Sensitivity and specificity of subgingival bacteria in predicting preterm birth- a pilot cohort study

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Abstract

Objective: Preterm birth (PTB) increases the risk of adverse outcomes for new born infants. Subgingival bacteria are implicated in causing PTB. The aim of the present study was to assess the accuracy of some subgingival gram positive and gram negative bacteria detected by routine lab procedures in predicting PTB.

Methodology: Pregnant Saudi women (n= 170) visiting King Fahad hospital, Dammam, Saudi Arabia, were included in a pilot cohort study. Plaque was collected in the 2nd trimester and screened for subgingival anaerobes using Vitek2. Pregnancy outcome (preterm/ full term birth) was assessed at delivery. Sensitivity, specificity and positive and negative likelihood ratios were calculated for the identified bacteria to predict PTB.

Results: Data about time of delivery was available for 94 subjects and 22 (23.4%) had PTB. Three gram negative and 4 gram positive subgingival bacteria had sensitivity ≥ 95% with two of each having negative likelihood ratios ≤0.10. Three gram positive bacteria had specificity > 95% with only one having positive likelihood ratio >2.

Conclusion: Subgingival bacteria identified using readily available lab techniques in the plaque of pregnant Saudi women in their 2nd trimester have useful potential to rule out PTB.

Key words: preterm birth; subgingival bacteria; sensitivity; specificity

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Introduction

Preterm birth (PTB) is a serious condition in which infants are born after less than 37 weeks of gestation. It is caused by a number of factors including environmental exposures, medical history of the pregnant mother, genetic, psychosocial and socioeconomic factors. PTB increases the risk of mortality and morbidity in newborn infants causing complications in the respiratory, gastrointestinal, immunologic and nervous systems. [1] Periodontal bacteria have been implicated in increasing the risk of PTB through translocation into the blood stream and causing metastasic infection. [2] Evidence suggests that periodontal microorganisms are capable of entering the uterine cavity leading to the production of pro-inflammatory cytokines hence increasing the risk of PTB. [3]

Several bacterial species were related to PTB, including salivary Lactobacilli, [4] Actinomyces naeslundii genospecies and Lactobacillus casei, [5] Porphyromonas gingivalis, Tannerella forsythia, Prevotella intermedia and Prevotella nigrescens, [6] Porphyromonas gingivalis, [7, 8] Aggregatibacter actinomycetemcomitans, [9] Eikenella corrodens and the orange and red complexes. [6, 10] Some studies described specific groups of pathogens related to periodontal diseases such as the purple, yellow, green, orange and red microbial complexes or Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia, Prevotella intermedia, Campylobacter rectus and Fusobacterium nucleatum. [11, 12] However, other studies [13, 14] did not find a relation between PTB and these organisms. Thus several subgingival bacteria may be implicated in PTB although there is no clear evidence that one of them is responsible alone for the condition.

Some researchers investigated the association between PTB and subgingival bacteria. [14] Other investigators assessed the decrease in the risk of PTB after treatment of periodontitis. [15] The identification of some subgingival bacteria in pregnant women can be used to predict PTB. Then, pregnant women who are at increased risk of PTB may be closely monitored and supportive measures can be provided to them.

Existing methods that are used to predict PTB include fetal fibronectin, fetal breathing movements and transvaginal sonographic cervical length. [16] Some studies reported that maternal salivary cortisol can also be used to predict PTB. [17] Other studies depended on markers such as neutrophil to lymphocyte ratio [18] and dual biomarker model of albumin/ vitamin D- binding protein. [19] Assessing the presence of specific subgingival bacteria has the advantages of being non-labor intensive, non-invasive and not requiring the use of sophisticated technology and may thus be more tolerable to pregnant women than these other methods. Studies assessing the ability of subgingival bacteria to predict PTB by measuring their sensitivity and specificity are rather limited.

