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RESEARCH ARTICLE

Serum Cytokine Levels in Term and Preterm Deliveries Relating to the Periodontal Health of Mothers: A Pilot Study

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ABSTRACT

Background: The aim of the study was to evaluate serum-levels of interleukin-1, beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) at birth and compare the values in case of preterm birth and normal birth groups of mothers considering the mothers' periodontal status.

Materials and methods: Blood samples from 81 women (preterm birth, 41 women, and term birth, 40 women) were collected within half an hour of after delivery. Serum levels of IL-1β and TNF-α were measured. Periodontal status was characterized by bleeding on probing (BOP) and probing depth (PD).

Results: The frequency of BOP differed significantly between preterm and term groups; however, mean PD did not show a significant difference. Serum IL-1β levels were significantly higher in the preterm birth group. The levels TNF-α were slightly bigger in the term birth group, the difference was significant. The rank correlation showed a significant negative relationship between serum IL-1β and TNF-α level and birth weight and the length of pregnancy, and also between BOP frequency and the length of pregnancy.

Conclusion: Within the limitations of the study, it was found that IL-1β and TNF-α levels were higher when the delivery occurred preterm and the birth weight was smaller; however, a significant increase of cytokines in the serum in connection with maternal periodontal disease was not detected. Periodontics of mothers was not associated with preterm birth in the sample.

Keywords: Cytokine, IL-1β, Pregnancy, Periodontal disease, Preterm birth, TNF-α.

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INTRODUCTION

There is still some controversy concerning whether preterm birth and low birth weight has a relationship with periodontal inflammation of pregnant women. The results of some reviews do not agree with the hypothesis,[1-3] while others found evidence or supporting data for the connection between preterm delivery and periodontitis.[4-7] The reason for the controversy may be that the studies differed in protocol, patients' number, social circumstances, ethnic, and education diversity of subjects and also in the criteria of periodontitis.[8] However, research in this field is important since the number of preterm deliveries did not substantially decrease in the last decades.[9]

Periodontal disease may lead to adverse pregnancy outcomes. A possible mechanism: molecular pathways eliciting this microbial effect on pregnancy have been suggested to involve microbial lipopolysaccharide (LPS) and toll like receptor (TLR) pathway, resulting in the release of primary inflammatory mediators, such as interleukin-1 (IL-1), IL-6 and tumor necrosis factor-alpha (TNF-α) and secondarily PGE2, which is capable to induce uterine contraction and modulating placenta blood flow, and in human can mediate cervical dilatation and premature labor.[10]

Cytokines play an important role in the host inflammatory response against infection, including periodontal infection. They take part in different biological actions on immune and nonimmune target cells,[11] and on tissues of the fetoplacental unit. The proinflammatory cytokines, like IL-1, IL-6 and TNF-α stimulate prostaglandin synthesis by via the human placenta and chorioamnion. The normal physiology of pregnancy is also regulated by prostaglandins. Gibbs et al[12] summarized the evidence supporting the role of these inflammatory mediators in human labor. Elevated levels of inflammatory mediators may play a role in premature delivery. These inflammatory mediators are produced in the periodontal tissues

as a consequence of periodontopathogenic bacterial invasion into the periodontal tissues,[13,14] and may enter the blood stream reaching the fetoplacental unit thus causing inflammatory reactions, like chorioamnionitis and a possible effect on the progress and outcome of the pregnancy.[15] In an animal study, it was found that IL-1β and TNF-α had a primary role in provoking preterm labor in connection with inflammation.[16]

Aim of the study was to estimate the role of hematogen spreading of inflammatory mediators in preterm birth through the evaluation of the levels of IL-1β and TNF-α in maternal serum and compare these data with the severity of the periodontal disease characterized by sulcus probing depth and bleeding on probing of mothers in a Hungarian group of women, and through that to search for evidence of a relationship between preterm birth and periodontal infection.

MATERIALS AND METHODS

The study protocol was approved by the Regional and Institutional Human Medical Biological Research Ethics Committee of the University of Szeged, Hungary. After receiving information about the study, mothers participated voluntarily. All the women signed a written informed consent before starting the examinations.

