Salivary and plasma inflammatory mediators and secretory status in preterm delivery women with periodontitis – a cross sectional study

Salivarni i inflamatorni medijatori plazme i sekretorni status prevremeno porođenih žena sa periodontitisom – studija preseka

Ljubinka Nikolić*, Saša Čakić†‡, Neda Perunović†, Emina Čolak§, Jelena-Kotur Stevuljević†, Saša Janković†, Milanko Djurić**, Darko Plećaš*††

Clinical Center of Serbia, Clinic for Gynecology and Obstetrics, *Department for Hematology and Transfusion Laboratory, ‡‡Department for Fertility Control, §Institute of Medical Biochemistry, Belgrade, Serbia; University of Belgrade, †Faculty of Dental Medicine, ‡Clinic of Periodontology and Oral Medicine; Faculty of Pharmacy, †Department of Medical Biochemistry; ‡Clinic of Medicine, Belgrade, Serbia; University of Novi Sad, **Clinic for Dentistry, Novi Sad, Serbia

Abstract

Background/Aim. Preterm birth is defined as a delivery prior to the completed 37th week of gestation. Literature data suggested that periodontal processes may influence to the feto-placental unit and induce preterm delivery. The degree of the periodontal disease is influenced by secretor status. Pro-inflammatory cytokines are involved in periodontitis as well as in delivery. The combined influence of these factors on the risk of preterm birth has not been explored. The aim of our study was to investigate the associations between periodontal diseases, secretor status, and interleukin-1-β (IL1-ß) and prostaglandine E2 (PGE2) levels in women delivered preterm.

Methods. The study included 56 pre-term delivery women and 56 women delivered at term as a control group, aged between 17 and 41 years. Periodontal examination, blood and saliva sampling were performed within 48 hours following delivery. Secretor phenotype was determined by hemagglutination inhibition method. The concentrations of IL1-ß and PGE2 were measured by high sensitivity Enzyme-linked Immunosorbent Assay (ELISA).

Results. In the pre-term birth group there were 66.1% of women with periodontitis, while in the control one there were 12.5% ($p < 0.01$). Concentrations of IL1-ß and PGE2 in plasma were significantly higher in the non-secretor group of women who gave birth pre-term and had periodontitis comparing to other groups. There was a significant correlation between salivary and plasma levels of PGE2 and IL1-ß in the preterm birth group ($R = 0.416$, $p = 0.017$ and $R = -0.592$, $p < 0.001$, respectively). There were no such correlations in women who delivered at term.

Conclusion. Our results support the hypothesis that non-secretor phenotype and periodontitis are at least in part responsible for pathogenesis of preterm birth. This probability of negative impact of non-secretor status cannot be ignored. These findings support the need for additional research into the biology of human parturition.

Key words: premature birth; periodontitis; interleukin-1beta; dinoprostone; saliva; plasma.

Apstrakt

Uvod/Cilj. Premrveni porođaj se definiše kao porođaj pre navršene 37 nedelje gestacije. Podaci iz literature govore u prilog tome da periodontalni procesi mogu uticati na feto-placentalnu jedinicu i indukovati preterminski porođaj. Sekretorni status može uticati na stepen periodontalne bolesti. Proinflamatorni citokini imaju uticaj na periodontitis kao i na porođaj. Kombinovani uticaj ovih faktora rizika za premrveni porođaj nije dovoljno istražen. Cilj ove studije je bio da istraži povezanost između periodontalne bolesti, sekretornog statusa, nivoa interleukina 1-ß (IL1-ß) i prostaglandina E2 (PGE2) kod žena koje su imale premrveni porođaj. Metode. Studijom je bilo obuhvaćeno 56 žena, koje su imale premrveni porođaj i 56 žena u kontrolnoj grupi koje su se porodile u terminu, starosti između 17 i 41 godine. Periodontalni pregled, uzorkovanje krvi i salive je izvršeno u prvih 48 sati po porođaju. Sekretorni status je

Correspondence to: Ljubinka Nikolić, Clinical Center of Serbia, Clinic for Gynecology and Obstetrics, Department for Hematology and Transfusion Laboratory, Višegradska 26, 11 000 Belgrade, Serbia. Email: ljubinka3.9nikolic@gmail.com; ljubinka.nikolic@kcs.ac.rs
Introduction

Preterm birth (PTB) is defined as a delivery prior to the completed 37th week of gestation. Two-thirds of PTBs are spontaneous. The global prevalence rate of preterm birth is ranging from 5% to 13.3% in low-income countries. PTB increases (as a percentage of all deliveries) with increasing altitude. This is associated with multiple pathological processes such as medical conditions of the mother or fetus, multiple pregnancies, genetic influences, male fetus, environmental exposure, infertility treatments, behavioral and socioeconomic factors, and iatrogenic prematurity. Intra-amniotic infection has been causally linked to PTB. Intra-amniotic infection induces the production of pro-inflammatory cytokines involved in term delivery, including tumor necrosis factor-alpha (TNF-α), interleukin (IL)-8, IL-6, IL-1β, and prostaglandine E2 (PGE2). In some cases of PTB microorganisms cannot be detected by cultivation and other microbiology techniques despite high levels of cytokines in amniotic fluid. Literature data suggest that in cases of PTB with sterile intra-amniotic inflammation, cytokines are produced in distant part of the body due to infection and inflammation, cross the placental barrier, and when they reach appropriate quantities stimulate labor. This statement is in accordance with Miller’s focal infection theory published in 1891.

Microbiological, immunological and animal model studies suggested that periodontal processes may influence to the feto-placental unit and induce preterm delivery (PTD).

Periodontal diseases

Periodontal diseases are infectious diseases that result in the inflammation of the specialized tissues that both surround and support the teeth. Diseases are multifactorial and they are initiated by bacterial colonization of the dentogingival environment, sustained by the presence of dental biofilm and host immune defense. According to the Armitage, there are two major categories of periodontal diseases: gingivitis – non-destructive and reversible gingival inflammation, and periodontitis – destructive inflammation of teeth supporting tissues.

