

The Prevalence and Association of Preterm Birth with Periodontal Disease in the Eastern Province of Saudi Arabia: Microbiological and Immunochemistry Evaluations

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Abstract

Background: Recent evidence suggests that periodontal disease may be a risk factor for both premature and low-birth weight babies. This study was designed to evaluate the nature of the relationship and the prevalence of preterm birth and periodontal disease in a sample population of women from the Eastern Province of Saudi Arabia. **Methods:** A total of 196 patients were divided into two groups. Group I (Test) included 80 women between 28–36 weeks of gestation with idiopathic preterm labor, who later delivered live infants whose birth weighing <2500g. Group II (Control) included 116 women with gestation periods ≥ 37 weeks delivered live infants weighing ≥ 2500 g. Microbiological analyses made and interleukin 6 (IL-6) levels were measured in the gingival cervicular fluid using a Vitek 2 automated bacterial identification system and an enzyme-linked immunosorbent assay (ELISA) for all patients. **Results:** The following data were obtained: Plaque index, probing pocket depth and clinical attachment level revealed a statistically significant difference between the two groups ($P < 0.05$) except for the gingival index (GI) and bleeding on probing (BoP) ($P > 0.05$). There were increased levels of IL-6 in the gingival crevicular fluid as well as increased Gram-negative bacteria in the preterm birth compared to the full term pregnancies. **Conclusion:** The prevalence of preterm birth was 8.2% in the test population from the Eastern Province of Saudi Arabia. Additionally, there was a correlation between maternal periodontal disease and the preterm birth among Saudi mothers in the Eastern Province suggesting that periodontal disease may be regarded as a true risk factor for preterm birth infants.

Key Words Preterm Birth, Periodontal diseases, IL-6, Periodontopathogens

Introduction

Periodontitis is considered a continuous pathogenic and inflammatory oral challenge at a systemic level, due to ulceration of the sulcular epithelium surface in the periodontal pockets and the lack of balance in the resulting immune response. As a result, microorganisms and their by-products are able to reach the other parts of the organism, creating lesions at different levels [1]. Moreover, some bacterial species, such as *Aggregatibacter actinomycetemcomitans* and *Porphyromona gingivalis*, can invade cells and tissues directly. Of particular concern is the fact that periodontopathogens can be introduced into the blood stream and cause infections after colonizing sites in the body remote from the oral cavity, a process known as “bacterial translocation” that is responsible for metastatic infections) [2].

Recent studies suggest that microorganisms are capable of entering the uterine cavity via blood spread [3, 4]. Periodontal disease, especially those forms that include a chronic low-grade infection, has been identified as a contributing factor to preterm birth. In addition, pregnant patients with periodontal disease and microbial vaginosis also share similar social and socio-demographic risk factors, suggesting a common pathophysiology [3]. Moreover, periodontal disease and changes of the microbial oral flora have been linked to preterm birth via the production of pro-inflammatory cytokines in the uterus [4].

In a 2006 study, Offenbacher et al. [5] reported a preterm birth incidence rate of 28.6% in women with moderate to severe periodontal disease compared to 11.2% preterm birth rate among women without periodontal diseases who maintained good oral health during their pregnancy. In a prior study, Jeffcoat et al., [6] reported that there was association between the advanced periodontal diseases and the preterm birth, with an odds ratio of 4.45 for preterm delivery less than 37 weeks, increasing to an odds ratio of 7.07 for delivery before 32 weeks gestation.

It is generally accepted that the treatment of periodontal diseases can reduce the preterm birth ratio [7,8]. On this very point; Offenbacher et al. [7] evaluated the effects of non-surgical periodontal therapy of periodontal disease on preterm birth rates. They found a minimal improvement and no statistical difference when comparing the 13.1% preterm birth rate benefit with gestational ages at delivery of less than 37, 35 or 32 weeks to a preterm birth rate of just 11.5% in non-treated patients. Moreover, George et al. [8] demonstrated that periodontal treatment for pregnant patients prior to labor significantly lowered the preterm births and low birth weight ratio. Therefore, the aim of the present study was to determine the prevalence of preterm births and their possible relationship with periodontal disease in a test population of patients from the Eastern Province of Saudi Arabia.

