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SALIVARNI I INFLAMATORNII MEDIJATORI PLAZME I SEKRETORNII STATUS KOD PREVREMENOG POROĐAJA ŽENA SA PERIODONTITISOM – STUDIJA PRESEKA

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SALIVARNI I INFLAMATORNI MEDIJATORI PLAZME I SEKRETORNI STATUS KOD PREVREMENOG PoroĎAJA ŽENA SA PERIODONTITISOM – STUDIJA PRESEKA

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Abstract
Preterm birth is defined as a delivery prior to the completed 37th week of gestation. Literature data suggested that periodontal processes may influence 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 IL1-β and PGE2 levels in women delivered preterm.

Material and methods. The study included 56 preterm 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-β and PGE2 were measured by High Sensitivity enzyme-linked immunosorbent assay.

Results. In the pre-term birth group we had 66.1% of women with periodontitis, while in the control there were 12.5% (p<0.01). Concentrations of IL1-β 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 levels of PGE2 and IL1-β (R=0.416, p=0.017) and between the plasma concentrations of IL1-β and PGE2 (R= -0.592, p<0.001) in preterm birth group. In women who delivered at term these correlations were failed to be found.

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: Preterm birth, Periodontitis, IL-1β, PGE2, Saliva, Secretor
Apstrakt

Materijali i metode
Studija obuhvata 56 žena koje su imale prevremen porođaj i 56 žena u kontrolnoj grupi koje su se porodile u terminu, uzrasta između 17 i 41 godine. Periodontalni pregled, uzorkovanje krvi i salive je usledilo u prvih 48 sati po porođaju. Sekretorni status je određen metodom inhibicije hemaglutinacije. Koncentracije IL1-β i PGE2 su merene visoko senzivinim ELISA testom.

Rezultati
U grupi prevremenih porođaja bilo je 66.1% žena sa periodontitisom, a u kontrolnoj grupi bilo 12.5% (p<0.01). Prevremeno porođene žene non-sekretori sa periodontitisom imale su u plazmi značajno više vrednosti IL 1-β i PGE 2 u odnosu na ostale grupe (p<0.01). U grupi prevremeno porođenih žena postoji značajna korelacija između salivarnih nivoa PGE2 i IL1-β (R=0.416, p=0.017) i između plazmatskih koncentracija IL1-β i PGE 2 (R= -0.592, p<0.001) . Ove korelacije ne postoje kod žena koje su imale terminski porođaj.

Zaključak
Naši rezultati podržavaju hipotezu da su susekretorni status i periodontitis bar delimično odgovorni za patogenezu preterminskog porođaja. Verovatnoća negativnog uticaja non-sekretora se ne sme ignorisati. Ovi zaključci ukazuju na potrebu za dodatnim istraživanjima porođaja.

Ključne reči: prevremeni porođaj, priodontitis, IL-1β, PGE2, Saliva, Sekretor
1. Introduction

Preterm birth (PTB) is defined as a delivery prior to the completed 37th week of gestation\(^1\). The global prevalence rate of preterm birth is ranging from 5% to 13.3\(^2\). PTB is the leading cause of perinatal morbidity and mortality, and two-thirds of PTBs occur after spontaneous preterm labor. PTB 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\(^3\). 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 (TNF)-\(\alpha\), IL-8, IL-6, IL-1\(\beta\), and 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\(^4\). This statement is in accordance with Miller’s focal infection theory published in 1891\(^5\).

Microbiological, immunological and animal model studies suggested that periodontal processes may influence to the feto-placental unit and induce preterm delivery (PTD)\(^6\).

1.1 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 multi-factorial and they are initiated by bacterial colonization of the dento-gingival environment, sustained by the presence of dental biofilm and host immune defense\(^7\). According to the Armitage, there are two major categories of periodontal diseases:

a) Gingivitis – non-destructive and reversible gingival inflammation

b) Periodontitis – destructive inflammation of teeth supporting tissues\(^8\).
1.2 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.