The aim of the present study was to assess the accuracy of subgingival bacteria that can be identified by readily available lab techniques in predicting PTB in a group of pregnant women in the Eastern Province of Saudi Arabia. The hypothesis of the study is that some gram positive and gram negative subgingival bacteria are associated with PTB and have sensitivity or specificity ≥95% to predict PTB.

Methods

The study protocol was approved by the Ethics Committee of the Deanship of Scientific Research, University of Dammam (#2013145) and by the Institutional Review Board of King Fahad Hospital, University of Dammam, Saudi Arabia (KFHU-EXEPD0058). Written informed consent was obtained from each subject before joining the study and the Helsinki declaration guidelines were followed.

Study Design

A prospective cohort study design was used where pregnant women were assessed for predictor variables (subgingival bacteria) in their 2nd trimester and followed till the end of pregnancy to assess the outcome (full/ preterm birth).

Setting

The study was conducted in Dammam, Saudi Arabia. Pregnant women were recruited from those visiting the Obstetrics Department of King Fahad Hospital in the period April 2013 to April 2014. The bacteriologic samples were analyzed in the Microbiology Diagnostic Laboratory, King Fahad Hospital.

Sample size

Sample size was calculated using the nomogram developed by Carley et al [20] based on the following assumptions: prevalence of PTB = 0.45 (personal communication with Head of Obstetrics Department), sensitivity= 0.95, confidence interval= 0.05. The sample size was calculated to be 170 subjects.

Inclusion Criteria

Participants were included in the study based on the following criteria: (1) being Saudis, (2) being non-smokers, (3) being free from systemic diseases, (4) having singleton gestation, (5) having history of PTB and (6) not using systemic antibiotics or non-steroidal anti-inflammatory drugs in the last 3 months before the study. After inclusion in the study and assessment of predictors (subgingival bacteria), subjects were followed till the end of their pregnancy as part of the routine care given to them. There was no indication of any intervention that might affect the risk of PTB. The following clinical parameters were measured: plaque index of Silness and Loe, [21] gingival index of Loe and Silness, [22] probing pocket depth and clinical attachment loss. [23] To reduce examiner variability, only one examiner, a periodontist, conducted the examination. The examiner made duplicate examinations of ten patients not included in the study at the beginning and midway during the study to ensure reliability. The intra examiner reliability measured by Kappa statistic was 0.87 and 0.8 for pocket depth and attachment loss in the beginning and 0.82 and 0.85 midway.

Variables

Outcome: preterm birth status

Study subjects were followed till the end of their term. Each woman was classified according to time of delivery into (1) full term birth (FTB), delivering live infants after 37 weeks gestation or (2) preterm birth (PTB), delivering live infants with idiopathic preterm birth after 28–36 weeks of gestation.

Predictors: subgingival bacteria

Plaque samples collection: A dentist collected subgingival plaque samples from the study subjects when they were in their 2nd trimester (6th month) using a standardized sterile paper strip #30. After isolation of teeth with cotton, the strip was inserted into the gingival crevice of each tooth and moved to collect plaque around the tooth. At least two anterior and two posterior teeth from each arch were sampled. Each strip was immediately inserted into a vial containing 0.5ml of reduced transport fluid (thioglycollate broth). [24] The vials were flooded with nitrogen and transported to the microbiology laboratory.

Culture

Samples were cultured on anaerobic modified Brucella agar AMBA (SPML, Saudi Arabia) and incubated anaerobically for 72 hours at 37°C. The remainder of the thioglycollate broth was also incubated in an anaerobic chamber and in case there was no growth from the plates at 72 hours, it was subcultured for organism isolation and identification.

Identification

Any potential anaerobe was confirmed using Gram stain reaction, the organism morphology and its aerotolerance. This was followed by identification using the VITEK2 automated system (Biomerieux, France) using sealed disposable ANC and Coryneform card #21347. Inoculation, reading, and interpretation of VITEK2 panels were performed according to the manufacturer's instructions. [25] The bacteriologist recorded the types of bacteria identified in an Excel sheet containing only the patient ID. Findings were recorded directly after sample collection (also in the 2nd trimester).