Material and Methods

The study population consisted of women who gave birth at the King Fahad Hospital between April 2013 and April 2014.

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This study included 196 pregnant women with a single gestation recruited from among patients admitted to the labor suite of Obstetrics Department of King Fahad Hospital, University of Dammam, Saudi Arabia. The enrolled women who volunteered and were selected for inclusion in this study had to present in the first stage of labor with intact membranes. A total of 80 subjects had definite preterm labor compared to 116 who experienced full-term labor. The patients were then assigned to one of two groups based on their gestation period:

Test Group (Group I): This group included 80 parturient women between 28–36 weeks of gestation with idiopathic preterm labor, who subsequently delivered live infants with a birth weight of less than 2500 g.

Control Group (Group II): This group consisted of 116 parturient women with a gestation ≥ 37 week who later delivered live infants weighting ≥ 2500 g.

To be selected for this study patients had to meet all of the following criteria: 1.) free from any systemic diseases, 2.) non-smoker, and 3.) no use of systemic antibiotics or non-steroidal anti-inflammatory medications within the 3-months period prior to the start of the study. Conversely, the exclusion criteria included the following: 1.) patients whose baby was stillborn, 2.) deliveries involving induced labor, and 3.) mothers who elected not to participate in the study.

The research protocol was reviewed and approved by the Ethical Committee of the Deanship Scientific Research, University of Dammam and supported under Grant No. 2013145. This study also was approved by Institutional Review Board, King Fahad Hospital, University of Dammam, Saudi Arabia (KFHU-EXEPD0058). Following approval, a written informed consent was obtained from each patient in order for any patient to participate in this study.

The following clinical examinations were performed by a single, calibrated, examiner using the UNC-15 periodontal probe (Hu-Friedy, USA); plaque index (PI) [9], gingival index (GI) [10], pocket probing depth (PPD), and clinical attachment level (CAL) [11] as well as bleeding on probing (BoP) [12].

Microbiological Analysis

Sample collection

Subgingival plaque and gingival crevicular fluid (GCF) each were collected with an individual standardized sterile paper strip #30. After isolation of the teeth with cotton, a strip was inserted into the gingival crevice of mesial and distal of different areas (1mm deep) of the most severe sites in each patient and left in situ for 10 seconds. After the collection, each microbial sample was placed immediately into a vial containing 0.5 ml of reduced transport fluid (Thioglycollate broth) [13]. The vials were flooded with nitrogen and transported to the Microbiology Diagnostic Laboratory, King Fahad Hospital, University of Dammam for analysis within 40 minutes of the samples collection.

Sample processing and bacterial identification

The samples, transported in thioglycollate broth, were subcultured on anaerobic modified Brucella agar AMBA (SPML, Saudi Arabia) and incubated anaerobically for 72 hours at 37°C without opening. The remainder of the thioglycollate broth was also incubated in an anaerobic chamber. In cases where there was no growth from the plates at the 72-hour point, samples were subcultured for organism isolation and identification. Any potential anaerobe was confirmed using Gram stain reaction, the organism morphology, and its aerotolerance followed by identification by the VITEK 2 automated system (Biomerieux, France) using sealed disposable ANC and Coryneform cards number 21347. Inoculation, reading, and interpretation of VITEK 2 panels were performed according to the manufacturer's instructions (Figure 1). The VITEK 2 instrument identifies bacterial growth based on kinetic analysis of the fluorescence, turbidity, and colorimetric tests for enzymatic activities, carbon source, and antibiotic susceptibility. The inoculum prepared for card inoculation was a bacterial suspension growth on AMBA adjusted to a McFarland turbidity standard of 3.0 in sterile saline. The 30-well cards with chromogenic substrates and modified conventional tests were automatically filled by a vacuum device, sealed, and then loaded into the VITEK 2 instrument. The card was subjected to a kinetic fluorescence measurement every 15 minutes by the VITEK 2 reader-incubator module, and the results were interpreted by the ID-GPC associated computer using VITEK AES software (Version 6.01) through comparison of the biochemical profile of the strains tested against the biochemical profiles of the strains present in the software database [14]. All cards used were automatically discarded into a waste container. Any anaerobe isolated was then stored in an Eppendorf tube with 1.0 ml Brain heart infusion with 12% glycerol at -80°C.