Gingivitis

Gingivitis is a reversible and nondestructive gingival inflammation related to a non-specific bacterial challenge. Dental plaque is the principal etiologic factor in gingivitis. It is characterized by inflammation, edema, erythema and bleeding of the gingival marginal portion. Studies reported prevalence of gingivitis in around 80% children and adolescents. Gingivitis is, therefore, the form of periodontal disease most commonly found. Among pregnant women incidence of gingivitis is even greater. Based on clinical observation, the frequency of gingivitis in pregnant women ranges from 35% to 100%. This variation may be a reflection of both the population studied and the clinical parameters used. Hormonal changes during pregnancy influence periodontal tissues through different mechanisms and alter maternal immune response. Increased circulating levels of progesterone in pregnancy can cause dilatation of gingival capillaries, increased capillary permeability, and gingival exudate. The onset of increased gingival inflammation observed in the second month of gestation, peaks in the eighth month, and coincides with an increase in the circulating levels of hormones. Prostaglandin concentration within the gingiva and gingival fluid also increases dramatically with the occurrence of gingival inflammation. When gingivitis is persistent, it can further leading to periodontitis.

Periodontitis

Periodontitis (PD) is a destructive inflammatory disease of the supporting tissues of the teeth initiated by polymicrobial biofilm. PD is a result of a chronic immune and inflammatory response following infection with a complex microbiome. Typical for the disease is formation of periodontal pockets and a chronic destructive inflammation which impacts the whole organism. Synergistic relationship between periodontal pathogens and their endotoxins induces chronic oral infection; enhance humoral immune response and production of inflammatory markers. Pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) and prostaglandin (prostaglandine 1 – PGE1 and PGE2) are produced in response to infection. Vascular permeability is also increased – allowing the diffusion of cytokines into the blood flow which may have systemic effects on the host. During the second and third trimester of pregnancy, the gingival/periodontal inflammation often becomes more severe. Published data show that cytokines produced in periodontal tissues are enabling to promote inflammation in feto-placental unit. Analysis of amniotic fluid obtained at the time of preterm delivery supports the hypothesis that pro-inflammatory cytokines are involved in preterm birth.
birth without chorio-amnionitis shows elevated levels of inflammatory cytokines \(^{13}\). Maternal periodontal infections provide a chronic reservoir of inflammatory mediators and cytokines (TNF-\(\alpha\), IL-1, IL-6, and PGE2) that could adversely affect pregnancy outcome \(^{14}\).

According to literature data, degree of periodontal inflammation correlates with cytokines levels \(^{15}\). Furthermore, it has been shown that the degree of the periodontal disease is influenced by secretor status \(^{16,17}\).

**Secretor status**

The secretor status is regulated by the fucosyltransferase2 (FUT2) gene. Individuals who express blood group antigens on cells surface, and in the saliva and other body fluids are termed secretors. Blood group antigens in non-secretors are present on cells surface but not in body fluids. Blood group antigens are oligosaccharides. Blood group substances in secretors body fluids (A, B, H, Lewis b, Lewis y) are glycoproteins. In saliva the blood reactive antigens are found primarily on mucins. Blood type antigens and other oligosaccharides act as receptors for bacterial adhesion and regulate the oral bacteria – oral microbiome. Binding of pathogens to these receptors activates a distinct signaling pathway that shapes the immune response. Therefore, secretor/non-secretor phenotypes are associated with some metabolic and infectious diseases. Recent evidence suggests that non-secretors are at increased risk of carrying some pathogenic microorganisms in their body \(^{18}\). In accordance with these data, non-secretors are at increased risk of inflammatory diseases, pre-cancerous and cancerous lesions along with periodontitis \(^{19}\).

Overall, the study findings are inconsistent and the combined influence of these factors on the risk of PTB has not been explored.

Given the established link between periodontitis and secretor status as well as the association between inflammatory mediators, periodontitis, and preterm birth we hypothesized that in some instances PTB risk could be associated with the co-occurrence of increased cytokine levels and secretor status in women with periodontitis. More specifically, elevated levels of IL-1 \(\beta\) and PGE2 occur in combination with non-secretor status.

Therefore, the purpose of our study was to investigate the associations between periodontal diseases, secretor status, and IL-1 \(\beta\) and PGE2 levels and risk of PTB.

**Methods**

This study included 112 women (56 preterm delivery women and 56 women that delivered at term as a control) aged between 17 and 41 years. Women were enrolled from August 2012 to March 2014. All women had their delivery in the Clinic for Gynecology and Obstetrics, Clinical Center of Serbia in Belgrade.

The study was conducted after obtaining approval from the Ethical Committee of the Faculty of Medicine, University of Belgrade and the Clinical Center of Serbia. Written informed consent from all the participants were obtained in accordance with the Helsinki Declaration, revised in 2000.

Blood sampling, saliva sampling, and periodontal examination were performed within 48 hours following delivery. Random sample of control mothers was selected from the birth register simultaneously as the cases. Only mothers with a singleton gestation were included in the study. Data for mothers and newborns were collected from medical records.

Gestational age was estimated by the last menstrual period and ultrasound examination.

Delivery prior to complete 37 weeks of gestation was considered as PTB.

Exclusion criteria included the following: multiple pregnancies, assisted reproductive technique, fetal congenital disease, diabetes, preeclampsia, intra-amniotic infection during pregnancy and clinical signs of infection (body temperature over 38°C).

Blood and saliva sampling were performed just before periodontal examinations.

**Blood sampling**

A 4-mL of venous blood samples from antecubital fossa were collected in EDTA Vacutainer\(^{6}\) tube (Becton Dickinson, UK). Blood samples were centrifuged at 3000 rpm for 15 min and the plasma was aliquoted. The plasma samples were frozen at -70°C until further analysis. The remaining content of tube was used for blood group determination.