Data Analysis

Sample description included comparison of the PTB and FTB women according to age, plaque, gingival indices, probing pocket depths and clinical attachment loss using t test. Bacteria were grouped into (1) gram positive (Actinomyces meyeri, Clostridium bifermentans, Clostridium clostridioforme, Clostridium perfringens, Clostridium subterminate, Eggerthella lenta, Peptoniphilus asaccharolyticus or Peptostreptococcus anaerobius) or (2) gram negative subgingival bacteria (Prevotella melaninogenica, Prevotella oralis or Veillonella parvula) and the prevalence of each was calculated.

Univariate logistic regression models were developed to assess the association between

the dependent variable (PTB) and the independent variables (various subgingival bacteria) and the models were adjusted for age. Sensitivity, specificity, positive and negative likelihood ratios were calculated as measures of accuracy to predict PTB. The following cut points were used for the interpretation of the likelihood ratios: [26]

LR-: Decrease in PTB likelihood	No change in PTB likelihood	LR+: Increase in PTB likelihood
<0.1: large and conclusive		>10: large and conclusive
0.1- <0.2: moderate	1	>5-10: moderate
0.2- <0.5: small		>2-5: small
0.5-<1: minimal		>1-2: minimal

Data was analyzed using SPSS version 17.0 (SPSS, Chicago, III). MedCalc (MedCalc Software bvba, Belgium) was used to calculate sensitivity, specificity and likelihood ratios. The level of significance was set at P≤ 0.05.

Results

Data about delivery status was obtained from 94 women out of 170 (55.3%). The remaining 76 women were lost to show-up for examination at the expected time of delivery during the study period. Of those, 22 had PTB (23.4%) and 72 carried their infants to full term. The mean (SD) age of women with PTB was 30.2 (2.6) years and that of FTB women was 32.1 (2.1) years with a statistically significant difference between both groups (P= 0.001). There were no differences between the two groups in plaque or gingival indices (2.3 compared to 1.4 and 1.3 compared to 1.2, P= 0.07 and 0.86). Women with PTB had more severe periodontal disease than those with FTB (probing pocket depth= 6.0 and 2.4, clinical attachment loss= 1.9 and 1.6, P<0.0001 and 0.003) (Table 1).

Table 1: Distribution of age, gingival index, plaque index, probing pocket depth, clinical attachment loss in the study groups

	PTB (n= 22)	FTB (n= 72)	P value
Age in years	30.2 (2.6)	32.1 (2.1)	0.004*
Plaque index	2.3 (1.6)	1.4 (2.1)	0.07
Gingival index	1.3 (2.4)	1.2 (2.3)	0.86
Probing pocket depth	6.0 (0.4)	2.4 (0.3)	<0.0001*
Clinical attachment loss	1.9 (0.3)	1.6 (0.4)	0.003*

PTB: preterm birth, FTB: full term birth, *: statistically significant at P≤ 0.05

