

Association between *Porphyromonas gingivalis* bacteria in infra-gingival plaque and premature labor with low birth weight

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Abstract

Context: There are some discrepancies regarding the association between periodontal disease and preterm in the literature. Considering all periodontal pathogens, *Porphyromonas gingivalis* (Pg) is the most invasive bacterium in connective tissues, and the impact of its presence in the diverse microbial community of periodontal pathogens with a lower frequency is much greater than expected.

Aim: This study investigated the association of Pg in infra-gingival plaque of preterm and low birth weight (PTLBW) mothers.

Setting and Design: This descriptive correlational study was conducted on 89 women, who were referred to Imam Khomeini Hospital in Sari, Iran, in 2017.

Materials and Methods: The samples were assigned by purposive method with 30 mothers had PTLBW newborns and 59 mothers had normal term and normal birth weight (NTNBW) neonates. The probing depth, bleeding on probing clinical attachment levels, plaque index, and gingival index were measured. A sample from the periodontal pocket was collected to distinguish the Pg in subgingival plaque by polymerase chain reaction.

Statistical Analysis Used: The data were analyzed using descriptive statistics and the Mann–Whitney U and Chi-square test.

Results: According to the periodontal indexes, 37.3% and 26.7% of the mothers in the NTNBW and PTLBW were diagnosed with periodontitis, respectively. Furthermore, Pg was detected in 30% and 20.3% of the cases in the PTLBW of NTLBW mothers. The amount of Pg in infra-gingival plaque was significantly higher in the PTLBW mothers than in the NTNBW ($P = 0.016$).

Conclusion: The results indicated that the quantity of Pg could be considered a risk factor for PTLBW delivery.

Keywords: Low birth weight, Periodontitis, *Porphyromonas gingivalis*, Preterm birth

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Received: 10 June 2021; **Revised:** 03 July 2022; **Accepted:** 06 July 2022; **Published:** 05 September 2022

Access this article online	
Quick Response Code:	Website: www.jnmsjournal.org
	DOI: 10.4103/jnms.jnms_93_21

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How to cite this article: Maboudi A, Ahmadi A, Heidari M, Rafiei A, Rahmani Z, Moosazadeh M, et al. Association between *Porphyromonas gingivalis* bacteria in infra-gingival plaque and premature labor with low birth weight. *J Nurs Midwifery Sci* 2022;9:237-40.

INTRODUCTION

Preterm and low birth weight (PTLBW) is a major medical problem that has been identified as the notable cause of neonatal mortality and long-term sequelae.^[1,2] The prevalence rates of PTLBW have been estimated at 5%–7% and 19% in developed and developing countries, respectively; considering this, around 15.5% of newborns are born with PTLBW each year, and more than 95.6% of them are born in developing countries. In Iran, the rate of PTLBW incidence is estimated at about 8%.^[3]

The etiology of more than 50% of premature deliveries is unknown; however, the results of extensive studies have shown the effect of nongenital maternal infection as the cause of PTLBW.^[4,5] In recent decades, the findings of epidemiological studies have demonstrated the connection between maternal periodontal diseases and PTLBW.^[6] Based on the results of European studies, the risk of a low birth weight (LBW) neonate is 3.2 times more in women with periodontal diseases, and the rate of delivering a premature newborn is 3.4 times higher in women with periodontal diseases than in women without periodontal diseases.^[7] Periodontal diseases, including periodontitis and gingivitis, are caused by the overgrowth of putative periodontal bacteria and determined by an immune-inflammatory response of the periodontal tissues. These diseases are characterized by dental attachment loss, periodontal pocket formation, bone resorption, and chronic tissue destructive inflammation.^[8]

Considering all periodontal pathogens, *Porphyromonas gingivalis* (Pg) is the most invasive bacterium in connective tissues, and the impact of its presence in the diverse microbial community of periodontal pathogens with a lower frequency is much greater than expected. Even with <0.01% of the total microbiota in the periodontal tissue, it can lead to the loss of connective tissue and ultimately tooth loss. Real-time polymerase chain reaction (PCR) offers a sensitive, efficient, and reliable approach to quantitation. The application of this system would lead to the accurate measurement of a large number of samples at one time and makes it possible to determine the amount of Pg present in the plaque samples directly without culturing.^[9-12]

Due to the fact that periodontitis is one of the possible causes of preterm labor, early diagnosis of the disease is highly crucial. Moreover, since some studies rule out a link between periodontitis, preterm labor, and LBW.^[13,14] Other studies of periodontitis consider it a risk factor for this problem.^[15,16]

This study aimed to investigate the association of Pg in infra-p; gingival plaque with preterm labor and LBW.