1.3 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 prostaglandins (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\textsuperscript{12}. Analysis of amniotic fluid obtained at the time of preterm birth without chorio-amnionitis shows elevated levels of inflammatory cytokines\textsuperscript{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\textsuperscript{14}. According to literature data, degree of periodontal inflammation correlates with cytokines levels\textsuperscript{15}. Furthermore, it has been shown that the degree of the periodontal disease is influenced by secretor status\textsuperscript{16,17}.

\subsection*{1.4 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\textsuperscript{18}. In accordance with these data, non-secretors are at increased risk of inflammatory diseases, pre-cancerous and cancerous lesions along with periodontitis\textsuperscript{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 β and PGE2 levels and risk of PTB.

2. Materials and 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 Centre of Serbia.

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 of 1975, as 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 is 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.

2.1 Blood Sampling
A 4-ml of venous blood samples from antecubital fossa were collected in EDTA Vacutainer® 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.
2.2 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 h of collection at 3500 rpm for 20 min at 4°C to obtain a cleared supernatant. Two thirds of supernatant were aliquotted, and stored at -70°C for further ELISA testing. The remaining amount of saliva samples was incubated in boiling water bath during 10 min, centrifuged (at 3500 rpm for 10 min), and supernatant was separated and stored at -20°C for further secretor status testing.

2.3 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 periodontitis20.

2.4 Determination of blood group and secretor status
ABO and Rh blood groups were determined in fresh blood EDTA-samples by standard hemagglutination methods21. Red blood cells were suspended in a 2-3% (v/v) saline solution; 50 uL of this suspension was mixed in tubes with 50 uL of specific antisera, then incubated for 10 min at room temperature, and the results were read by naked eye after centrifugation at 2000 rpm for 1 min.
Secretor and non-secretor phenotypes were evaluated by boiled saliva samples using the Hemagglutination Inhibition Assay-test21. 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) respectively; tubes were incubated for 10 minutes at room temperature, after that, 50 uL 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 2000 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 was 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.

2.5 Determination of IL-1ß and PGE2
The concentrations of IL-1ß and PGE2 were measured by commercially available high sensitivity enzyme-linked immunosorbent 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.

2.6 Statistical analysis
Numerical data were presented as mean ± 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.
3. Results

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

Tested groups were homogenous comparing to age and parity. ABO and Rh representation as well as secretor status does 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 is noted that the representation of periodontitis is 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 representation of gingivitis in PTB and FTB group does not show a statistically significant difference (p>0.05).

By analyzing the periodontal status and the secretor status we can note that in the group with a healthy periodontium we have only 11.4% of non-secretors while in the group with periodontitis we have 31.8% of NON-secretors which is a statistically significant difference and this is shown in the Table 2.

At baseline, there is no difference between number of preterm and term birth subjects according to their secretor status (Table 1.). However, there is significantly greater number (83.3%) of non-secretors preterm birth subjects with periodontitis compared to other periodontal disease categories. In 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-1β and PGE2 values in plasma of preterm birth group compared to FTB group. Differences in other parameters in blood, so as in saliva did not reach statistical significance (Table 4.).

According to Spearman correlation, in the PTB group there was a significant association between salivary PGE2 and salivary IL-1β (R=0.416, p=0.017). The significant negative/inversely correlations was identified between plasma concentrations of IL-1β and PGE2 (R= -0.592, p<0.001) in PTB group. In women who delivered at term these
correlations were failed to be found. In 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).

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

4. Discussion

Consistent with the hypothesis, we found an increased amount of IL1-ß and PGE2 in plasma samples obtained from non-secretor women delivered preterm. In addition, there was a strong correlation between IL1-ß and PGE2 levels in PTB compared with 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-secretor 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 ratios 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 ratios 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 ratio22.

Numerous cohort/cross-sectional studies have been shown more and less strong association between PTB and periodontitis23,24.