**Salivary sampling**

Unstimulated whole saliva was collected in 10 mL glass tubes. No antiseptic mouth rinse was used prior to collection. Collected saliva samples were centrifuged within 1 hour of collection at 3,500 rpm for 20 min and the supernatant was separated and stored at -20°C for further secretor status testing.

**Periodontal examination**

The full-mouth periodontal measurements were performed in six sites per tooth by one same experienced examiner. Periodontal measurements include following periodontal clinical parameters: Probing Depth (PD), Clinical Attachment Level (CAL), Bleeding on Probing (BOP), Visible Plaque Accumulation (PI). According to the classification of periodontal diseases, periodontal status was defined as: healthy periodontium, gingivitis, and periodontitis \(^{20}\).

**Determination of blood group and secretor status**

ABO and Rh blood groups were determined in fresh blood ethylenediaminetetracetic acid (EDTA)-samples by standard hemagglutination methods \(^{21}\). Red blood cells were suspended in a 2–3\% (v/v) saline solution; 50 \(\mu\)L of this suspension was mixed in tubes with 50 \(\mu\)L of specific antisera, then incubated.
for 10 min at room temperature, and the results were read by naked eye after centrifugation at 2,000 rpm for 1 min.

Secretor and non-secretor phenotypes were evaluated by boiled saliva samples using the Hemagglutination Inhibition Assay test 21. For each patient 3 tubes were prepared; 50 uL of boiled saliva samples was mixed with 50 uL diluted commercial antisera (anti-A, anti-B, and anti-H); tubes were incubated for 10 minutes at room temperature, after that, 50 μL of corresponding erythrocytes (A, B, O) were added to the test mixture and all the test tubes were agitated and left at room temperature for another 10 minutes. Results were read by naked eye after centrifugation at 2,000 rpm for 1 min. Negative reaction for agglutination is interpreted as positive for secretor status. Positive reaction for agglutination means a negative test which has proven that the person is non-secretor.

The commercial antisera used for determination of blood groups and secretor status were the following: 1) monoclonal anti-A, anti-B, anti-AB, and anti-D (Lorne, UK) and 2) Anti-H lectin (CE Immunodiagnostics, Germany). All assays included appropriate known controls. Each aliquot of saliva and blood samples was used only once in an assay, and then discarded.

**Determination of IL-1β and PGE2**

The concentrations of IL-1β and PGE2 were measured by commercially available high sensitivity enzyme-linked immnosorbent assay (ELISA) eBioscience kits, Vienna, Austria, and EIA kit Enzo Life Science, Germany. The microplates were read according to the manufacturer’s recommended time frame using an automated plate reader: Sunrise, Tecan Dorset, UK.

**Statistical analysis**

Numerical data were presented as mean ± standard deviation (SD) for normally distributed data or median with interquartile range for non-normally distributed data, while categorical variables were presented as frequencies or percentages. Distribution of periodontal status among PTB and FTB groups was assessed using Fisher's exact test. Inter-group comparisons of age, and biochemical parameters was performed using Mann-Whitney test. Depending on the data types, differences between independent samples were assessed using χ²-test, Fisher test, Student’s t-test, Kruskal-Wallis, Mann-Whitney U test, while differences between the related groups were examined by Wilcoxon test. The correlations between clinical parameters and laboratory parameters as well as between saliva and plasma levels of biomarkers amongst PTB and FTB were tested with the Spearman's rank correlation test. The statistical analysis was performed using commercial software SPSS 20.0, Inc., Chicago, IL; p values < 0.05 were considered to be significant.

**Results**

The demographic and clinical characteristics of patients who gave preterm birth and full term birth are displayed in Table 1.

Table 1  

| Parameter                                      | Preterm birth | Term birth | p   |
|------------------------------------------------|---------------|------------|-----|
| Maternal age (years), mean ± SD                | 30.7 ± 5.5    | 27.0 ± 3.9 | ns  |
| Maternal (ABO) blood type, n (%):              |               |            |     |
| O                                              | 16 (28.6)     | 14 (25.0)  | ns  |
| A                                              | 24 (42.8)     | 28 (50.0)  | ns  |
| B                                              | 14 (25.0)     | 12 (21.4)  | ns  |
| AB                                             | 2 (3.6)       | 2 (3.6)    | ns  |
| Maternal RhD factor, n (%):                     |               |            |     |
| positive                                       | 46 (82.1)     | 50 (89.3)  | ns  |
| negative                                       | 10(17.9)      | 6 (10.7)   | ns  |
| Maternal secretory status, n (%):              |               |            |     |
| secretor                                       | 44 (78.6)     | 44 (78.6)  | ns  |
| non-secretor                                   | 12 (21.4)     | 12 (21.4)  | ns  |
| Gingival status, n (%):                        |               |            |     |
| healthy periodontium                            | 4 (7.1)       | 31 (55.3)  | < 0.001 |
| gingivitis                                     | 15 (26.8)     | 18 (32.1)  | ns  |
| periodontitis                                  | 37 (66.1)     | 7 (12.5)   | < 0.001 |
| Infant gender, n (%):                          |               |            |     |
| female                                         | 24 (42.8)     | 34 (60.7)  | < 0.05 |
| male                                           | 32 (57.2)     | 22 (39.3)  | < 0.01 |
| Infant birth length (cm) mean ± SD             | 44.96 ± 3.1   | 50.68 ± 1.2| < 0.05 |
| Infant birth weight (g) mean ± SD              | 1862 ± 381    | 3300 ± 208 | < 0.01 |
| Apgar score, mean ± SD                         | 8.02 ± 1.5    | 9.02 ± 0.1 | < 0.01 |
| Apgar score 9–10/1 min                         | 32 (57.2)     | 56 (100)   | < 0.01 |
| Apgar score < 7/1 min                          | 5 (8.9)       | 0 (0)      | < 0.01 |
| Parity                                         |               |            |     |
| primiparous                                    | 35 (62.5)     | 34 (60.7)  | ns  |
| multiparous                                    | 21 (37.5)     | 22 (39.3)  | ns  |

SD – standard deviation; ns – non significant.

