Original Article

Porphyromonas gingivalis in dental plaque and serum C-reactive protein levels in pregnancy

Nada M. Souccar[1], Marita Chakhtoura[1], Joseph G. Ghafari[2], Alexander M. Abdelnoor[1]

[1]*Department of Microbiology and Immunology, Faculty of Medicine, American University of Beirut, Lebanon*
[2]*Division of Orthodontics and Dentofacial Orthopedics, Department of Otolaryngology-Head and Neck Surgery, Faculty of Medicine, American University of Beirut, Lebanon*

Abstract

Background: The periodontopathogen *Porphyromonas gingivalis (Pg)* has been reported as a risk factor for preterm labour. Its pathogenesis and role in pregnancy have not been investigated in Lebanon. Elevated C-reactive protein (CRP) levels in pregnant women with periodontitis also appear to mediate preterm labour.

Methodology: The study included 20 pregnant women with periodontitis and 20 with normal periodontium. PCR was done for *Pg* detection in oral plaque and vaginal samples. Serum CRP levels were determined by ELISA.

Results: *Pg* was detected in the oral plaque of 13 of 20 pregnant subjects with clinical periodontitis (patients) and 2 of 20 controls with a healthy periodontium. Vaginal swabs were all *Pg*-negative, ruling out systemic infection. Serum CRP levels were elevated in 12 of 20 patients and 8 of 20 controls. None of the participants experienced preterm labour.

Conclusions: This is the first report that implicates *Pg* in Lebanese periodontitis patients. Preliminary results do not indicate a relationship among *Pg*, periodontitis, CRP levels and preterm labour.

Key words: C-reactive protein, periodontitis, *Porphyromonas gingivalis*, preterm labour

J Infect Dev Ctries 2010; 4(6):362-366.

(Received 15 March 2010 - Accepted 22 March 2010)

Introduction

Porphyromonas gingivalis (Pg), a Gram-negative non motile, asaccharolytic obligate anaerobic cocco-bacillus, is one of the most studied periodontal pathogens. *Pg* possesses a number of virulence factors including the lipopolysaccharide (LPS) component of its cell wall and the tissue-damaging enzymes it produces [1].

The detection of *Pg* from subgingival samples is related to periodontal inflammation, increased probing depth, poor oral hygiene and attachment loss [2]. Although the pathogen can be horizontally transmitted, the patient's own oral flora seems to be the main source of re-emerging periodontal disease after treatment [3,4]. *Pg* is generally detected by culture, especially when antibiotic sensitivity is needed, or by molecular methods, mainly polymerase chain reaction (PCR), which provides an excellent detection threshold and is highly specific [5].

Maternal periodontal disease has been reported as a risk factor for preterm labour in the United States [6]; however, this association was not observed in studies of Asian emigrants in European and Sri Lankan women [7,8].

Elevated levels of serum C-reactive protein (CRP) are detected in low-grade inflammation such as periodontitis [9]. As mentioned by van Winkelhoff and Slots [10], van Winkelhoff *et al*. failed to isolate *Pg* from the vagina of pregnant women with periodontitis; however, its presence in a higher vaginal location could not be ruled out. If *Pg* is absent in the vagina, products of causative agents of periodontitis such as *Pg* are thought to trigger the release of cytokines which in turn signal increased production of acute phase reactants such as CRP by the liver [11,12]. Reports on the role of elevated CRP levels in pregnant women with periodontitis and preterm labour are not conclusive. Several reports indicated that elevated CRP levels in pregnant women with periodontitis appear to mediate preterm labour [9,13-15]. While Ghezzi *et al.* [16] did not find a relationship between elevated circulating CRP levels and preterm labour, they reported an

Table 1. Inclusion and exclusion criteria used for patient enrollment in the study

Inclusion criteria	Exclusion criteria
- Between 18 and 39 years of age (inclusive)	- Unable to provide informed consent or comply with study protocol
- At least 20 teeth	- At medical risk as a result of participation
- No vaginosis	- Have multiple fetuses as diagnosed by ultrasound
- No urinary tract infection	- Require antibiotic treatment for any medical/dental reason
- Periodontal disease	

association between amniotic fluid CRP levels and preterm delivery.