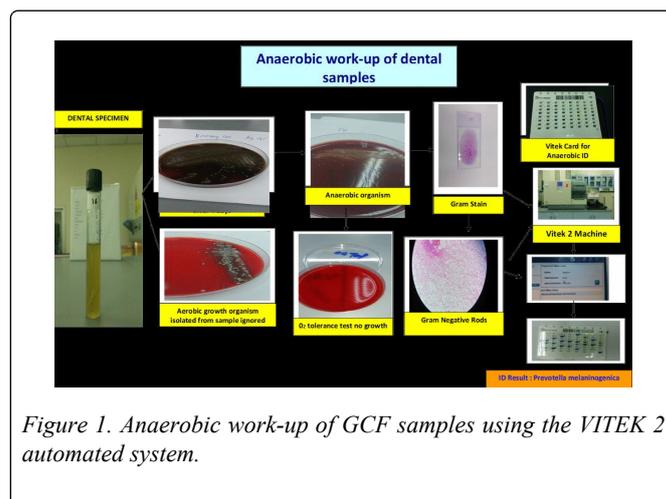


Figure 1. Anaerobic work-up of GCF samples using the VITEK 2 automated system.

Quality control and storage of the isolates

The isolates and super master stock of control ATCC strains were stored in cryovials containing Brain Heart Infusion (BHI) broth with 12% glycerol (SPML, Saudi Arabia) at -80 °C. As recommended by Clinical and Laboratory Standards Institute (CLSI), *B. fragilis* ATCC 25285 was used as the quality control (QC) strain. The strains were taken out and the cryovial and allowed to thaw slightly at room temperature before preparing a subculture with an aseptic technique onto

AMBA incubated anaerobically at 37°C. After 48 hours of incubation, the culture plates were examined to determine if the morphological characteristics were comparable to the desired organism and also to look for the presence of pure growth. Purity plates were included from each bacterial suspension for bacterial identification by VITEK 2.

Immunochemical analysis

For IL-6 analysis, GCF was collected from the mesiobuccal sulcus at three sites (two anterior regions and one posterior region) of the mouth, using Harco Periopaper (Harco, Irvine, CA, USA). Each strip was measured for fluid volume with a calibrated Periotron 8000 (Harco) [15] and placed in a sterile Eppendorf tube. GCF sample were then used to measure IL-6 using enzyme-linked immunosorbent assays (ELISA, BD OptEIA, BD Biosciences, San Diego, US).

Statistical analyses

Data was analyzed using SPSS version 16.0 (SPSS, Chicago, III). Descriptive statistics were generated for all variables. The baseline values to assess the homogeneity of the groups (excluding the relative values) consisted of using the unpaired t-test for each of the tests used. The paired t-test was used for the statistical evaluation of changes in each group. For comparisons between groups, the unpaired t-test was performed. Binary univariate logistic regression models were developed to assess the association between PTB as a dependent variable and independent variables which were the bacterial species that showed significant difference between the two groups in the X2 comparison. The level of significance was established at $P \leq 0.05$.

Results

The prevalence of preterm birth was 8.2% (101) for the 1238 singleton live births that admitted to the labor suite of Obstetrics Department of King Fahad Hospital, University of Dammam, Saudi Arabia during the period of this study (April, 2013 to April, 2014).