Nikolić IJ, et al. Vojnosanit Pregl 2020; 77(3): 247–255.
Table 2
Number of secretors and non-secretors women according to their periodontal status

| Secretor status | Healthy periodontium | Gingivitis | Periodontitis |
|-----------------|----------------------|------------|--------------|
| Non-secretor    | 4 (11.4)             | 6 (18.2)   | 14 (31.8)*   |
| Secretor        | 31 (88.6)            | 27 (81.8)  | 30 (68.2)*   |

*Chi-square test, $\chi^2 = 5.00; p < 0.05$.

Table 3
Number of subjects in the preterm birth (PTB) and full term birth (FTB) groups according to the periodontal and secretory status

| Periodontal status | PTB secretors n (%) | PTB non-secretors n (%) | FTB secretors n (%) | FTB non-secretors n (%) |
|--------------------|---------------------|-------------------------|---------------------|-------------------------|
| Periodontitis      | 27 (61.4)           | 10 (83.3)*              | 3 (6.8)             | 4 (33.3)                |
| Gingivitis         | 14 (29.5)           | 14 (31.8)               | 4 (33.3)            |                         |
| Healthy periodontium| 4 (9.1)             | 0 (0.0)                 | 27 (61.4)*          | 4 (33.3)                |

*Chi-square test, $\chi^2 = 43.6; p < 0.001$.

Table 4
Inflammation markers and basic hematological parameters in the preterm and term birth subgroups

| Parameter                  | Preterm birth | Full term birth | $p$  |
|----------------------------|---------------|-----------------|------|
|                            | Median (25th–75th percentile) | Median (25th–75th percentile) |     |
| IL-1ß, sal (pg/mL)         | 10.837 (9.882–11.570) | 11.778 (5.690–12.094) | ns   |
| IL-1ß, pl (pg/mL)          | 0.0125 (0.0115–0.0141) | 0.0099 (0.0075–0.0133) | < 0.01 |
| PGE2, sal (pg/mL)          | 279 (62–567)   | 327 (215–423)   | ns   |
| PGE2, pl (pg/mL)           | 967 (107–1267) | 461 (36.1–1600) | < 0.01 |
| hsCRP, pl (mg/L)           | 20.4 (7.43–36.7) | 19.0 (8.4–47.6) | ns   |
| WBCx10^9/L                 | 15.2 (13.4–16.8) | 15.6 (12.7–18.2) | ns   |
| Hb (g/L)                   | 112 (95.6–118)  | 111 (101–122)   | ns   |
| Plt x10^9/L                | 204 (202–265)   | 218 (201–293)   | ns   |

sal – saliva; pl – plasma; WBC – white blood cells; Hb – hemoglobin; Plt – platelets; IL-1ß – interleukin-1ß; PGE2 – prostaglandine E2; hsCRP – high sensitivity C-reactive protein; ns – non significant.

Tested groups were homogenous comparing to age and parity. ABO and Rh representation as well as secretor status did not show the difference between the tested group and the control.

In the entire research group there were 44/112 (39.3%) women with periodontitis. It was noted that the prevalence of periodontitis was significantly higher ($p < 0.001$) in the PTB group in comparison to the control group of women delivered at term (66.1% and 12.5%, respectively). The prevalence of gingivitis in the PTB and the FTB group did not show a statistically significant difference ($p > 0.05$).

Analysis of the periodontal status and the secretory status showed that there were only 11.4% of non-secretors in the group with a healthy periodontium, while in the group with periodontitis there were 31.8% of non-secretors which was a statistically significant difference (Table 2).

At baseline, there was no difference between number of preterm and term birth subjects according to their secretory status (Table 1). However, there was significantly greater number (83.3%) of non-secretors preterm birth subjects with periodontitis compared to other periodontal disease categories. In the PTB non-secretors mothers there were no subjects with healthy periodontium. Also, full term birth secretors had the highest number of subjects (61.4%) with healthy periodontium ($p < 0.001$) (Table 3).

When we compared inflammation markers in plasma of women who gave preterm birth with women with term birth we found significantly higher IL-1ß and PGE2 values in plasma of the preterm birth group compared to the FTB group. Differences in other parameters in blood, as well as in saliva did not reach statistical significance (Table 4).

According to Spearman’s correlation, in the PTB group there was a significant association between salivary levels of PGE2 and IL-1ß ($R = 0.416, p = 0.017$). The significant negative/inverse correlation was identified between plasma concentrations of IL-1ß and PGE2 ($R = -0.592, p < 0.001$) in the PTB group. These correlations were not found in women who delivered at term. In the FTB group there were significant correlation between salivary and plasma levels of PGE2.
and maternal age ($R = 0.428$, $p = 0.0009$ and $R = -0.289$, $p = 0.03$, respectively) (Table 5).

### Table 5

| Parameters | PTB ($R$, $p$) | FTB ($R$, $p$) |
|------------|----------------|----------------|
| sal IL-1β – sal PGE2 | $R = 0.416$ | $R = 0.336$ |
| pl IL-1β – pl PGE2 | $R = -0.592$ | $R = 0.138$ |
| sal PGE2 – Age | $R = -0.265$ | $R = -0.289$ |
| pl PGE2 – Age | $R = 0.131$ | $R = 0.428$ |

$R$ – Spearman’s correlation coefficient; PTB – preterm birth; FTB – full term birth; sal IL-1β – saliva interleukin-1β; pl IL-1β – plasma interleukin-1β; sal PGE2 – saliva prostaglandine E2; pl PGE2 – plasma prostaglandine E2

At baseline, the mean IL-1β and PGE2 values in the two subsets of patients (secretors and non-secretors) were not significantly different (Table 6). However, mean IL-1β level in the non-secretor PTB subgroup with periodontitis was significantly higher than in other groups ($p < 0.001$) (Figure 1).