There are a few proposed pathways by which periodontitis might affect pre-term birth25:

1) Directly when perio-pathogens 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 response to mothers oral pathogens26,
4) By mothers enhanced antigraft response\textsuperscript{27};
5) Genetically, by heritable factors\textsuperscript{28}.

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

The representation of periodontitis in both groups was 39.3% which is in accordance to data presented by Lieff and coworkers, who have found that in the population of pregnant women we will have around 40% of periodontitis\textsuperscript{31}.

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 and coworkers there was no significant association between disease of the periodontium and preterm birth but the study cannot exclude an association between periodontal disease and intra-amniotic inflammation\textsuperscript{32}.

Preterm birth, as well as periodontitis, is characterized in an increased level of inflammatory markers and among them IL-1\textsubscript{\beta} and PGE\textsubscript{2}\textsuperscript{4,15}.

IL-1\textsubscript{\beta} is a pro-inflammatory cytokine and is expressed by many cells including macrophage, NK cells, monocytes, and neutrophils. It belongs to the IL-1 family cluster that includes the IL-1\textsubscript{a}, and IL1-RN genes. IL-1\textsubscript{\alpha} and IL-1\textsubscript{\beta} 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 IL1-\beta has to be processed into mature bioactive form of IL1-\beta and is usually proteolitically mediated by inflammatory cysteine protease caspase-1\textsuperscript{33}. IL-1\textsubscript{\beta} is one of the cytokine in the inflammatory cascade resulting in increased production of cyclooxygenase (COX)-2 and prostaglandins (PGs)\textsuperscript{10,33}. Prostaglandins act as long-term
mediators of inflammation. IL-1β and PGE2 are involved in biochemical processes in inflammation along with delivery\textsuperscript{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\textsuperscript{34}. IL-1β will activate an inflammatory cascade that leads to increased concentrations of PGE2 that are required for onset of delivery. PGs are the most effective mediators for cervical dilatation in women and stimulation of labor. In the myometrium PGs 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\textsuperscript{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 and coworkers 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\textsuperscript{35}.

IL-1β and PGE2 are increased in infections of the periodontium tissue. Many authors have showed that salivary IL-1b levels between subjects with periodontitis were significantly greater than those detected for healthy controls. A study performed by Kinney, and Rathnayake, reported that levels of IL-1β correlated with periodontium status. Moreover, salivary IL-1b of IL1-β correlated significantly with clinical degree of periodontal inflammation\textsuperscript{15,36}. Certain number of research papers show that in patients with periodontitis there is a faster deterioration and rejection of allografts\textsuperscript{23}.

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\textsuperscript{37}.
Published data has shown that IL-1β enhances the host antigraft adaptive response and suggests that IL1-ß may have an inherited condition that causes a hyperactivity, which in turn may be responsible for the PTB\textsuperscript{38}.

Vamvakopoulos and coworkers have demonstrated an association between IL-1β and chronic rejection at the genetic level in heart graft recipients. The risk of rejection was a 20-fold increase in patients with both the IL-1β (π3953) C allele and the IL1RN1 allele\textsuperscript{39}.

Peter Medawar 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 \textsuperscript{40}. That tolerance is compromised in PTB.

Many authors reported that levels of IL-1β, IL-6 and PGE2 in the blood samples showed higher levels between the preterm delivery women and healthy control groups 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 the level of IL-1β concentration is an independent predictor of preterm delivery in patients with threatened preterm labor\textsuperscript{41}.

Consistently with the reported results, in our study group plasma levels of IL-1β and PGE2 were significantly higher in PTB group in comparison to FTB group. Differences in plasma CRP concentrations among PTB and FTB group did not reach statistical significance. This data is in accordance with results of Michalowicz and coworkers who have suggested that in pregnant women levels of CRP were not associated with infant birth weight or a risk for preterm birth\textsuperscript{42}.