Figure 1 shows the prevalence of various subgingival bacteria identified in the subgingival plaque of the study subjects. The most prevalent gram positive organisms were Clostridium bifermentans (14 cases, 14.9%), Peptoniphilus asaccarolyticus (8 cases, 8.5%) followed by Clostridium clostridioforme and Clostridium perfringens (6 cases, 6.4% for each). The most frequently isolated gram negative bacteria were Prevotella melaninogenica (7 cases, 7.4%). In general, these gram positive bacteria were identified in samples from 35 (37.2%) of women whereas gram negative bacteria were identified in 12 (12.8%).
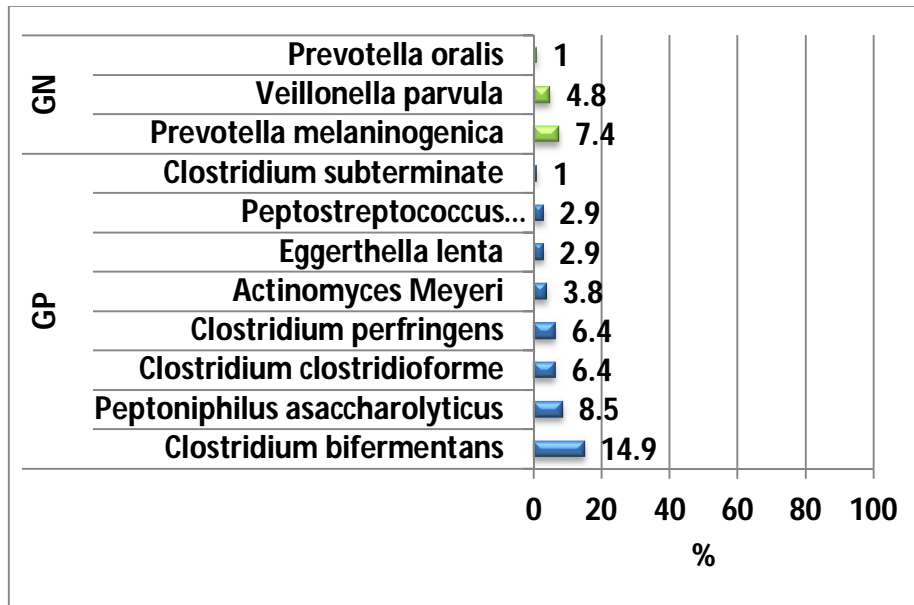
Figure 1: Subgingival bacteria identified in plaque of the study subjects (GP: gram positive, GN: gram negative)

Table 2 shows the association between various subgingival bacteria and PTB using age-adjusted regression models. Among the three identified gram negative bacteria, Veillonella parvula was significantly associated with higher odds of PTB (odds ratio= 11.2) although the estimate was not precise (95% confidence interval= 1.1, 115.0).

Among the eight gram positive bacteria, Actinomyces meyeri and Clostridium bifermentans were significantly associated with higher odds of PTB (11.2 and 5.1), with the estimate of Clostridium bifermentans showing greater precision (95% confidence interval = 1.5, 17.5).

Table 2: Logistic regression models for the association of subgingival bacteria with PTB

	Wald X^2	P value	OR¶	95% C.I.
Gram negative				
Prevotella oralis	0	1.00	55.7	0, -
Veillonella parvula	4.1	0.04*	11.2	1.1, 115.0
Prevotella melaninogenica	0	0.99	-	-
Gram positive				
Clostridium subterminate	0	1.00	55.4	0, -
Peptostreptococcus anaerobius	0	0.99	0	0, -
Eggerthella lenta	0.7	0.40	3.4	0.2, 56.4
Actinomyces meyeri	4.2	0.04*	11.2	1.1, 114.0
Clostridium perfringens	0.3	0.56	0.5	0.1, 4.6
Clostridium clostridioforme	1.5	0.22	2.7	0.6, 13.1
Peptoniphilus asaccharolticus	0. 8	0.38	0.4	0.1, 3.2
Clostridium bifermentans	6.8	0.009*	5.1	1.5, 17.5

OR: odds ratio adjusted for age, 95% C.I.: 95% confidence interval, *: Statistically significant at P≤ 0.05

Table 3 shows the sensitivity, specificity and likelihood ratios of various subgingival bacteria. All gram negative subgingival bacteria identified in this study had high sensitivity (≥95%); Prevotella oralis, Veillonella parvulis and Prevotella melaninogenica in addition to 4 gram positive bacteria; Clostridium subterminate, Eggerthella lenta, Actinomyces meyeri and Clostridium clostridioforme.