Study Population

Only women with singleton pregnancies, who voluntered and had delivery at the Department of Obstetrics and Gynecology, Faculty of Medicine, University of Szeged were included in the study. Women were systemically healthy, without any known systemic diseases, which may play a role in preterm birth. They had at least 16 teeth. Women having alcohol and drug abuse, or being younger then 17 years were excluded. Those who would require antibiotic cover for dental treatment or those receiving antibiotics at the time of the dental examination were not enrolled either. Women enrolled into the study did not receive antibiotic treatment 4 weeks before the delivery.

Mothers were divided into two groups: term mothers (40 women, average age 30.8 years) and preterm mothers (41 women, average age 30.4 years), according to the newborn's weight and time of delivery. The preterm group consisted of those mothers whose baby's weight was <2500 gm or the delivery occurred before 37 completed weeks of pregnancy.[17]

A structured questionnaire was used to collect information about patients' education levels, type of work, and place of residence. Education levels were categorized as follows: primary school, technical school, grammar school, and higher education. The occupation of the patient was defined as manual worker, intellectuals or 'other occupation', e.g. shop assistant, office staff, and housewife.

Collecting Blood Samples and Laboratory Analyses

Blood samples from each woman were collected within 30 minutes of delivery. Five milliliters of blood were collected from each woman in a BD Vacutainer tube (SST™ II Advance, Ref 367955, BD Plymouth PL6 7BP, UK). After clotting, the blood samples were centrifuged for 10 minutes at 3000 revolutions per minute (RPM) to separate serum from blood cells. Serum samples were stored at −80°C till the time of laboratory analyses.

Serum IL-1β and TNF-α levels were determined by High Sensitivity ELISA kits (Quantikine HS, R&D Systems, Minneapolis, USA) according to the manufacturer's instructions. Briefly, these assays employ the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-1β/human TNF-α has been precoated onto a microplate. Standards and samples were pipetted into the wells and any IL-1β/human TNF-α present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-1β/human TNF-α was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells. After an incubation period, an amplifier solution was added to the wells and color developed in proportion to the amount of IL-1β/human TNF-α bound in the initial step. The color development was stopped and the intensity of the color was measured.

The standards ranged from 0 to 8 pg/ml human IL-1β/IL-1F2 and from 0 to 32 pg/ml human TNF-α. The minimum detectable doses (MDD) were 0.039, 0.057 and 0.106 pg/ml for human IL-1β/IL-1F2 and human TNF-α, respectively. All absorbance values were read in an ELISA plate reader and the concentration of the samples was calculated by Microsoft Excel. All patient's and standard samples were run in duplicate. The mean values of measurements were used for statistical analyses.

Periodontal Examinations

Periodontal examinations were carried out within 3 days of delivery, with the patients sitting in a dental chair at the department of obstetrics and gynecology. A Michigan periodontal probe (Hu-Friedy, USA) was used for measuring probing depth (PD) to the nearest millimeter. It was measured from the gingival margin to the most apical point of the sulcus/pocket, at six sites of each

tooth, i.e. mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual and distolingual, with the exception of third molars. Bleeding on probing (BOP) was considered as positive if it occurred within 15 seconds after assessing probing depth at any site of the examined tooth.

Examinations were completed by a dentist specialized in periodontology, and before the study was started the examiner re-examined 10 patients after a 30 minutes time period had lapsed to test her reproducibility. Relating the periodontal status (probing depth, BOP) the intraclass correlation coefficient was 0.94 or greater.

Periodontitis had the criteria of having ≥ 4 mm probing depth, at least at one site of the teeth and BOP at ≥ 50% of teeth. A woman without these criteria was regarded as periodontally healthy.

STATISTICAL ANALYSIS

Univariate analysis of the data included descriptive statistics and testing continuous data for normal distribution. Because of the lack of normality the Mann-Whitney test was used to compare the preterm and control groups and Spearman's rho test to assess correlations. For categorical data, standard Chi-squared test as well as Fisher's exact p was applied. For the differences in the amount of inflammatory mediators two-way analysis of variance (ANOVA) was used with the two factors being preterm birth and periodontal disease. Statistical significance was set at the $p < 0.05$ level.