PGE2 in saliva of PTB mothers shows a significant positive correlation with IL-1β in saliva, while plasma level of PGE2 shows 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 (3-4h), therefore it will only show a positive correlation only if tested on the place of its secretion and not in plasma where it is distant to the inflammatory lesion. Here the correlation
becomes negative because PGE2 is a long term activator of the inflammatory pathway and it remains high even after it’s descend to plasma, but the levels of IL-1β decrease due to its short half-life\(^{43}\).

Higher levels of IL-1β and PGE2 in the PTB group could be explained by the effect of the inflammations in the periodontal tissue, hyperactive IL-1β\(^{36}\), reaction of the rejection of allograft\(^{23,39}\) or hereditary genetic factors\(^{28,37}\).

The recognition that heritable factors play a role in PTB\(^{37}\) is compatible with the notion that extent of periodontitis is influenced by secretor status which is to a large extent inherited and stable\(^{17,28}\).

Secretor 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\(^{18}\).

In secretors salivary blood group antigens agglutinate oral pathogens and thus enable multiple functions of saliva such as rinsing, bacterial clearance and antimicrobial defense\(^{19}\). In non-secretors there are no soluble blood group substances, oral pathogens recognize histo-blood group antigens on cells surface as attachment factors, form poly microbial gingival/subgingival biofilm, and cause gingival infection and induction of periodontitis resulting in an increased level of TNF-alpha, IL-1β, IL-6, and PGE2\(^ {10,15,18}\).

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 infertility and success of assisted reproductive techniques\(^ {44}\).

Furthermore, oligosaharides 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 of necrotizing enterocolitis deaths in newborns\(^ {45}\).
According to literature data it is conceivable that the non-secretors with a lower level of iso-antibodies and immunoglobulins may have a lower resistance to infection and thereafter higher rate of periodontitis\textsuperscript{19}. Results of Rocha suggest an active role of mucin glycoproteins in the innate immune regulation of periodontal bacterial colonization and disease progression\textsuperscript{16}. Tabasum and coworkers reported 22.2\% non-secretors in the chronic periodontitis group\textsuperscript{46}. 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\textsuperscript{7,9}.

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\textsuperscript{22}.

5. 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. PTB is a complex syndrome and there are other unknown factors responsible for the PTB. 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 between periodontitis, secretor status and adverse pregnancy outcomes. A randomized clinical trial will be necessary to appropriately test our hypothesis and conclude whether non-secretor status have impact on adverse pregnancy outcome.

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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.


Figure 1. Quantitative comparison of median blood IL-1b concentration in study group with periodontitis according to birth term and secretor status, *,** P<0.05, 0.01, respectively vs. preterm non-secretor group, ††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 PTB non-secretor group differed significantly from other groups at level P < 0.01.
Table 1.
Demographic and clinical characteristics of patients with preterm and term delivery