None of the gram negative bacteria had specificity >95% whereas 3 of the gram positive bacteria had specificity >95% although their sensitivity was low; Peptostreptococcus anaerobius, Clostridium perfringens and Peptoniphilus asaccharolyticus.

Four out of the 7 subgingival bacteria with sensitivity >95% had LR- ≤0.1; Prevotella oralis, Prevotella melaninogenica, Clostridium subterminate and Actinomyces meyeri. None of the 3 subgingival bacteria with specificity >95% had LR+ >5 and only one had LR+>2 (Peptoniphilus asaccharolyticus). Clostridium bifermentas which had the most precise estimate of association with PTB had a sensitivity of 91.2% and specificity of 34.8%.

Table 3: Sensitivity, specificity and positive and negative likelihood ratios of subgingival anaerobes in prediction of PTB

Subgingival bacteria	N: FTB/ PTB (72/22)	Sn (95% C.I.)	Sp (95% C.I.)	LR+	LR-
Gram negative bacteria					
Prevotella oralis	0/ 1	100 (95.4- 100)	4.3 (0.7- 22)	1.1	0
Veillonella parvula	1/ 3	97.5 (91.2- 99.6)	13 (2.9- 33.6)	1.1	0.2
Prevotella melaninogenica	0/ 8	100 (95.4- 100)	34.8 (16.4- 57.3)	1.5	0
Gram positive bacteria					
Clostridium subterminate	0/ 1	100 (95.4- 100)	4.3 (0.7- 22)	1.1	0
Peptostreptococcus anaerobius	3/ 0	3.7 (0.8- 10.6)	100 (85- 100)	-	0.9
Eggerthella lenta	1/ 1	97.5 (91.2- 99.6)	4.3 (0.7- 22)	1.0	0.6
Actinomyces meyeri	1/ 3	98.7 (93.2- 99.8)	13 (2.9- 33.6)	1.1	0.1
Clostridium perfringens	6/ 1	7.5 (2.8- 15.6)	95.7 (78- 99.3)	1.7	0.9
Clostridium clostridioforme	4/ 3	95 (87.7- 98.6)	13 (2.9- 33.6)	1.1	0.4
Peptoniphilus asaccharolyticus	8/ 1	10 (4.4- 18.8)	95.7 (78- 99.3)	2.3	0.9
Clostridium bifermentans	6/ 7	91.2 (82.8- 96.4)	34.8 (16.4- 57.3)	1.4	0.3

FTB: full term birth, PTB: preterm birth, Sn: Sensitivity, Sp: Specificity, C.I.: confidence interval, LR+: positive likelihood ratio, LR-: negative likelihood ratio

Discussion

The results of the present study support the hypothesis that some subgingival bacteria are associated with PTB and that they have high sensitivity and/ or specificity to predict it. In the present study, and based on the indices measured, PTB women had more severe periodontal disease. [27] Previous studies also reported more severe periodontal diseases among women with PTB. [28- 31] Infection, as in the case of periodontitis, is associated with PTB because of the presence of less "optimal" bacteria.

Among all the subjects included in the present study, the most prevalent subgingival bacteria were Clostridium bifermentans, followed by Peptoniphilus asaccharolyticus and Prevotella melaninogenica. In two studies including subjects from different countries, Haffajee et al [32, 33] reported that there were differences in the prevalence of subgingival bacterial species by country. For example, Prevotella melaninogenica was identified in about 6% of Chilean and Swedish subjects but not in subjects from other countries. Also, Veillonella parvula was detected in higher percentage among Americans in comparison to Swedish subjects (7.0% and 4.5%). Another study reported that subgingival bacterial profile changes over the course of pregnancy as well as after delivery. [34]

The role of the gram negative subgingival anaerobes in the causation of PTB has been confirmed by others. [9] Most studies, however, focused on what Socransky et al described as the orange complex (Peptostreptococcus micros, Prevotella nigrescence, Fusobacterium nucleatum and Prevotella intermedia) and red complex (Campylobacter rectus, Tannerella forsythia, Treponema denticola, Porphyromonas gingivalis), [11, 35] none of which were identified in the present study. These organisms could either be totally absent among the study subjects or could be incorrectly missed by the ANC card of Vitek2.