RESULTS

The preterm and term birth groups did not differ in relation to age and social background, but differed in terms of place of residence (Table 1). Altogether only 5 mothers admitted smoking during pregnancy, their number in both groups was very small (two in preterm group, and three in control group). Birth weight and weeks of pregnancy are shown in Table 2; there were significant differences according to the grouping of the mothers.

Table 3 shows the number of mothers who had BOP ≥ 50% of the teeth and had PD ≥ 4 mm at least at one site. The periodontitis characterized by having a probing depth ≥ 4 mm and BOP ≥ 50%, did not differ significantly between the preterm and term mothers, although the frequency of these criteria occurred more often in the preterm group. The frequency of BOP and the mean PD were bigger in the preterm group than in the term group [45 *vs* 27%, (p = 0.006) and 1.87 *vs* 1.76 mm].

Serum IL-1β level was significantly higher (p = 0.000), while TNF-α levels were smaller (p = 0.026) in the preterm birth group, although the difference between the TNF-α levels was minimal (Table 4). In the Table 5, the serum cytokine levels are shown according to the presence or non presence of periodontal disease. With the exception of one value (TNF-α = 3.10 pg/ml) the cytokine levels were higher in patients without periodontitis. Only the IL-1β level showed significant difference (p = 0.003) between the preterm and term birth groups.

Table 1: Characteristics of mothers in the preterm birth and control groups

	Preterm birth (n = 41)	*Term birth (n = 40)*	*All (n = 81)*	*p-value*
Age				
Mean	30.4	30.8	30.5	0.72
Minimum	17.9	19.4	17.9	
Maximum	43.5	40.0	43.5	
Educational level of mothers				
Primary school	4 (9.8%)	4 (10%)	8	0.68
Technical school	8 (19.5%)	4 (10%)	12	
Grammar school	12 (29.3%)	13 (32.5%)	25	
Higher education	17 (41.5%)	19 (47.5%)	36	
Occupation of mothers				
Unemployed	6 (14.6%)	2 (5.1%)	8 (10%)	0.52
Manual worker	8 (19.5%)	10 (25.6%)	18 (22.5%)	
Other	2 (4.9%)	1 (2.6%)	3 (3.8%)	
Intellectual	25 (61%)	26 (66.7%)	51 (63.8%)	
Place of residence				
City	26 (63.4%)	34 (87.2%)	60 (75%)	0.02*
Village	15 (36.6%)	5 (12.5%)	20 (25%)	
Smoking				
No	39 (95.1%)	37 (92.5%)	76 (93.8%)	0.62
Yes	2 (4.9%)	3 (7.5%)	5 (6.2%)	

For the differences between preterm and control groups, Fisher's test was used; Significance level: *p < 0.05

Table 2: Data of deliveries in the preterm birth and control groups

	Preterm birth (n = 41)	*Term birth (n = 40)*	*All (n = 81)*	*p-value*
Birth weight (gm ± SD)	2315.6 ± 596	3507.0 ± 408	2903.9 ± 786	0.000***
Minimum	980	2640	980	
Maximum	2880	4390	4390	
Weeks of pregnancy (±SD)	33.9 ± 2.5	39.5 ± 1.2	36.6 ± 3.4	0.000***
Minimum	26	37	26	
Maximum	36	42	42	

Significance level: ***p < 0.001

Table 3: Periodontal status in the preterm birth and control groups

	Preterm birth (n = 41)	*Term birth (n = 40)*	*All (n = 81)*	*p-value*
BIO ≥ 50%				
No	25 (61%)	32 (80%)	57 (70.4%)	0.08
Yes	16 (39%)	8 (20%)	24 (29.6%)	
PD ≥4 mm				
No	24 (58.5%)	25 (62.5%)	49 (60.5%)	0.82
Yes	17 (41.5%)	15 (37.5%)	32 (39.5%)	
BOP ≥50% + PD ≥4 mm				
No	28 (68.3%)	33 (82.5%)	61 (75.3%)	0.19
Yes	13 (31.7%)	7 (17.5%)	20 (24.7%)	
Mean PD (mm ± SD)	1.87 ± 0.52	1.76 ± 0.45	1.81 ± 0.49	0.08
BOP frequency (% ± SD)	45 ± 31	27 ± 26	36.6 ± 3.4	0.006**

