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: **Benzoyl-Dl-Arginine-Naphthylamide (Bana) Test, Screening Test for Mother, Risk for Delivery, Pre-Term and Low Birth Weight**

I. 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 [1]. PLBW is increasing extensively and becoming an important problem in both developing and developed countries [2]. 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 [3]. The increase of estrogen and progesterone concentration in plasma stimulates bacterial growth and is associated with periodontal disease progression [5].

Researchers showed that between 18 and 50 % of all pre-term 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 [5]. 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

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among the subgingival microflora most frequently associated with periodontal disease [6].

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 [7]. In our previous study done in South Africa but with a very small number of participants with pre-term delivery infants [8], 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.

II. MATERIAL AND METHOD

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 [9] and scored from 0 to 3. Gingival inflammation (GI) was scored from 0 to 3 according to the stage of gingival inflammation [10]. 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 manufacture'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 55^0 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 [11].

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.

III. RESULTS

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).

Table I: Distribution of Age (±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

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).

Table II: 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)

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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).

Table III: 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 IV: 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 V: 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.

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 (See 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).

Table VI: Relationship between BANA and Birth weight

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

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

Table VI: Relationship between BANA and Birth weight

BANA (n)	Weight of the new born		
	<2500g	>2500g	Total
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 VII: Relationship between BANA and Gestation Term

BANA (n)	Gestation Term in Weeks			
	<37 weeks	37-39 weeks	>39weeks	Total
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.

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).

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

BANA (n)	Underweight and Preterm		
	Yes	No	Total
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

IV. DISCUSSION

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

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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 (±SD) PI was 2.24 (±0.617). These scores are too high compared to our previous study [8] done in South Africa (Cape Town), where 68.5 % had a PI=0, with a mean (SD) of 0.4 (±0.47). Tilakaratne et al. reported a mean PI of 0.69 at the third trimester of preganancy [12]. The high PI in this study may be due to the bad oral hygiene of the population study.

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. These scores are high comparing with our previous study [8] where the mean (±SD) GI was 1.10 (±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 [13], which may cause dilatation of gingival capillaries, gingival permeability, exudates and therefore gingival inflammation [14]. The mean (±SD) PD was 4.20 (±0.735) mm. 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 [8].

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 [12,15,16].

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 [17]. 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 [8].

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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