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Preterm birth</th>
<th>Term birth</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, years, mean ± SD</td>
<td>30.7±5.5</td>
<td>27.0±3.9</td>
<td>Ns</td>
</tr>
<tr>
<td>Maternal blood type (ABO), n (%):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>16 (28.6)</td>
<td>14 (25.0)</td>
<td>Ns</td>
</tr>
<tr>
<td>A</td>
<td>24 (42.8)</td>
<td>28 (50.0)</td>
<td>Ns</td>
</tr>
<tr>
<td>B</td>
<td>14 (25.0)</td>
<td>12 (21.4)</td>
<td>Ns</td>
</tr>
<tr>
<td>AB</td>
<td>2 (3.6)</td>
<td>2 (3.6)</td>
<td>Ns</td>
</tr>
<tr>
<td>Maternal RhD factor, n (%):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>46 (82.1)</td>
<td>50 (89.3)</td>
<td>Ns</td>
</tr>
<tr>
<td>Negative</td>
<td>10 (17.9)</td>
<td>6 (10.7)</td>
<td>Ns</td>
</tr>
<tr>
<td>Maternal secretory status, n (%):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secretor</td>
<td>44 (78.6)</td>
<td>44 (78.6)</td>
<td>Ns</td>
</tr>
<tr>
<td>Non-secretor</td>
<td>12 (21.4)</td>
<td>12 (21.4)</td>
<td>Ns</td>
</tr>
<tr>
<td>Gingival status, n (%):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy periodontium</td>
<td>4 (7.1)</td>
<td>31 (55.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gingivitis</td>
<td>15 (26.8)</td>
<td>18 (32.1)</td>
<td>Ns</td>
</tr>
<tr>
<td>Periodontitis</td>
<td>37 (66.1)</td>
<td>7 (12.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Infant gender, n (%):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>24 (42.8)</td>
<td>34 (60.7)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Male</td>
<td>32 (57.2)</td>
<td>22 (39.3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Infant birth length (cm) mean ± SD</td>
<td>44.96±3.1</td>
<td>50.68±1.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Infant birth weight (g) mean ± SD</td>
<td>1862±381</td>
<td>3300±208</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Apgar score, mean ± SD</td>
<td>8.02±1.5</td>
<td>9.02±0.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Apgar score 9-10/1 min</td>
<td>32 (57.2)</td>
<td>56 (100)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Apgar score &lt;7/1 min</td>
<td>5 (8.9)</td>
<td>0 (0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td>35 (62.5)</td>
<td>34 (60.7)</td>
<td>Ns</td>
</tr>
<tr>
<td>Multiparous</td>
<td>21 (37.5)</td>
<td>22 (39.3)</td>
<td>Ns</td>
</tr>
</tbody>
</table>
Table 2.
Number of secretors and non-secretors women according to their periodontal status

<table>
<thead>
<tr>
<th>Secretor status</th>
<th>Periodontal status</th>
<th>Healthy periodontium n (%)</th>
<th>Gingivitis n (%)</th>
<th>Periodontitis n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NON-secretor</td>
<td></td>
<td>4 (11.4)</td>
<td>6 (18.2)</td>
<td>14 (31.8)*</td>
</tr>
<tr>
<td>Secretor</td>
<td></td>
<td>31 (88.6)</td>
<td>27 (81.8)</td>
<td>30 (68.2)*</td>
</tr>
</tbody>
</table>

Legend: *chi-square test $\chi^2=5.00$, p<0.05

Table 3.
Number of subjects in study group (PTB and FTB) according the periodontal and secretor status

<table>
<thead>
<tr>
<th>Periodontal status</th>
<th>PTB Sekretors n(%)</th>
<th>PTB non-Sekretors n(%)</th>
<th>FTB Sekretors n(%)</th>
<th>FTB non-Sekretors n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Periodontitis</td>
<td>27 (61.4)</td>
<td>10 (83.3)*</td>
<td>3 (6.8)</td>
<td>4 (33.3)</td>
</tr>
<tr>
<td>Gingivitis</td>
<td>14 (29.5)</td>
<td>2 (16.7)</td>
<td>14 (31.8)</td>
<td>4 (33.3)</td>
</tr>
<tr>
<td>Healthy periodontium</td>
<td>4 (9.1)</td>
<td>0 (0.0)</td>
<td>27 (61.4)*</td>
<td>4 (33.3)</td>
</tr>
</tbody>
</table>