### Table 6

| Parameter | Non-secretor | Secretor | $p$ |
|-----------|--------------|----------|-----|
| Age (years), median (range) | 35 (33–37) | 29.5 (23–31) | ns |
| IL-1β, sal, pg/mL | 11.203 (10.834–11953) | 10.598 (5.690–11.953) | ns |
| IL-1β, pl, pg/mL | 0.0149 (0.0126–0.0173) | 0.0126 (0.0089–0.0137) | 0.081 |
| PGE2, sal, pg/mL | 506 (279–733) | 327 (62–514) | ns |
| PGE2, pl, pg/mL | 454 (107–800) | 1117 (142–1433) | ns |
| hsCRP, pl, mg/L | 22.6 (8.4–46.7) | 19.0 (7.3–40.6) | ns |
| WBC ×10^9/L | 12.5 (9.8–15.2) | 16.0 (13.4–19.1) | ns |
| Hb, g/L | 104.8 (95.6–114.0) | 111.5 (98.0–123.2) | ns |
| Plt ×10^9/L | 183 (162–204) | 220 (204–279) | 0.040 |

Note: Results are expressed as medians (25th – 75th percentile); sal – saliva; pl – plasma, WBC – white blood cells; Hb – hemoglobin; Plt – platelets; IL-1β – interleukin-1β; hsCRP – high sensitivity C-reactive protein; ns – non significant.

**Discussion**

Consistent with the hypothesis, we found an increased amount of IL1-β and PGE2 in plasma samples obtained from non-secretor preterm delivery women. In addition, there was a strong correlation between IL1-β and PGE2 levels in the PTB group compared with the control (FTB) subjects. These data support the hypothesis that non-secretor phenotype and periodontitis are at least in part responsible for pathogenesis of PTB and the probability of negative impact of non-secretory status cannot be ignored.

There are various risk factors for preterm birth, out of which a previous preterm birth is one of the most important (odds ratio 4.5–7.1). This risk factor likely reflects persistent genetic and epigenetic components. Nullparity and prior cesarean birth are important risk factors for spontaneous preterm birth, but with small associations (odds ratio 1.4–2.4). The gender of the unborn baby also seems to play a role in the process of being born prematurely with low risk odds ratio 2.2.

Numerous cohort/cross-sectional studies have been shown more and less strong association between PTB and periodontitis 23, 24.

There are a few proposed pathways by which periodontitis might affect pre-term birth 25: 1) directly when periodopathogens invade the fetal-placental unit subsequently stimulating local inflammation; 2) indirectly when inflammatory mediators circulate from periodontal burden and synergistically increase local inflammation; 3) by fetal inflammatory re-

---

Fig. 1 – Quantitative comparison of median blood interleukin-1β (IL-1β) concentration in the study group with periodontitis according to birth term and secretor status.

**$\ast p < 0.05$, $\ast\ast p < 0.01$, respectively vs. preterm non-secretor group; $\dagger\dagger p < 0.01$ vs. preterm secretor group according to Kruskal-Wallis and subsequent Mann-Whitney U test [the box represents the first and third quartiles (rectangular boxes); the line within the box is the median, and vertical bars show the 95% confidence interval]. Values obtained from the preterm birth (PTB) non-secretor group differed significantly from other groups at level $p < 0.01$.**

---

Nikolić Ij, et al. Vojnosanit Pregl 2020; 77(3): 247–255.
response to mother’s oral pathogens 26, 4) by mother’s enhanced antigeft response 27, 5) genetically, by heritable factors 28.

Periodontitis was diagnosed in 66.1% of women in the PTB group. Similar to our findings Dörbudak et al. 29 have reported that periodontitis was diagnosed in 20% of normal cases and in 83% of preterm birth ones. These differences could be attributed partially to inconsistent definitions of periodontal disease and different definitions of adverse pregnancy outcomes. The data of Jarjoura et al. 30 on 83 PTB cases and 120 controls support the notion that periodontitis is independently associated with PTB and low birth weight.

The representation of periodontitis in both groups of our patients was 39.3% which is in accordance with data presented by Lieff et al. 31 who found that in the population of pregnant women there were around 40% cases of periodontitis.

However, some cohort and case-control studies did not find a significant association between PTB and periodontitis. In the prospective study of 273 women performed by Soucy-Giguère et al. 32, there was no significant association between disease of the periodontium and preterm birth but the study could not exclude an association between periodontal disease and intra-amniotic inflammation.

Preterm birth, as well as periodontitis, is characterized by increased levels of inflammatory markers and among them IL-1β and PGE2 4, 15.

IL-1β is a pro-inflammatory cytokine and is expressed by many cells including macrophage, natural killer cell (NK) cells, monocytes, and neutrophils. It belongs to the IL-1 family cluster that includes IL-1α, and IL-1RN genes. IL-1α and IL-1β participate in the regulation of immune response, inflammatory reactions, and haematopoiesis. During systemic inflammation IL-1 induction in the hypothalamus may regulate neuroendocrine functions. The inactive precursor IL-1β has to be processed into mature bioactive form of IL1-β and is usually proteolytically mediated by inflammatory cysteine protease caspase-1 33. IL-1β is one of cytokines in the inflammatory cascade resulting in increased production of cyclooxygenase-2 (COX-2) and prostaglandins 10, 33. Prostaglandins act as long-term mediators of inflammation. IL-1β and PGE2 are involved in biochemical processes in inflammation along with delivery 10, 11.