MATERIALS AND METHODS

This descriptive correlational study was conducted on 89 mothers of live newborns who were referred to Imam Khomeini Hospital in Sari, Iran, in 2017. Based on previous studies and the prediction of a correlation coefficient of 0.25^[15] with a 95% confidence level and power of 0.80%, the sample size was estimated 88. The samples were assigned by purposive method with 30 mothers had PTLBW newborns and 59 mothers had normal term and normal birth weight (NTNBW) neonates. Therefore, the postpartum mothers with NTNBW (BW of >2500 gr and gestational age 37–42 weeks) and postpartum mothers with PTLBW were included (BW of <2500 g and gestational age of <37 weeks) whom experiencing the first or second vaginal delivery, and being in the age range of 18–40 years old, were entered into the study. Exclusion criteria included a history of previous preterm and LBW, twin or higher-order multiple pregnancy, vaginitis in pregnancy, medical problems affecting body weight (untreated thyroid disease), drug-related hypertension, type 1 or 2 diabetes mellitus, addiction, smokers, nutritional problems, being on special diet, chronic illness, kidney disease, anemia, heart problems, thrombotic thrombocytopenic purpura, and idiopathic thrombocytopenic purpura; history of periodontal treatment; history of previous urogenital infection, placenta problems, polyhydramnios and body mass index (BMI) ≥ 30 kg/m.^[11]

The data about the demographic and general information and the weight of newborns were obtained from the medical records. Clinical examination (1 day after delivery) was performed in the hospital by a general dentist and a trained examiner. The bleeding on probe (BOP), clinical attachment loss (CAL), gingival index (GI) by Loe and Silness,^[14,16] plaque index (PI) by Silness and Loe,^[17] and probing depth (PD) were evaluated in six teeth based on the Ramfjord method^[18] using a manual periodontal probe (Williams probe, Medisporex Company, Pakistan).

In gingivitis, the gums became red and edematous and bled during procedures, such as probing with a periodontal probe, and the GI was used. While healthy gums lacked these symptoms and had a firm, coral-pink consistency, and no bleeding following probatation. Periodontitis was also diagnosed by involving at least one site with a PD of ≥ 3 mm and a CAL of ≥ 2 mm of clinical connections.^[18,19]

For microbial analysis, the subgingival plaque was removed from the selected area and the samples were gathered from the deepest pockets of each quadrant. To this aim, the paper

cones were inserted into the gingival crevice and left there for 30 s. The papers were transferred to a sterile microtube containing phosphate-buffered saline and immediately frozen at -20°C . The bacterial DNA was extracted and purified using G-spin™ Total DNA Extraction Mini Kit (iNtRON biotechnology DR) according to the manufacturer's instructions. The recovered DNA was quantitatively and qualitatively evaluated by agarose gel electrophoresis and nano spectrophotometer methods, respectively. The DNA of *Pg* was used to determine the standard curve, showing a correlation coefficient of ≥ 0.99 . To analyze the DNA by PCR, the 16S rRNA sequences of the genus *Pg* were selected from the NCBI Taxonomy Homepage of the National Center for Biotechnology Information.^[19] The research objectives and procedures were explained to all participants, and they signed the informed consent form. The study protocol was reviewed and approved by the Ethics Committees of Mazandaran University of Medical Sciences (No: IR.MAZUMS.REC.96.3154).

The SPSS (version 22; SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The quantitative data were presented as mean and standard deviation, whereas the qualitative data were expressed as frequency and percentage. Pearson correlation coefficient was used to determine the correlation between variables. A Chi-square test was utilized to compare the prevalence of *Pg* in variables. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

This study was conducted on 89 women (mean age of 29.0 ± 3.28 years) that gave birth to neonates, including 30 mothers of PTLBW newborns (mean age of 29.02 ± 5.48 years) and 59 mothers of NTN BW neonates (mean age of 28.1 ± 4.97 years). The mean values of age showed that there was no significant difference between the two groups in this regard ($P = 0.27$).