The sensitivity of values of Prevotella oralis, Veillonella parvula, Prevotella melaninogenica, Clostridium subterminate, Eggerthella lenta and Actinomyces meyeri were ≥95%. These high sensitivity values are useful to rule out conditions when the test results are negative. [36] The negative likelihood ratios are helpful in demonstrating the usefulness of the studied bacteria. These likelihood ratios do not change when the prevalence of PTB changes so they can support the applicability of the test (subgingival bacteria identification) in different settings. For example, the negative likelihood ratios of Prevotella oralis, Prevotella melaninogenica, Clostridium subterminate and Actinomyces meyeri were all ≤0.1 which means that their absence indicates large and conclusive decrease in the likelihood of PTB. [37] It is interesting that among the three bacteria that were associated with significantly higher odds of PTB and though the most prevalent, the accuracy of Clostridium

bifermentas was not high enough to usefully predict PTB. Veillonella parvula and Actinomyces meyeri had similarly high sensitivity although the LR- of Actinomyces meyeri was lower than that of Veillonella parvula indicating the former's greater accuracy to rule out disease. This emphasizes that the presence of association between some subgingival bacteria and PTB does not necessarily indicate that these bacteria would be useful in predicting PTB and that it is important to use sensitivity, specificity and likelihood ratios to decide the usefulness of certain markers/ tests for prediction of disease.

Because of the generally lower specificity of most microorganisms in the present study, the identification of these subgingival bacteria would not have a great "rule in" value. The highest positive likelihood ratio was calculated for Peptoniphilus asaccharolyticus (LR+= 2.3) signifying only a small increase in the likelihood of the disease when the test is positive and the organism is detected. [37] These bacteria with LR- ≤ 0.1 can rule out PTB if they are absent in plaque during the 2nd trimester to a greater extent than the ability of any of the subgingival bacteria to rule in disease if they are present.

Boots et al [16] reported in a meta-analysis of 72 studies the accuracy of methods used to predict PTB in the short term. They found that the sensitivity of transvaginal sonographic cervical length (TVS CL), fetal breathing movement (FBM) and fetal fibronectin (fFN) was 77%, 75% and 62% with specificity= 88%, 93% and 81% respectively. The highest positive likelihood ratio was for FBM (LR+= 10.4) and the lowest negative likelihood ratio was for TVS CL (LR-= 0.26). If the aim is to rule out suspected cases, the reported values for negative LRs, within the confines of the small sample size in our study, show promising results that may be superior to TVS CL.

In view of the small number of subjects with PTB in our study, our results should be interpreted with caution and it should be considered as pilot for larger future studies. We did not assess the effect of alcohol as confounder since cultural and religious factors prohibit its consumption and would interfere with valid reporting of its use if this occurred. One limitation of the study is the small sample size due to the loss of subject to follow up where data was available for only 55% of those

initially examined. Whereas this represents a considerable loss, the association between predictor(s) and outcome was statistically and clinically significant so that type 2 error is not a concern in the study. Further studies including subjects from other countries and women at different stages of pregnancy are needed before our findings can be generalized to other populations. These promising findings pave the way for further studies including larger samples to assess the sensitivity and specificity of the subgingival bacteria identified in our study. It would be interesting to investigate in future studies the impact of periodontal disease severity on the association between these subgingival bacteria and PTB and whether controlling the number/ occurrence of these bacteria may potentially decrease the risk of PTB. In addition, another direction for research may include comparison of the accuracies of subgingival bacterial screening with other tests for predicting PTB.

