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 *(Figure1)*. 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.

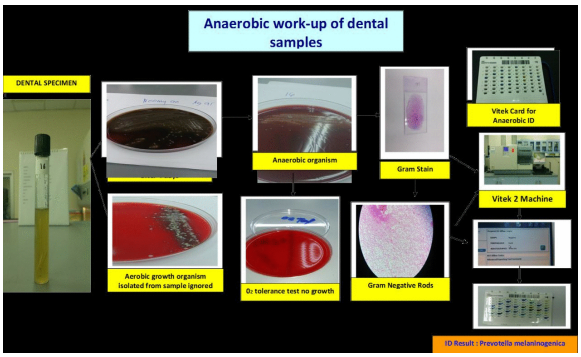

Figure 1. Anaerobic work-up of GCF samples using the VITEK 2 automated system.

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

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. 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).

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 ß, 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 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 exits 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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