Ethnic differences have been linked to the pathogenesis of periodontitis [17,18]. Although this disease is common among Lebanese, its pathogenesis and role in pregnancy have not been investigated in Lebanon. The aims of this study were to determine the following: i) the prevalence of *Pg* in dental plaque and the vagina of patients; ii) the serum CRP levels of the patients; iii) whether a correlation exists between periodontitis and preterm labour; and iv) whether a correlation exists between elevated CRP levels and preterm labour.

Materials and methods

Subjects

The study included 40 pregnant women in their third trimester: 20 with periodontitis and 20 with normal periodontium. They were informed about the study aims and the samples to be collected. The women who agreed and met the inclusion and exclusion criteria (Table 1) signed the consent form approved by the American University of Beirut Institutional Review Board. The present study was conducted in compliance with the Helsinki Declaration. None of the patient or control groups experienced preterm labour in previous pregnancies.

Periodontal disease was diagnosed clinically when bleeding on probing (BOP) existed in 35% or more of all tooth sites, and /or patients had at least one site on four different teeth with pocket depth ≥ 4 mm and clinical attachment loss ≥ 2 mm. Their oral status was evaluated for caries and periodontal disease. Probing depth, clinical attachment level (CAL), plaque index (PI), and the mean gingivitis score (BOP) were measured on all present teeth. The same investigator performed all periodontal examinations.

Specimens

Plaque samples, vaginal swabs, and blood were obtained from each subject. Plaque samples were collected from the deepest pockets of each quadrant. The area to be sampled was cleaned from the supragingival plaque and dried with cotton pellets, then isolated from saliva with cotton rolls. Sterile paper points (Maillefer, Konstanz, Germany) were inserted in the selected pockets and left for 20 seconds. Next they were transferred to a sterile 1.5 ml microcentrifuge tube containing 0.5 ml phosphate buffered saline (PBS). Vagina samples were taken by running a sterile cotton swab at the entry of the vagina. The swab was dipped in 0.5 ml sterile PBS. Five milliliters of blood were collected from each patient in a plain tube, allowed to clot, and the serum transferred to a sterile container. All samples were stored at -20ºC until used.

Detection of Pg *by PCR*

DNA was extracted from the plaque and vagina specimens using GFX genomic blood DNA purification kit (Amersham Biosciences, GE healthcare, Buckinghamshire, UK). The recovered DNA was tested by PCR for *IS1126*, an insertion sequence specific to *Pg*, as described by Park *et al*. [19] using PI1 (5' - CCC GGC TTA TGA CGT GAT TTC TCT - 3') and PI2 (5' - CTG TTG CGT TTG TGC CCT TGT GC - 3') as primers. These primers amplify the 693-bp fragment of *IS1126.* Briefly, in a 50-µl total volume, 2.5 µl of Taq polymerase, 0.2 µM each of dNTP, 0.3 µM of each primer, 100 ng of DNA, and 1.5mM of $MgCl_2$ were mixed with PCR buffer. The amplifications were performed in a thermal cycler (Thermoelectron Corporation, Waltham, MA, USA). The program selected involved 30 cycles of denaturation at 94°C for 30 seconds, annealing at 45°C for 30 seconds, and extension at 72°C for 1 minute, with an initial denaturation at 94°C for 5 minutes and a final extension at 72°C for 5 minutes. The PCR products were analysed by agarose electrophoresis.

Determination of serum CRP levels by ELISA

Serum CRP levels were determined using the High Sensitivity C-Reactive Protein Enzyme

Table 2. Baseline socio-economic and dental characteristics of pregnant women

Parameter	Periodontal disease	Periodontal health	Significance P≤ 0.05
Age (years)	34.3 ± 5.36	26.10 ± 4.57	0.017
Number of pregnancies	2.3 ± 1.62	1.53 ± 0.79	NS
Smoking	26.5%	23%	NS
Educational level	Low	Low	NS
Probing depth (mm)	5.3 ± 3.2	3.2 ± 1.5	0.027
Clinical attachment loss	4.2 ± 2.3	1.2 ± 0.8	0.021
Bleeding on probing	182/ 240 sites (75.8%)	74/ 240 sites (30.83%)	0.011