Spontaneous delivery at term is characterized by the expression of inflammasome components, which may participate in the activation of caspase-1 and lead to the cleavage and release of mature IL-1β by the chorio-amniotic membranes. These results support the participation of the inflammasome in the mechanisms responsible for spontaneous parturition at term 34. IL-1β will activate an inflammatory cascade that leads to increased concentrations of PGE2 that are required for onset of delivery. Prostaglandins are the most effective mediators for cervical dilatation in women and stimulation of labor. In the myometrium prostaglandins contribute to increased uterine contractions and in cervix cause degradation of the extracellular matrix resulting in effacement and dilatation. PGE2 has been shown to be a key step for the activation of labor 33.

Intrauterine infection induces an intra-amniotic inflammatory response involving the activation of a number of cytokines among them IL-1β and PGE2 which, in turn, may trigger preterm contractions, cervical ripening and rupture of the membranes, and induce PTB. Zhumakanova et al. 35 reported that increasing of level IL-1β, IL-6 and TNF-α in serum during pregnancy can be used as a nonspecific marker in women at risk of preterm birth.

IL-1β and PGE2 are increased in infections of the periodontium tissue. Many authors have shown that saliva IL-1β levels in subjects with periodontitis were significantly greater than those detected in healthy controls. Studies performed by Kinney et al. 36, and Rathnayake et al. 15, showed that levels of IL-1β correlated with periodontium status. Moreover, salivary IL-1β of IL-1-B correlated significantly with clinical degree of periodontal inflammation. Certain number of research papers show that in patients with periodontitis there is a faster deterioration and rejection of allografts 21.

It has been demonstrated that production of IL-1β and prostaglandins is increased during rejection and that these molecules are able to interfere with graft function 37.

Published data has shown that IL-1β enhances the host antigrift adaptive response and suggests that IL-1β may have an inherited condition that causes a hyperactivity, which in turn may be responsible for PTB 38.

Vamvakopoulos et al. 39 have demonstrated an association between IL-1β and chronic rejection at the genetic level in heart graft recipients. The risk of rejection was 20-fold increased in patients with both the IL-1β (α3953) C allele and the IL1RN1 allele.

Medawar 40 first posed the theory of the fetus-as-allograft nearly 60 years ago explaining the normal course of pregnancy by maternal-fetal interface, antigenic inertness of the fetus and maternal immune tolerance of foreign tissue. That tolerance is compromised in PTB.

Many authors reported that levels of IL-1β, IL-6 and PGE2 in the blood samples were higher in the preterm delivery women than in the healthy control group and that IL-1β and PGE2 levels in maternal blood were higher among those with severe disease of the periodontium in the PTD group. According to Kedzierska-Markowicz et al. 41 the level of IL-1β concentration is an independent predictor of preterm delivery in patients with threatened preterm labor.

Consistently with the reported results, in our study groups plasma levels of IL-1β and PGE2 were significantly higher in the PTB group in comparison to the FTB group.

Differences in plasma levels of C-reactive protein (CRP) between the PTB patients and the FTB ones did not reach statistical significance. This data is in accordance with results of Michalowicz et al. 42 who have suggested that in pregnant women levels of CRP were not associated with infant birth weight or a risk for preterm birth.

PGE2 level in saliva of PTB mothers showed a significant positive correlation with IL-1β in saliva, while plasma level of PGE2 showed a significant negative correlation to the plasma level of IL-1β. It may be related to the fact that the half-life of IL-1β is very short (3–4 h), therefore it will show a positive correlation only if tested on the place of its secretion and not in plasma where it is distant from the in-
flammary lesion. Here the correlation becomes negative because PGE2 is a long-term activator of the inflammatory pathway and it remains high even after its descend in plasma, but the levels of IL-1β decrease due to its short half-life.

Higher levels of IL-1β and PGE2 in the PTB group could be explained by the effect of inflammation in the periodontal tissue, hyperactive IL-1β, reaction of the rejection of allograft or hereditary genetic factors. The recognition that heritable factors play a role in PTB is compatible with the notion that extent of periodontitis is influenced by secretory status which is to a large extent inherited and stable. Secretory status of an individual is genetically determined by a pair of allomorphic genes: Se and se with Se dominant over se. Approximately 80% of the population has the secretor (Se) gene. In secretors salivary blood group antigens agglutinate oral pathogens and thus enable multiple functions of saliva such as rinsing, bacterial clearance and antimicrobial defense. In non-secretors there are no soluble blood group substances, oral pathogens recognize histo-blood group antigens on cells surface as attachment factors, form polymicrobial substances, oral pathogens recognize histo-blood group antigens on cells surface as attachment factors, form polymicrobial gingival/subgingival biofilm, and cause gingival infections on cells surface as attachment factors. Thus, it remains high even after its descend in plasma, because PGE2 is a long-term activator of the inflammatory pathway and it remains high even after its descend in plasma.

Blood group oligosaccharide structures are also important for blastocyst adhesion and resistance to microbial invasion. Recent studies suggest intrauterine selection against non-secretor embryo carried by a secretor mother. This data could have practical importance in assessing the risk of infection and success of assisted reproductive techniques.

Furthermore, oligosaccharides, glycans, found in the breast milk of secretor mothers protect newborns from pathogens and play important role in development of the neonatal immune system. In the preterm infant they show protective effect against gut immaturity. Low salivary blood group oligosaccharides were associated with 10-fold increased odds against gut immaturity. Low salivary blood group oligosaccharides were associated with 10-fold increased odds against gut immaturity.

According to literature data it is conceivable that non-secretors with a lower level of iso-antibodies and immunoglobulins may have a lower resistance to infection and thereafter higher rate of periodontitis. Results of Rocha et al. suggest an active role of mucin glycoproteins in the innate immune regulation of periodontal bacterial colonization and disease progression. Tabasum and Nayak reported 22.2% non-secretors in the chronic periodontitis group. The higher results in our group could be attributed to placental hormones that might affect the clinical and biological features of periodontal infections during pregnancy.