A total of 196 subjects, 80 in Group I (Test) and 116 in Group II (Controls) enrolled in the study, the gestational age of the preterm birth (tests) ranged from 28 to 36 weeks, with a mean of 33.4 ± 3.2 weeks, while the gestational age of the term birth (Group II controls) varied from 37 to 42 weeks, with a mean of 39.3 ± 2.3 weeks. In addition, the entire test group had a history of a previous preterm birth.

The results of the present study demonstrate that the Gingival Index (Group I, 1.3 ± 2.4 ; Group II controls, 1.2 ± 2.3), bleeding on probing (controls 34.3 ± 22.1 ; test 36.4 ± 21.3), plaque index (test, 2.3 ± 1.6 ; controls 1.4 ± 2.1), probing pocket depth (test, 4.6 ± 0.4 ; controls, 2.4 ± 0.3) and the clinical attachment level (test, 1.9 ± 0.3 ; controls, 1.6 ± 0.4) revealed a statistically significant difference between the Group I and Group II ($P < 0.05$) except for the Gingival Index and Bleeding on Probing were no significant differences were found ($P > 0.05$). In addition, there was increased level of IL-6 in gingival crevicular fluid, 2.63 ± 0.15 pg/ml for the preterm birth group compared to the control group 1.67 ± 0.21 pg/ml ($p < 0.05$) (Table 1). Moreover, the prevalence of periodontal

disease was 33 (28.5%) in the term patients while 50 (62.5 %) in the preterm patients ($P < 0.05$) (Table 1).

Table 1. Periodontal clinical parameters and IL-6 levels in the gingival cervicular fluid for the full-term and preterm birth groups. GI=Gingival Index, PI=Plaque Index, BoP= Bleeding on Probing, PPD=Probing Pocket Depth, CAL=Clinical Attachment Loss, *=significant difference, NS=Non-significant difference.

	Control	Test	P value
	n(116)	n (80)	
GI, mean (SD)	1.2 ± 2.3	1.3 ± 2.4	.456 NS
PI, mean (SD)	1.4 ± 2.1	2.3 ± 1.6	.001*
% sites with BoP, mean (SD)	34.3 ± 22.1	36.4 ± 21.3	.567 NS
PPD, (mm); mean (SD)	2.4 ± 0.34	6 ± 0.4	.003*
CAL (mm); mean (SD)	1.6 ± 0.4	1.9 ± 0.3	.003*
Prevalent Periodontitis (n) (%)	33 (28.5)	50 (62.5)	.002*
IL-6 (pg/ml)	$.67 \pm 0.21$	2.63 ± 0.15	.0001*

Table 2 shows the comparison of the prevalence of various species of subgingival bacteria between full term and preterm birth groups.

Table 2. Comparison between full-term and preterm birth groups regarding prevalence of subgingival bacteria. *: Statistically significant at $P \leq 0.05$, GPR: Gram positive rods, GPC: Gram positive cocci, GNR: Gram negative rods, GNC: Gram negative cocci.

Periodontal bacteria	Full-term (N= 116)	Preterm birth (N= 80)	P value
Actinomyces meyeri (GPR)	2 (1.7)	6 (7.5)	0.04*
Clostridium bifermentans(GPR)	12 (10.4)	17 (21.3)	0.005*
Clostridium clostridioforme (GPR)	7 (6.03)	6 (7.5)	0.35
Clostridium perfringens (GPR)	10 (8.6)	4 (5)	1.00
Clostridium subterminale(GPR)	0	3 (3.8)	0.23
Eggerthella lenta(GPR)	2 (1.8)	3 (3.8)	0.42
Peptoniphilus Asaccharolyticus(GPC)	13 (11.2)	4 (5)	0.68
Peptostreptococcus anaerobius(GPC)	5 (4.3)	0	1.00
Prevotella melaninogenica(GNR)	0	33 (41.3)	<0.0001*
Prevotellaoralis(GNR)	0	3 (3.8)	0.23
Veillonella(GNC)	2 (1.7)	9 (11.3)	0.04*
Gram negative organism	2 (1.7)	45 (56.3)	<0.0001*
Gram positive organism	51 (43.9)	43 (53.7)	0.02*