NS = Non Significant
-*IS1126*; insertion sequence specific for *Porphyromonas gingivalis.* CRP; C-Reactive Protein
-CRP level greater than 8.2 mg/l is considered elevated

ImmunoAssay kit (BioCheck Inc, Vintage Park, California, USA) according to the manufacturer's instructions. Specimens were run in duplicates. Absorbance values were read at 450nm. CRP concentrations in mg/l were then calculated using a quadratic regression curve. Based on the manufacturer's instructions, all values greater than 8.2 mg/l (norm 0.068- 8.2 mg/l) were considered elevated.

Statistical analysis

Differences between groups were tested with the two-tailed Student's *t*-test ($p \leq 0.05$).

Results

Population and dental analysis

There were no significant differences between the two groups concerning the number of pregnancies, smoking, and level of education. None of the patients or controls experienced preterm labour.

All dental parameters had a statistically significant difference between patient and control groups ($P \leq 0.05$). The average values of tested indices for the patient group indicated a moderate periodontitis. Some women in the control group had gingivitis and thus presented false pockets and bleeding on probing (Table 2).

Detection of Pg

Pg was detected in the dental plaque of 13 (65%) patients and two (10%) controls (Table 3). The agarose electrophoretic pattern obtained using specimens obtained from five patients are shown in Figure 1. The vaginal specimens were all negative for *IS1126*.

CRP levels

Nine of the 13 *IS1126*-positive patients and one of the two *IS1126*-positive controls had elevated CRP levels. Three of the *IS1126*-negative patients and seven of the 18 *IS1126*-negative controls had elevated CRP levels (Table 3).

Discussion

The pregnant women enrolled in this study were all healthy except for periodontal disease. This selection avoided any bias related to previous oral infections that would have necessitated antibiotic intake; thus the differences between the study and control groups emerge with greater significance regarding periodontal condition.

Bleeding on probing is a sign of gingival inflammation. Both groups presented elevated bleeding indices, compatible with their hormonal status and poor hygiene. Since both groups were of low educational level, these observations further emphasize low socioeconomic status as a risk factor for periodontal disease [20].

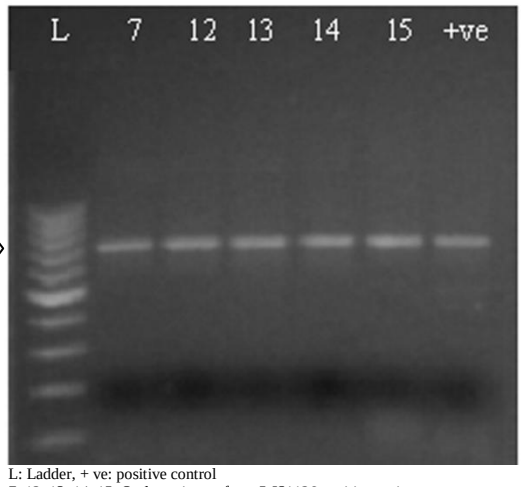
The mesial surfaces of the teeth present periodontal pockets more frequently than the buccal, distal or lingual surfaces. This prevalence is, in part, related to tooth anatomy and the presence of grooves that facilitate plaque accumulation [21,22]. In this

Table 3. *IS1126*-status and CRP levels in *IS1126*-positive and -negative patients and controls

Group	Number of subjects		Number of subjects with elevated CRP	
	IS1126-positive	*IS1126*-negative	*IS1126*-positive	*IS1126*-negative
Patients	13/20 (65%)	7/20 (35%)	9/13 (69.2%)	3/7 (42.8%)
Controls	2/20 (10%)	18/20 (90%)	1/2 (50%)	7/18 (39%)

-*IS1126*; insertion sequence specific for *Porphyromonas gingivalis.* CRP; C-Reactive Protein
-CRP level greater than 8.2 mg/l is considered elevated

Figure 1. Gel electrophoresis: *IS1126* amplicons obtained by PCR



L: Ladder, + ve: positive control
7, 12, 13, 14, 15: Oral specimens from 5 *IS1126*-positive patients

study, the plaque samples were taken from the deepest pockets, which were most often located on the mesial surface of maxillary and mandibular molars. While this sampling method proved to be effective, not all the periodontitis patients tested positive for *Pg*. Thirteen of 20 (65%) patients and two of 20 (10%) healthy controls were *Pg*-positive. These results concur with those of Griffen *et al*. [23] who reported that 103 of 130 (79%) patients and 46 of 181 (25%) controls were *Pg*-positive. They concluded that their data implicate *Pg* in periodontitis.

