%) | 52 (24.1%) | 22 (10.2%) | 6 (2.8%) | 216   |
| Total (%)   | 57 (14.5%) | 170 (43.1%) | 119 (30.2%) | 33 (8.4%)  | 15 (3.8%) | 394   |

At a level of 5%, a significant association (p-value=0.019) was found between infant weight and BANA.

Table 6b Relationship between BANA and birth weight

| BANA (n) | Weight of the new born |          |          | Total |
|---------|-------------------------|----------|----------|-------|
|         | <2500g                  | >2500g   |          |       |
| Negative (%) | 36 (16.7%) | 180 (83.3%) | 216 (100.0%) |       |
| Positive (%) | 21 (11.8%) | 157 (88.2%) | 178 (100.0%) |       |
| Total (%)   | 57 (14.5%) | 337 (85.5%) | 394 (100.0%) |       |

At a level of 5%, a significant association (p-value=0.196) was found between weight of the new born and BANA.

Table 7 Relationship between BANA and gestation term

| BANA (n) | Gestation Term in Weeks |          |          | Total |
|---------|-------------------------|----------|----------|-------|
|         | <37 weeks | 37-39 weeks | >39 weeks |       |
| Negative (%) | 20 (17.20%) | 29 (25.0%) | 67 (57.8%) | 116 (100%) |
| Positive (%) | 29 (18.4%) | 23 (14.6%) | 106 (67.1%) | 158 (100%) |
| Total (%)   | 49 (17.9%) | 52 (19.0%) | 173 (63.10%) | 274 (100%) |

At a level of 5%, there is no significant association (p-value=0.090) between gestation term and BANA.

Table 8 Association between BANA and Preterm delivery of Low Birth Weight (PLBW)

| BANA (n) | Underweight and Preterm |          |          | Total |
|---------|-------------------------|----------|----------|-------|
|         | Yes                     | No       |          |       |
| Negative (%) | 8 (4.0%) | 194 (96.0%) | 202 (100%) |       |
| Positive (%) | 20 (8.1%) | 228 (91.9%) | 248 (100%) |       |
| Total (%)   | 28 (6.2%) | 422 (93.8%) | 450 (100%) |       |

P-value=0.073

More than 89 % of participants had a PD measuring between 4 and 6 mm and only 10.4 % measuring between 1 and 3 mm. The high measurement of PD is in relationship with the high scores of PI and GI in this study. In our previous study, the mean PD was 3.2 (±0.75) mm and 66 % of the study population had a PD measuring between 1 and 3 mm and 33.1 % had a PD measuring between 4 and 6 mm.

Two hundred forty eight (55.11 %) of the population showed a BANA negative test while 202 (44.88 %) of them showed a positive BANA test. A strong relationship (p-
value=0.000) was revealed between PI, GI, PD and BANA test. The more the scores of PI, GI and PD increased, the more the number of patients with BANA positive increased and the other way around observation was found in mothers with BANA negative (Table 3, 4 and 5). These findings demonstrate a strong relationship between BANA and the clinical indices used to indicate periodontal disease as it was found in previous studies (Figueiredo et al., 2000; Alexandre et al., 2010).

A significant relationship (p = 0.019) was observed between BANA and Birth weight (Table 6a), but no significant relationship was found between BANA and Birth weight when all the mothers who delivered infants with >2500g were pooled together (Table 6b). There was no significant relationship (p-value=0.090) between BANA and gestation term (Table 7), although mothers who delivered babies with <37 weeks had more BANA positive than BANA negative. No significant relationship (p-value=0.073) was found between BANA and Preterm delivery of Low Birth Weight (PLBW), although mothers with PLBW had more BANA positive than the normal ones (Table 8). A study performed in Taiwan on 19 (7%) preterm deliveries among 268 subjects found that BANA-positive was associated with preterm births after controlling for other risk factors (Chan et al., 2010).

In our previous study where we had only two mothers who delivered PLBW infants out of 66, a significant relationship was shown between BANA and PLBW (Africa et al., 2009).

In conclusion, findings of this study showed that BANA cannot be used for now as screening test for mother at risk for preterm delivery, while waiting for a multicenter study which will help to verify the cause of these discrepancies of results. In the other hand we can recommend BANA to be used as a routine test for the detection of periodontal disease due it strong relationship with clinical indices used to indicate periodontal disease.

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