The frequencies of periodontitis in the PTLBW group were lower than in the NTN BW group, though the differences were not significant. The frequencies of gingivitis in the PTLBW group were higher than in the NTN BW group, though the differences were not significant. Furthermore, no association was observed between maternal periodontal PD, GI, PI, BOP, and CAL statuses and PTLBW [Table 1].

The agarose electrophoretic pattern obtained from the patients' samples is shown in Figure 1, which illustrates 1650 basebands. According to the results of the real-time PCR, the value of copy number was 1.8×10^5 and 1.9×10^5 in the NTN BW and PTLBW groups, respectively,

representing a statistically significant difference ($P = 0.016$). It was revealed that the women who were PCR-positive had higher PI, PD, and CAL; nevertheless, this difference was not statistically significant [Table 2].

The findings of our study revealed no statistical differences in the periodontal indices in mothers with PTLBW and NTN BW. Likewise, the findings of another study demonstrated no association between any periodontal pathogens, such as *Pg*, and preterm birth.^[20] However, our results indicated that there was an association between the quantities of *Pg* in infra-gingival plaque and PTLBW. Hence, it was demonstrated that periodontal therapy before labor can be effective and resulted in a decrease in PTLBW labor due to the reducing the number of *Pg* because of its potential to cause PTLBW.^[19]

This study described the application of the real-time PCR method to enumerate the *Pg* genotype in subgingival

Table 1: Comparison of maternal periodontal status between preterm low birth weight and normal term normal birth weight

	NTNBW	PTLBW	P
Periodontal status, n (%)			
Gingivitis	19 (32.2)	11 (36.7)	0.6
Periodontitis	22 (37.3)	8 (26.7)	
Healthy	18 (30.5)	11 (36.7)	
Periodontal index (mean±SD)			
GI	1.24±0.48	1.13±0.66	0.70
PI	1.82±0.66	1.68±0.64	0.43
BOP	27.02±31.86	28.04±33.66	0.90
CAL	1.20±0.63	1.55±0.5	0.70
PD	0.47±1.60	1.76±0.84	0.33

PI: Plaque index, GI: Gingival index, BOP: Bleeding on probe, PD: Probing depth, CAL: Clinical attachment level, SD: Standard deviation, NTN BW: Normal term normal birth weight, PTLBW: Preterm low birth weight



Figure 1: Gel electrophoresis: 16S rRNA amplicons for *Porphyromonas gingivalis* in infra-gingival obtained by PCR. PCR: Polymerase chain reaction

Table 2: Association between periodontal parameters and the presence or absence of Porphyromonas gingivalis

Periodontal parameters	Mean±SD		P
	Negative PCR	Positive PCR	
GI	1.23±0.5	1.10±0.68	0.26
PI	1.75±0.61	1.84±0.79	0.56
PD	1.60±0.46	1.89±0.99	0.38
BOP	28.22±31.87	24.59±34.27	0.46
CAL	0.45±1.08	1.05±1.88	0.13

PI: Plaque index, GI: Gingival index, BOP: Bleeding on probe, PD: Probing depth, CAL: Clinical attachment level, SD: Standard deviation, PCR: Polymerase chain reaction, SD: Standard deviation

plaque, which is a more accurate method. Quantitative measurements of bacteria are necessary because of the importance of the abundance of the organism concerning the destructive nature of periodontal diseases.

One of the limitations of this study was related to the limited number of women with PTLBW neonates lacking systemic problems. In addition, the transfer and storage of samples in appropriate conditions was one of the challenges in our study. Therefore, further examinations are required with a detailed classification of periodontitis, based on complexity, severity, distribution, and extent to assess the actual impact of the periodontal status on pregnancy.

Based on the results of this study, there was an association between Pg in infra-gingival plaque and PTLBW. Accordingly, the quantity of Pg could be considered a risk factor for PTLBW delivery. Furthermore, we can conclude that periodontal therapy before labor can be effective and resulted in a decrease in PTLBW labor due to the reducing the number of Pg because of its potential to cause PTLBW.

Conflicts of interest

There are no conflicts of interest.

Author's contributions

All authors Contributed to conceiving the idea of the study, collecting data, as well as analyzing and editing the manuscript.

Financial support and sponsorship

Nil.

Acknowledgment

The authors would like to manifest gratitude to the Vice-President of Research and Technology, Mazandaran University of Medical Sciences for financial support.

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