The fact that some periodontally healthy controls were *Pg*-positive is in agreement with studies that demonstrated the presence of periodontopathogens in even healthy adolescents and young adults [5].

The reports that periodontitis in pregnancy leads to preterm labour suggest that the causative agent is not confined to the oral cavity. Rather, it becomes systemic and exerts its effect in the uterus. Hu *et al*. [24] reported that in a mouse model infected with *Pg*, remote lesions from the site of infection were observed. They suggested that activation of the kinin system is involved in allowing *Pg* to disseminate. The inability to detect *Pg* in the vagina argues against a systemic infection. This concurs with the report of van Winkelhoff and Slots [10] who failed to isolate *Pg* from the vagina of pregnant women with periodontitis, but its presence in a higher vaginal location is possible. If *Pg* is absent in the vagina, products of causative agents of periodontitis such as *Pg* are thought to trigger the release of cytokines such as IL-6, which in turn signal increased production of acute phase reactants such as CRP by the liver [11,12].

CRP is an important biological marker of inflammation. It has been reported that periodontal disease accompanied by elevated CRP levels is associated with adverse pregnancy outcome [16]. Twelve of 20 (60%) patients had elevated CRP levels. In a study of 1,351 women, Miller [25] reported that CRP levels were higher in healthy pregnant women than non-pregnant women. Concurring with this report, eight of 20 (40%) healthy pregnant controls had elevated levels of CRP. This finding suggests that an elevated CRP level in pregnancy is a normal finding and not related to periodontitis.

Although the number of participants is small, the fact that none of the patients or controls experienced preterm delivery tends to indicate the absence of a relationship between periodontitis and preterm labour. Lebanese women fit the known profile of other Caucasian women in terms of the detection of *Pg* as a causative agent of periodontitis.

Acknowledgments

The authors thank Dr. Rabih Chahine for helping to recruit the study subjects and Dr. Mohamed Itani for his valuable periodontal evaluation. We appreciate the American University of Beirut Funding Agency for supporting this study.

References

1. Kinane DF, Mooney J, Ebersole JL (1999) Humoral immune response to *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in periodontal disease. Periodontol 2000 20: 289–340.
2. Takeuchi Y, Umeda M, Sakamoto M, Benno Y, Huang Y, Ishikawa I (2001) *Treponema socranskii*, *Treponema denticola*, and *Porphyromons gingivalis* are associated with severity of periodontal tissue destruction. J Periodontol 72: 1354-1363.
3. Asikainen S, Chen C, Slots J (1996) Likelihood of transmitting *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in families with periodontitis. Oral Microbiol Immunol 11: 387-394.
4. van Steenbergen TJ, Petit MD, Scholte LH, van der Velden U, de Graaff J (1993) Transmission of *Porphyromonas gingivalis* between spouses. J Clin Periodontol 20: 340-345.
5. Sanz M, Lau L, Herrera D, Morillo JM, Silva A (2004) Methods of detection of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Tannerella forsythensis* in periodontal microbiology, with special emphasis on advanced molecular techniques: a review. J Clin Periodontol 31: 1034–1047.
6. Offenbacher S, Beck JD, Lieff S, Slade G (1998) Role of periodontitis in systemic health: spontaneous preterm birth. J Dent Educ 62: 852-858
7. Davenport ES, Williams CE, Sterne JA, Murad S, Sivapathasundram V, Curtis MA (2002) Maternal