Three species were more prevalent in the full term group than in the preterm birth group (*Clostridium perfringens*, *Peptoniphilus asaccharolyticus* and *Peptostreptococcus anaerobius*) although the differences between both groups were not statistically significant ($P = 1.00, 0.68$ and 1.00). In

the remaining species surveyed in the study, the prevalence was higher in the preterm birth than in the full term birth. The difference between the two groups was statistically significant in the case of *Actinomyces meyeri*, *Clostridium bifermentans*, *Prevotella melaninogenica* and *Veillonella* ($P=0.04$, 0.005 , <0.0001 and 0.04). When all Gram negative were considered together, a statistically significant higher prevalence was found in preterm than in full term groups (56.3% and 1.7% , <0.0001). Similarly, when all Gram positive organisms were included, the difference between full and preterm groups was statistically significant (53.7% and 43.9% , $P= 0.02$).

Table 3 shows the association of having Gram positive or Gram negative bacteria in addition to age with preterm birth.

Table 3: Association between age and presence of gram positive and gram negative subgingival bacteria and preterm birth.

Variables	Univariate regression				Multivariate regression			
	Wald X2	P value	OR	CI	Wald X2	P value	OR	CI
Age	6.64	0.01*	0.76	0.62, 0.94	1.70	0.19	0.83	0.63, 1.10
Gram negative organism	15.19	<0.0001*	17.00	8.33, 26.49	15.07	<0.0001*	10.15	9.78, 25.30
Gram positive organism	5.02	0.03*	3.08	1.15, 8.23	4.44	0.04*	4.67	1.11, 19.62

Discussion

The prevalence of preterm birth in this study was 8.2% which is less than that of the global range (16%) [16], but higher than the 4% rate reported by Davenport et al. in East London in 1998 [17]. Moreover, preterm births (PTB) affect almost 12-13% of pregnancies in the United States and 5-9% in Europe and developed countries [18]. These differences may be attributed to the small sample size of the Saudi Arabian population compared to larger sample sizes in other reports.

The present study supports the earlier findings regarding the risk of having preterm birth in mothers with periodontal disease [18, 20-22]. There is highly association between the periodontal diseases and the preterm birth explained by the direct and/or indirect effect of periodontopathogens on the developing fetus. In addition, the biologic mechanism initiating by the Gram-negative bacterial endotoxins present in the periodontal diseases. These gram negative endotoxins can stimulate the production of the pro-inflammatory cytokines and prostaglandin. Some cytokines such as IL-1 β , IL-6, and TNF- α , as well as prostaglandins in appropriate quantities, are able to stimulate labor. However, the presence of Gram-negative bacteria and the elevated IL-6 levels in the preterm birth group compared to the normal pregnant in the present study support this biologic mechanism. Moreover, bacterial identification offers an inexpensive and fast lab technique for predicting PTB. The presence of several cards for the detection of various types of microorganisms helps in screening against a variety of species and decreases the cost thus increasing the efficiency of using subgingival to predict PTB. Women who are at risk who test negative can be ruled out leaving a considerably smaller proportion for monitoring and/ or further testing using more intensive methods.

Patients with a history of a previous preterm birth are considered to be at a higher risk to experience a subsequent preterm birth but the reasons for this remain unknown. In

Higher odds of preterm birth were associated with Gram negative and gram positive bacteria (OR=17.00 and 3.08). when age was considered in conjunction with the presence of Gram negative and Gram positive bacteria in association with preterm birth, only the presence of bacteria showed significant associations ($P< 0.0001$ and 0.04). The presence of Gram negative bacteria was associated with ten times the odds of preterm birth whereas the presence of Gram positive bacteria was associated with about 5 times the odds of preterm birth (OR=10.15 and 4.67).