Legend: *Chi-square test; $\chi^2=43.6$, P<0.001
Table 4.
Inflammation markers and basic hematological parameters in preterm and term birth subgroups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Preterm Birth</th>
<th>Full Term Birth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (25th–75th percentile)</td>
<td>Median (25th–75th percentile)</td>
</tr>
<tr>
<td>IL-1ß, sal, pg/ml</td>
<td>10.837 (9.882-11.570)</td>
<td>11.778 (5.690-12.094)</td>
</tr>
<tr>
<td>IL-1ß, pl, pg/ml</td>
<td>0.0125 (0.0115-0.0141)</td>
<td>0.0099 (0.0075-0.0133)</td>
</tr>
<tr>
<td>PGE2.sal, pg/ml</td>
<td>279 (62-567)</td>
<td>327 (215-423)</td>
</tr>
<tr>
<td>PGE2, pl, pg/ml</td>
<td>967 (107-1267)</td>
<td>461(36.1-1600)</td>
</tr>
<tr>
<td>pl hsCRP, mg/L</td>
<td>20.4 (7.43-36.7)</td>
<td>19.0 (8.4-47.6)</td>
</tr>
<tr>
<td>WBCx10⁹/L</td>
<td>15.2 (13.4-16.8)</td>
<td>15.6 (12.7-18.2)</td>
</tr>
<tr>
<td>Hb, g/L</td>
<td>112 (95.6-118)</td>
<td>111 (101-122)</td>
</tr>
<tr>
<td>Plt x10⁹/L</td>
<td>204 (202-265)</td>
<td>218 (201-293)</td>
</tr>
</tbody>
</table>

Legend: sal-saliva, pl-plasma; WBC-White blood cells; Hb-Hemoglobin; Plt-Platelets;
Table 5.

Correlation of laboratory parameters in PTB and FTB group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PTB</th>
<th>FTB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R, p</td>
<td>R, p</td>
</tr>
<tr>
<td>sal IL-1b - sal PGE2</td>
<td>0.416</td>
<td>0.336</td>
</tr>
<tr>
<td></td>
<td>0.017</td>
<td>0.09</td>
</tr>
<tr>
<td>pl IL-1b - pl PGE2</td>
<td>0.592</td>
<td>0.138</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>0.48</td>
</tr>
<tr>
<td>sal PGE2 – Age</td>
<td>0.34</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>0.06</td>
<td>0.289</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

Legend: Sal-saliva, IL-1b- IL1 ß, pl-plasma, R-Spearman correlation coefficient, p-statistical significance, PTB-preterm birth, FTB-full term birth
Table 6.
Inflammation markers and basic hematological parameters in a sub-groups according to secretory status

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NON-secretor</th>
<th>Secretor</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE *</td>
<td>35 (33-37)</td>
<td>29.5 (23-31)</td>
<td>Ns</td>
</tr>
<tr>
<td>IL-1β, sal, pg/ml*</td>
<td>11.203 (10.834-11953)</td>
<td>10.598 (5.690-11.953)</td>
<td>Ns</td>
</tr>
<tr>
<td>IL-1β, pl, pg/ml*</td>
<td>0.0149 (0.0126-0.0173)</td>
<td>0.0126 (0.0089-0.0137)</td>
<td>0.081</td>
</tr>
<tr>
<td>PGE2.sal, pg/ml*</td>
<td>506 (279-733)</td>
<td>327 (62-514)</td>
<td>Ns</td>
</tr>
<tr>
<td>PGE2, pl, pg/ml*</td>
<td>454 (107-800)</td>
<td>1117 (142-1433)</td>
<td>Ns</td>
</tr>
<tr>
<td>hsCRP, pl, mg/L*</td>
<td>22.6 (8.4-46.7)</td>
<td>19.0 (7.3-40.6)</td>
<td>Ns</td>
</tr>
<tr>
<td>WBCx10e9/L*</td>
<td>12.5 (9.8-15.2)</td>
<td>16.0 (13.4-19.1)</td>
<td>Ns</td>
</tr>
<tr>
<td>HGB, g/L*</td>
<td>104.8 (95.6-114.0)</td>
<td>111.5 (98.0-123.2)</td>
<td>Ns</td>
</tr>
<tr>
<td>PLTx10e9/L*</td>
<td>183 (162-204)</td>
<td>220 (204-279)</td>
<td>0.040</td>
</tr>
</tbody>
</table>

Legend: *Medians (25th – 75th percentile); sal-saliva, pl-plasma, WBC-White blood cells; Hb-Hemoglobin; Plt-Platelets;

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