periodontal disease and preterm low birthweight: case-control study. J Dent Res 81: 313-318.
8. Rajapakse PS, Nagarathne M, Chandrasekra KB, Dasanayake AP (2005) Periodontal disease and prematurity among non-smoking Sri Lankan women. J Dent Res 84: 274-277.
9. Ioannidou E, Malekzadeh T, Dongari-Bagtzoglou A (2006) Effect of periodontal treatment on serum C-reactive protein levels: a systematic review and meta-analysis. J Periodontol 77: 1635-1642.
10. van Winkelhoff AJ, Slots J (1999) *Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis* in nonoral infections. Periodontol 2000 20: 122-135.
11. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO 3rd, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC Jr, Taubert K, Tracy RP, Vinicor F (2003) Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 107: 499-511.
12. Dasanayake AP, Li Y, Wiener H, Ruby JD, Lee MJ (2005) Salivary *Actinomyces naeslundii* genospecies 2 and *Lactobacillus casei* levels predict pregnancy outcomes. J Periodontol 76: 171-177.
13. Pitiphat W, Gillman MW, Joshipura KJ, Williams PL, Douglass CW, Rich-Edwards JW (2005) Plasma C-reactive protein in early pregnancy and preterm delivery. Am J Epidemiol 162: 1108-1113.
14. Hvilsom GB, Thorsen P, Jeune B, Bakketeig LS (2002) C-reactive protein: a serological marker for preterm delivery? Acta Obstet Gynecol Scand 81: 424-429.
15. Offenbacher S, Lin D, Strauss R, McKaig R, Irving J, Barros SP, Moss K, Barrow DA, Hefti A, Beck JD (2006) Effects of periodontal therapy during pregnancy on periodontal status, biologic parameters, and pregnancy outcomes: a pilot study. J Periodontol 77: 2011-2024.
16. Ghezzi F, Franchi M, Raio L, Di Naro E, Bossi G, D'Eril GV, Bolis P (2002) Elevated amniotic fluid C-reactive protein at the time of preterm delivery. Am J Obstet Gynecol 186: 268-273.
17. Borrell LN, Burt BA, Gillespie BW, Lynch J, Neighbors H (2002) Periodontitis in the United States: beyond black and white. J Public Health Dent 62: 92-101.
18. Borrell LN, Lynch J, Neighbors H, Burt BA, Gillespie BW (2002 Winter) Is there homogeneity in periodontal health between African Americans and Mexican Americans? Ethn Dis 12: 97-110.
19. Park OJ, Min KM, Choe SJ, Choi BK, Kim KK (2004) Use of Insertion Sequence Element IS*1126* in a Genotyping and Transmission Study of *Porphyromonas gingivalis.* J Clin Microbiol 42: 535–541.
20. Machuca G, Khoshfeiz O, Lacalle JR, Machuca C, Bullón P (1999) The influence of general health and socio-cultural variables on the periodontal condition of pregnant women. J Periodontol 70: 779-785.
21. Papapanou PN, Tonetti MS (2000) Diagnosis and epidemiology of periodontal osseous lesions. Periodontol 2000 22: 8-21.
22. Papapanou PN, Wennström JL, Gröndahl K (1988) Periodontal status in relation to age and tooth type. A cross-sectional radiographic study. J Clin Periodontol 15: 469-478.
23. Griffen AL, Becker MR, Lyons SR, Moeschberger ML, Leys EJ (1998) Prevalence of *Porphyromonas gingivalis* and periodontal health status. J Clin Microbiol 36: 3239-3242.
24. Hu S-W, Huang H-C, Lai Y-Y, Lin Y-Y (2006) Transvascular dissemination of Porphyromonas gingivalis from a sequestered site is dependent upon activation of the lallikrein/kinin pathway. J Periodontal Res 41: 200-207.
25. Miller EM (2009) Changes in serum immunity during pregnancy. Am J Hum Biol 21: 401-403.

Corresponding author

Alexander M. Abdelnoor, PhD
Department of Microbiology and Immunology
Faculty of Medicine
American University of Beirut
PO Box 11-0236, Riad El-Solh
Beirut 1107 2020
Lebanon
Phone: (+) 961 1 350000, ext 5120
Fax: (+) 961 1 744487
Email: aanoor@aub.edu.lb

Conflict of interests: No conflict of interests is declared.