Conclusion

Subgingival bacteria identification offers a relatively inexpensive and fast lab technique for predicting PTB. In our study, the most prevalent microorganism were Clostridium bifermentans followed by P.asacchrolyticus and P.melaninogenica. The negative likelihood ratios of the studied microorganism shows promising accuracy to rule out PTB in comparison to other methods used to predict pregnancy outcomes. Further studies including a larger sample size are needed to confirm the findings of the present study.

References:
1. Institute of Medicine (US) Committee on Understanding Premature Birth and Assuring Healthy Outcomes; Behrman RE, Butler AS, editors. Preterm Birth: Causes, Consequences, and Prevention. Washington (DC): National Academies Press (US); 2007.
2. Scannapieco FA. Systemic effects of periodontal diseases. Dent Clin North Am. 2005;49(3):533-50.
3. Romero R, Gotsch F, Pineles B, Kusanovic JP. Inflammation in pregnancy: its role in reproductive physiology, obstetrical complications, and fetal injury. Nutr Rev. 2007; 65: 194-202.
4. Durand R, Gunselman EL, Hodges JS, Diangelis AJ, Michalowicz BS. A pilot study of the association between cariogenic oral bacteria and preterm birth. Oral Dis. 2009;15(6):400-6.
5. Dasanayake AP, Li Y, Wiener H, Ruby JD, Lee MJ. Salivary Actinomyces naeslundii genospecies 2 and Lactobacillus casei levels predict pregnancy outcomes. J Periodontol. 2005;76(2):171-7.
6. Lin D, Moss K, Beck J, Hefti A, Offenbacher S. Persistently high levels of periodontal pathogens associated with preterm pregnancy outcome. J Periodontol. 2007; 78:833-41.
7. Katz J, Chegini N, Shiverick KT, Lamont RJ. Localization of P. gingivalis in preterm delivery placenta. J Dent Res. 2009; 88(6):575-8.
8. Ryu JI, Oh K, Yang H, Choi BK, Ha JE, Jin BH et al. Health behaviors, periodontal conditions, and periodontal pathogens in spontaneous preterm birth: a case-control study in Korea. J Periodontol. 2010; 81(6):855-63
9. Africa CW. Oral colonization of Gram-negative anaerobes as a risk factor for preterm delivery. Virulence 2011; 2(6):498-508.
10. Santa Cruz I, Herrera D, Martin C, Herrero A, Sanz M. Association between periodontal status and pre-term and/or low-birth weight in Spain: clinical and microbiological parameters. J Periodontal Res. 2013; 48(4):443-51.
11. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998;25:134–144.
12. Novak MJ, Novak KF, Hodges JS, Kirakodu S, Govindaswami M, Diangelis A, et al. Periodontal bacterial profiles in pregnant women: response to treatment and associations with birth outcomes in the obstetrics and periodontal therapy (OPT) study. J Periodontol. 2008; 79(10):1870-9.
13. Madianos PN, Lieff S, Murtha AP, Boggess KA, Auten RL Jr, Beck JD et al. Maternal periodontitis and prematurity

II: Maternal infection and foetal exposure. Ann Periodontol. 2001; 6:175-82.

14. Vettore MV, Lea˜o AT, LealMdo C, Feres M, Sheiham A. The relationship between periodontal disease and preterm low birthweight: clinical and microbiological results. J Periodont Res. 2008; 43: 615–626.

15. Pirie M, Linden G, Irwin C. Intrapregnancy non-surgical periodontal treatment and pregnancy outcome: a randomized controlled trial. J Periodontol. 2013; 84(10): 1391-400.

16. Boots AB, Sanchez-Ramos L, Bowers DM, Kaunitz AM, Zamora J, Schlattmann P. The short-term prediction of preterm birth: systematic review and diagnostic metaanalysis. Am J Obstet Gynecol. 2014; 210(1):54.e1-54.e10.