Despite much accumulated knowledge on individual etiological factors, the interactions among risk factors and the pathophysiology of preterm birth remain unclear and there is no biologic explanation for 2/3 of all preterm births.

Conclusion

Afore mentioned risk factors are surely a surrogate for genetic and epigenetic causes of preterm birth, and support the need for additional research into the biology of human parturition. The etiology of spontaneous PTB is still unknown because PTB is a complex syndrome with different co-factors, involving a complex interaction between genetic, immunological and environmental factors. We believe that the identification of genomic and proteomic markers may represent an added value in the further investigation of the association among periodontitis, secretory status and adverse pregnancy outcomes. A randomized clinical trial will be necessary to appropriately test our hypothesis and conclude whether non-secretory status have impact on adverse pregnancy outcome.

Acknowledgement

This study was conducted as a part of the projects No.175075 and No. 41008, financially supported by the Ministry of Education, Science, Technological Development of the Republic of Serbia.

References

1. Blowware H, Content S, Oestergaard MZ, Chen D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet 2012; 379(9832): 2162–72.

2. Beck S, Wijesingha D, Say L, Betran AP, Meriárdi M, Régues JH, et al. The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity. Bull World Health Organ 2010; 88(1): 31–8.

3. Shiozuki A, Youeda S, Nakabayashi M, Takeda Y, Takeda S, Sugimura M, et al. Multiple pregnancy, short cervix, part-time worker, steroid use, low educational level and male fetus are risk factors for preterm birth in Japan: a multicenter, prospective study. J Obstet Gynaecol Res 2014; 40(1): 53–61.

4. Ramen R, Miranda J, Chaouchaibong P, Chaiwongsong T, Kassanasic JP, Dong Z, et al. Sterile and microbiallyassociated intra-amniotic inflammation in preterm prelabor rupture of membranes. J Matern Fetal Neonatal Med 2015; 28: 1394–409.

5. Miller WD. The human mouth as a focus of infection. Dent Cosmos 1891; 33(9): 689–713.

6. Staudtmann PF, Eich S, Salié GE, Surbek D, Mohr S, Bünig W, et al. Increased periodontal inflammation in women with preterm premature rupture of membranes. Clin Oral Investig 2015; 19(6): 1537–46.

7. Oliveira Costa F, Soares Dutra Oliveira AM, Miranda Costa LO. In- terrelation Between Periodontal Disease and Preterm Birth, In: Erez O, editor. Preterm Birth. Rijeka: InTech; 2013; p. 3–39.

8. Armitage GC. Periodontal disease and pregnancy: discussion, conclusions and recommendations. Ann Periodontol 2001; 6(1): 189–92.

9. Robinson P, Schmerman M. Influence of Pregnancy on the Oral Cavity. Glob libr women’s med 2015; ISSN: 1756–2228, [about 5 p]. Available from: https://www.glwom.com [cited 2017 Jun 19].

10. Chukkapalli SS, Rivera-Kweh MF, Veleko IM, Chen H, Zheng D, Bhattacharya I, et al. Chronic oral infection with major periodontal bacteria Tannerella forsythia modulates systemic ather-
osclerosis risk factors and inflammatory markers. Pathog Dis 2015; 73(3): pii: ftt009.

11. Rath-Jürgeský A, Hasselbach L, Ocklitz E, Brückner S, Rao A, Gebring H, et al. Inter-individual differences in cytokine release in patients undergoing cardiac surgery with cardiopulmonary bypass. J Heart Lung Transplant 2001; 12(5): 80–8.

12. Ahati S, Villa A, Ciniz I. Oral environment, inflammation and preterm birth. J Pediatr Neonat Individual Med 2015; 4(2): e040239.

13. Saini R, Saini S, Saini SR. Periodontitis: A risk for delivery of premature baby and low-birth-weight infants. J Nat Sci Biol Med 2010; 1(1): 40–2.

14. Chaesaithong P, Remero R, Kozigzewski SJ, Dong Z, Yau L, Hassan SS, et al. A point of care test for the determination of amniotic fluid interleukin-6 and the chemokine CXCL1-10/PI-10. J Matern Fetal Neonatal Med 2015; 28(13): 1510–9.

15. Rathnayaka N, Akerman S, Klöze B, Landgren N, Jansson H, Tryselius Y, et al. Salivary biomarkers of oral health: a cross-sectional study. J Clin Periodontol 2013; 40(2): 140–7.

16. Raca Dde M, Zenitho EG, Van Dyke T, Silva KS, Costa FO, Soares RF. Differential expression of salivary glycoproteins in aggressive and chronic periodontitis. J Appl Oral Sci 2012; 20(2): 180–5.

17. Nikolić Lj, Janković Lj, Ćakić S, Maksijan A. Secretory status and periodontal disease. Bilt Transf 1996; 42(1): 23–6. (Serbian)

18. Narjadi D, Lepeleda J, Kremer PG, Zanger P. Staphylococcus aureus throat carriage is associated with ABO-secretor status. J Infect 2010; 63(4): 310–7.

19. Gantpat ST, Lietzerov A, Tsarbou B, Brounner M, Veenman EC, Wouters D. Complement activation by salivary agglutinin is secretor status dependent. Biol Chem 2015; 396(1): 35–43.

20. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999; 4(1): 1–6.

21. Blood grouping and typing. In: Walker RH, editor. Technical manual. 11th ed. Bethesda: American Association of Blood Banks; 1993. p. 621–38.

22. Ferrero DM, Larmin J, Jacobsson B, Di Renzo GC, Norman JE, Mar- tin JN Jr, et al. Cross-Country Individual Participant Analysis of 4.1 Million Singleton Births in 5 Countries with Very High Human Development Index Confirms Known Associations but Provides No Biologic Explanation for 2/3 of All Preterm Births. PLoS One. 2016; 11(9): e0162506.