Significance level: **p < 0.01

Table 4: Amount of inflammatory mediators in preterm birth and in control groups

	Preterm birth (n = 41)	*Term birth (n = 40)*	*All (n = 81)*	*p-value*
IL-1β (pg/ml ± SD)	1.71 ± 1.2	0.83 ± 1.2	1.27 ± 1.2	0.000***
TNF-α (pg/ml ± SD)	2.30 ± 1.4	2.33 ± 4.2	2.32 ± 3.1	0.02*

Significance level: *p < 0.05; ***p < 0.001

Table 5: Amount of inflammatory mediators in preterm birth and in control groups with and without periodontal inflammation

	Preterm birth (n = 41) BOP ≥ 50% + PD ≥ 4 mm		*Term birth (n = 40) BOP ≥ 50% + PD ≥ 4 mm*		
	No (n = 28)	*Yes (n = 13)*	*No (n = 33)*	*Yes (n = 7)*	*p-value*
IL-1β (pg/ml ± SD)	1.77 ± 1.2	1.57 ± 0.8	0.89 ± 1.31	0.52 ± 0.23	0.003**
TNF-α (pg/ml ± SD)	2.38 ± 1.6	2.13 ± 1.0	2.16 ± 4.4	3.10 ± 3.5	0.66

p-value characterizes significance of difference between preterm and control group based on two-way ANOVA test; Significance level: **p < 0.01

Table 6: Rank correlations between periodontal status, birth data and inflammatory mediator levels

	Birth weight	*Weeks of pregnancy*	*IL-1β*	*TNF-α*
BOP frequency (%)	–0.209	–0.300**	0.067	0.100
BOP ≥ 50%	–0.179	–0.233*	–0.022	0.115
PD ≥ 4 mm	–0.036	–0.017	–0.016	–0.017
BOP ≥ 50% + PD4 mm	–0.136	–0.203	0.008	0.135
Mean PD	–0.104	–0.112	–0.048	0.062
Birth weight	–	0.824***	–0.494***	–0.221*
Weeks of pregnancy	0.824***	–	–0.573***	–0.241*

The table contains the Spearman rho values: Significant level: *p <0.05, **p <0.01, ***p <0.001

The rank correlations between periodontal status and birth data (Table 6) showed that if BOP occurred more frequently then the length of the pregnancy was significantly shorter. Length of pregnancy and birth weight correlated negatively with BOP frequency and BOP ≥50%. The serum levels of inflammatory mediators, such

as the levels of IL-1β and TNF-α correlated also negatively with birth weight and the weeks of pregnancy. No significant correlation was detected between the serum level of cytokines and periodontal parameters (PD, BOP) in this study group.

DISCUSSION

The number of mothers living in a village or in a city differed significantly (p = 0.002), but in the southern part of Hungary, the living conditions in villages and cities are similar, therefore, this difference is negligible, and it did not influence the pregnancy outcome or other results. The number of women with a higher education and working as intellectuals was large, because in Szeged the university attracts many professionals to work.

The criteria for periodontitis (BOP ≥ 50% + PD ≥4 mm at least at one site together) were selected based on the authors' previous studies. Bleeding on probing was accepted as an obvious sign of periodontal inflammation.[18,19] The probing depth bigger than 4 mm is regarded as a 'critical probing depth',[20] because smaller probing depth belong to clinical variation.[21] During the examination, the actual status of the periodontium was examined and not the result of previous processes in the periodontium. Lang and Tonetti[22] suggested these parameters to be considered as important factors in risk assessment of patients' for recurrence of periodontitis.