1991 Steer [23] demonstrated that genitourinary tract infections should be considered as a risk factors preterm birth. But it is generally accepted that a history of a previous preterm birth is the most important predictor of the likelihood of preterm delivery in the index pregnancy of multiparous women [24-26]. The findings of the present study were consistent with the earlier reports demonstrated that an increased risk of preterm birth in multiparous women with previous preterm pregnancy [27-29]. Furthermore, it has been reported that the risk of a preterm delivery increased substantially in women with a history of more than one previous preterm birth [30-31]. That outcome aside, any preterm birth should prompt clinicians to search for other predisposing factors, such as the presence of undiagnosed periodontal diseases.

Importantly, in the present study there were increased IL-6 levels in the crevicular gingival fluid. These finding support the hypotheses of Offenbacher et al., [19] that gram-negative anaerobic periopathogens, their associated endotoxins, and pro-inflammatory mediators can have possible adverse effects on the developing fetus. Moreover, periodontal infections may lead to excessive production of the pro-inflammatory cytokines and prostaglandins, all of which are established biochemical mediators of parturition [32]. However; the observation of the elevated IL-6 levels in the present study was a consistent and reproducible finding in those subjects who experienced a preterm pregnancy.

It has been previously reported in several cohort studies that a positive association exists between the severity of periodontal disease and the incidence of preterm birth. In a study by Lopez et al. [32] the prevalence of preterm birth was 8.6% higher in pregnant with periodontal diseases compared to a prevalence of 2.5% in an otherwise periodontally healthy pregnant group. The preterm prevalence in the study by Jeffcoat et al. [33] was 4.4% for a sample population composed of 1313 primarily black pregnant women with

periodontal disease. The prevalence rose to 7.1% prior to 36 weeks of gestation, and up to 5.3% before the 32-week gestation period. The findings of this study also indicated that there was relationship between the preterm birth and the periodontal diseases.

To confirm the association between the severity of periodontal disease and the incidence of preterm birth, several studies [20, 32, 33] have shown that the basic periodontal treatment significantly reduces the incidence of the preterm birth. In a 2002 study by Lopez et al. [20] a reduction of approximately 30% was noted in the incidence of preterm, low birth weight (LBW) infants to an incidence rate of 13.5% in a group of women who received simple periodontal prophylaxis during pregnancy. These patients were compared with another group of pregnant women who did not receive such treatment before delivery and their preterm incidence rate was higher at 18.9%. A 1999 report of a randomized clinical trial from Chile [31] showed an incidence of preterm low birth weight of 1.8% in women with periodontal diseases who were treated before 28 weeks of gestation compared with a 10.1% incidence of preterm low birth weight in a control group of untreated women.

Conclusions

Based on the results obtained in this study, it can be concluded that:

- The prevalence of periodontal disease among the preterm birth pregnant patient's samples in Eastern Province, Saudi Arabia was high.
- The prevalence of preterm birth in Eastern Province, Saudi Arabia was 8.2%.
- There was a correlation between maternal periodontal disease and preterm birth among Saudi mothers in Eastern Province, Saudi Arabia, suggesting that periodontitis may be regarded as a true risk factor for the preterm birth.

Recommendations

- Additional epidemiological studies are required that involve a larger sample population along with data obtained from numerous hospitals in different areas in Eastern Province of Saudi Arabia.
- In order to confirm a direct association between periodontal disease and preterm birth, additional researches is needed to evaluate the effects of treatment of the periodontal disease on preterm birth rates.
- It is recommended that a periodontal screening and treatment of periodontal disease become a routine part of prenatal care for pregnant women.

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Competing Interests

The authors declare that they have no competing interests.

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