17. Soghra K, Zohreh S, Kobra AK, Reza MM. Single measurement of salivary estriol as a predictor of preterm birth. Pak J Biol Sci. 2014 May;17(5):730-4.

18. Kim MA, Lee YS, Seo K. Assessment of predictive markers for placental inflammatory response in preterm births. PLoS One. 2014 Oct 7;9(10):e107880.

19. Liong S, Di Quinzio MK, Fleming G, Permezel M, Rice GE, Georgiou HM. New biomarkers for the prediction of spontaneous preterm labour in symptomatic pregnant women: a comparison with fetal fibronectin. BJOG. 2015 Feb;122(3):370-9.

20. Carley S, Dosman S, Jones SR, Harrison M. Simple nomograms to calculate sample size in diagnostic studies. Emerg Med J. 2005; 22(3):180-1.

21. Silness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand. 1964; 22: 121-135

22. Löe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand. 1963; 21:533-551.

23. Ramfjord SP. The periodontal disease index (PDI). J Periodontol. 1967; 38: 602-610.

24. Ronald MA. Handbook of microbiological media, 2nd ed., New York/Tokyo: Boca Raton; 1997.

25. Schreckenberger PC, Celig DM, Janda WM. Clinical evaluation of the VITEK ANI card for identification of anaerobic bacteria. J Clin Microbiol. 1988; 26:225–230.

26. Ebell M, Barry H. Evidence- Based Medicine Course, College of Human Medicine, Michigan State University. Available at: http://omerad.msu.edu/ebm/Diagnosis/Diagnosis6.html

27. Alleman BW. Preterm birth: prediction, prevention, care. University of Iowa, 2014. Dissertation. Available at: http://ir.uiowa.edu/etd/4563.

28. Alchalabi HA, Al Habashneh R, Jabali OA, Khader YS. Association between periodontal disease and adverse pregnancy outcomes in a cohort of pregnant women in Jordan. Clin Exp Obstet Gynecol. 2013;40(3):399-402.

29. Baig SA, Khan N, Baqai T, Fatima A, Karim SA, Aziz S. Preterm birth and its associated risk factors. A study at tertiary care hospitals of Karachi, Pakistan. J Pak Med Assoc. 2013 Mar;63(3):414-8.

30. Al Habashneh R, Khader YS, Jabali OA, Alchalabi H. Prediction of preterm and low birth weight delivery by maternal periodontal parameters: receiver operating characteristic (ROC) curve analysis. Matern Child Health J. 2013 Feb;17(2):299-306.

31. Baskaradoss JK, Geevarghese A, Kutty VR. Maternal periodontal status and preterm delivery: a hospital based case-control study. J Periodontal Res. 2011 Oct;46(5):542-9.

32. Haffajee AD, Bogren A, Hasturk H, Feres M, Lopez NJ, Socransky SS. Subgingival microbiota of chronic periodontitis subjects from different geographic locations. J Clin Periodontol. 2004; 31(11):996-1002.

33. Haffajee AD, Japlit M, Bogren A, Kent Jr RL, Goodson JM, Socransky SS. Differences in the subgingival microbiota of Swedish and USA subjects who were periodontally healthy or exhibited minimal periodontal disease. J Clin Periodontol. 2005; 32: 33–39.

34. Adriaens LM, Alessandri R, Spörri S, Lang NP, Persson GR. Does pregnancy have an impact on the subgingival microbiota? J Periodontol. 2009; 80(1):72-81.

35. Davenport ES. Systemic disease and oral bacteria: Preterm low birth weight and the

role of oral bacteria. J Oral Microbiol. 2010; 2:5779.

36. Sackett D, Haynes R, Guyatt G, Tugwell P. Clinical Epidemiology: A Basic Science for Clinical Medicine. Boston, MA: Little Brown; 1992.

37. McGee S. Simplifying likelihood ratios. J Gen Intern Med. 2002; 17(8):646-9