23. Glimm P, Öztürk VÖ, Bozkurt E, Emingil G. Evaluation of the gingival inflammation in pregnancy and postpartum via 25-hydroxy-vitamin D3, prostat glandin E2 and TNF-α levels in saliva. Arch Oral Biol 2016; 63: 1–6.

24. Carbalda S, Tascheri S, Del Vabolom M, Frenzitti L, Weinstin R, Feranger E. Adverse pregnancy outcomes and periodontitis: A systematic review and meta-analysis exploring potential association. Quintessence Int 2016; 47(3): 193–204.

25. Wala M, Saini N. Relationship between periodontal diseases and preterm birth: Recent epidemiological and biological data. Int J Appl Basic Med Res 2015; 5(1): 2–6.

26. Boggess KA, Muiu K, Madianos P, Martha AP, Beck J, Offenbacher S. Fetal immune response to oral pathogens and risk of preterm birth. Am J Obstet Gynecol 2005; 193(3 Pt 2): 1121–6.

27. Madianos PN, Bobezić YA, Offenbacher S. Adverse pregnancy outcomes (APOs) and periodontal disease: pathogenic mechanisms. J Periodontol; 2013; 84(4 Suppl): S170–80.

28. Kamie H, Ishihara Y, Fuma D, Nius T, Kamiyo Y, Yukai T, et al. Interleukin-1 receptor gene variants are associated with aggressive periodontitis in the Japanese. Arch Oral Biol 2014; 59(7): 756–63.

29. Diörtlouad Ö, Eberthardi R, Uim M, Peresson GR. Periodontitis, a marker of risk in pregnancy for preterm birth. J Clin Periodontol 2005; 32(1): 45–52.

30. Jarjaru K, Devina PC, Preç-Delhyo A, Herreras-Abruña M, D’Alto M, Papapanou PN. Markers of periodontal infection and preterm birth. Am J Obstet Gynecol 2005; 192(2): 513–9.

31. Leiff S, Boggess KA, Murtha AP, Jared H, Madianos PN, Musi K, et al. The oral conditions and pregnancy study: periodontal status of a cohort of pregnant women. J Periodontol 2004; 75(1): 116–26.

32. Soucy-Giguère L, Titou A, Gauttier S, Morand M, Chandad F, Giguère Y, et al. Periodontal Disease and Adverse Pregnancy Outcomes: A Prospective Study in a Low-Risk Population. J Obstet Gynaecol Can 2016; 38(4): 346–50.

33. Gringhuis SI, Kaepstein TM, Winters BA, Theelen B, van der Vlist M, Boekhout T, et al. Dectin-1 is an extracellular pathogen sensor for the induction and processing of IL-13 via a noncanonical caspase-8 inflammasome. Nat Immunol 2012; 13(3): 246–54.

34. Remor A, Xu X, Pieço O, Chaesaithong P, Chaumaptong T, Undel R, et al. A Role for the Inflammasome in Spontaneous Labor at Term. Am J Reprod Immunol 2018; 79(6): e124403.

35. Zhamakhanova K, Abounoua B, Kazygkeeva A, Kazhribayeva K, Ermebova G. Some clinical and immunological aspects of preterm birth. Georgian Med News 2016; (253): 17–25. (Russian)

36. Kinney JS, Murselli T, Braun T, Ramseyer CA, Herr AE, Sugai JV, et al. Saliva/pathogen biomarker signatures and periodontal disease progression. J Dent Res 2011; 90(6): 752–8.

37. Karyalainen MK, Huuikke JM, Uutlu J, Suuskanni J, Laukkonen A, Verramo K, et al. A Potential Novel Spontaneous Preterm Birth Gene, AR, Identified by Linkage and Association Analysis of X Chromosomal Markers. PLoS One 2012; 7(12): e15378.

38. Amristiry R, Patel RP, Das S, Saraf J, Jothy A, Manshi A. Interleukin 1β (+1354, -511 and -31) polymorphism in chronic periodontitis patients from North India. Acta Odontol Scand 2015; 73(5): 343–7.

39. Vanvackopaoulos JE, Taylor CJ, Green C, McNeil K, Wallwork J, Goodman R, et al. Interleukin 1 and chronic rejection: possible genetic links in human heart allografts. Am J Transplant 2002; 2(1): 76–83.

40. Pawar PB. Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. Symp Soc Exp Biol 1953; 7: 320–41.

41. Kodzierska-Marawanik A, Krokotuk M, Biustia L, Głowacka E, Kruźmiński G. Evaluation of the correlation between IL-1β, IL-8, IFN-γ cytokine concentration in cervico-vaginal fluid and the risk of preterm delivery. Ginekol Pol 2015; 86(11): 821–6. (Polish)

42. Michalowicz BS, Nowak MJ, Hodges JS, DiAngeli A, Buchanan W, Papapanou PN, et al. Serum inflammatory mediators in pregnancy: changes after periodontal treatment and association with pregnancy outcomes. J Periodontol 2009; 80(11): 1731–41.

43. Loper-Castejon G, Brogou D. Understanding the mechanism of IL-1β secretion. Cytokine Growth Factor Rev 2011; 22(4): 189–95.

44. Gloria-Bottini F, Magrini A, Cazzoli E, Neri A, Pietroustiti A, Amante A, et al. ABH secretor genetic polymorphism: evidence of intraracine selection. Eur J Obstet Gynecol Reprod Biol 2011; 154(1): 20–3.

45. Lewis ZT, Totten SM, Smilowitz JT, Popovic M, Parker E, Lemay M, et al. Maternal fucosyltransferase 2 status affects the gut bifidobacterial communities of breastfed infants, Microbiome 2015; 3: 13.

46. Yahasson ST, Nayak RP. Salivary blood group antigens and microbial flora. Int J Dent Hyg 2011; 9(2): 117–21.

Received on November 6, 2017.
Revised on April 9, 2018.
Accepted on April 12, 2018.
Online First April, 2018.