Alveolar bone level was not assessed radiographically, because radiographic evaluation is not always reliable in the early stage of periodontitis. Since mothers were relative young great bone loss was not expected. Additionally, the size of the surface area of the pocket,[23] through which bacteria and their products can diffuse into the periodontal tissues, has been found to be more important than bone levels *per se*, as these indicate only the consequence of previous inflammation.

Further reason for the selection of these criteria was that the measurement of the probing depth is quicker in women close after delivery for whom sitting or lying in a dental chair might not be comfortable, then the measurement of clinical attachment loss (CAL). In this young age, the attachment loss is probably small, since periodontitis is more frequent in older ages.[24] Additionally, PD measurements are regarded useful in the assessment of the seriousness of periodontal disease.[25] Measurements of PD and CAL correlate well especially in younger population, and both are accepted as measurements of periodontal status.[8] Other criterion for periodontitis was BOP at ≥ 50% of the teeth was used because gingival bleeding shows well the seriousness of the inflammation.[25,26] In another[27] study, it was also found that BOP was an important periodontal finding in connection with preterm birth.

The detectable amount of IL-1β was significantly higher in the preterm group (p = 0.000), while the amount of TNF-alpha significantly lower (p = 0.026), although the mathematical value differed slightly and clinically, the difference was negligible. In the study of Hasegawa et al,[28] and the relationship between serum cytokine levels and periodontal status was examined. It was found that women with threatened preterm birth had worse periodontal status and serum cytokine levels of IL-6, IL-8 and IL-1 β were higher than in non-threatened preterm labor women, however, the serum level of TNF-α was significantly higher in the term birth group. It was concluded that higher level of cytokines might cause preterm birth through premature uterine contractions. Sert et al found that the serum levels of the systemic proinflammatory cytokines: IL-1β, IL-6, TNF-α were significantly higher in pregnancy relating to non-pregnant women's data.[29] They found significantly higher serum IL-1β level in the preterm low birth weight group than in term birth, preterm birth and nonpregnant group in case of periodontitis.

In our study, there was a significant relationship between serum TNF-α level and preterm birth, but there was no significant relationship between periodontal status and TNF-α level. In another study, TNF-α levels were significantly higher in the group of women who had more sites with mild periodontitis and had threatened premature labor (TPL), than those who had less periodontitis sites and belonged to the non-TPL group.[30] Similarly, Sert et al[29] found no significant difference among groups (term birth, preterm birth) relating TNF-α levels.

In the study of Michalowicz et al[31] periodontal treatment did not associate with the levels of serum inflammatory mediators, and did not significantly reduced serum IL-1β, IL-6, TNF-α level. On the contrary, Offenbacher et al[32] found a decrease in the amount of serum IL-6 after improving periodontal heath during pregnancy. It seems that there is no overall evidence for the decreasing level of inflammatory mediators in serum after periodontal treatment,[33] but in pregnancy the level of serum cytokines is higher,[29] and there may be a small rise in the level and due to this it reaches a threshold grade and causes contraction of the uterus and the beginning of labor.

Bleeding of probing frequency had a significant relationship with preterm birth (p = 0.006) showing the importance of gingival inflammation during pregnancy.[26] In this study group, there was no significant difference between the frequency of preterm birth and BOP ≥ 50%, PD ≥ 4 mm, neither BOP ≥ 50% + PD ≥ 4 mm, nor average PD. Although in the preterm birth group a greater percentage of the patients had PD ≥ 4 mm or BOP

≥ 50%, than in the term birth group, and frequency of BOP ≥ 50% + PD ≥ 4 mm was twice as big in preterm group than in term group, a significant difference was not found, probably due to the small sample size.

The hypothesis that in patients with a higher frequency of BOP and having deeper pockets higher levels of cytokines can be found in the serum was not supported by this investigation.

Further investigations are important, since the mean age of mothers at first delivery has increased in the last 40 years from 20 to 25 and 25 to 30 years or over 30 years,[34] when the frequency of chronic gingivitis and periodontitis increases.[35]

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