Bull. Group. Int. Rech. Sci. Stomatol. Odontol. 45, 18-28, 2003

THE SECRETION OF PROSTAGLANDIN E_2 AND INTERLEUKIN 1-BETA IN WOMEN WITH PERIODONTAL DISEASES AND PRETERM LOW-BIRTH-WEIGHT

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KEYWORDS: preterm labor, low birth weight, periodontitis, risk factors
MOTS CLES: accouchement prématuré, poids faible de naissance, périodontite, facteurs du risque

RESUME

La prématurité est une des causes principales de maladies des nouveaux-nés et de la mortalité post-natale. Les observations cliniques ont montré que la périodontite chez les femmes enceintes peut être le facteur du risque directe de l'accouchement prématuré dont l'influance est plus importante si l'on compare avec d'autres conditions. La présente étude avait pour le but de l'estimation du lien entre les pérodontopathies et le fait d'un accouchement prématuré d'un nouveau-né avec un poids faible de naissance dans la population des femmes polonaises et l'évaluation des concentrations PGE_2 et IL-1β dans le liquide gencival et le sérum sanguin chez les femmes avec accouchement prématuré (PLBW) par rapport au groupe de contrôle et la séparation des agents inflammatoires dans le sang après la stimulation avec lipopolysaccharide bactérien (LPS) dans ce deux groupes de femmes. Le groupe de recherche se composait de 84 femmes avec PLBW (femmes primipares-39,2%) entre 17 et 41 ans (moyenne 27,57). Le groupe de contrôle se composait des 44 femmes (47,7% des femmes primipares) entre 16 et 38 ans (moyenne 26,36) qui avait accouchés après semaine 37 de grossesse un nouveau-né du poids supérieure du 2500 g et toutes les grossesses précedentes se sont terminées ainsi. La concentration IL-1β et PGE_2 dans le sérum sanguin et dans le liquide gencival a été déterminée à l'aide du méthode immunoenzymatique.Dans la population examinée des femmes nous avons prouvé la plus grande probabilité de PLBW au dessus de 28 ans et en présence d'autres facteurs médicaux du risque connus de cette pathologie. Chez les femmes primipares au dessus de 28 ans, nous avons prouvé la probabilité de 4 fois plus élèvée d'un accouchement prématuré. Dans le cas où elles avaient une périodontite avancée et généralisée, nous avons prouvé la probabilité de 3,9 fois plus élèvée du PLBW par rapport aux femmes qui n'avaient pas de périodontophatie soit elles avaient la périodontophatie moins avancée. Chez toutes les femmes et chez les femmes primipares avec PLBW nous avons prouvé une concentration considérablement plus élèvée de PGE_2 et IL-1β dans le liquide gencival et en plus, chez les femmes primipares, PGE_2 dans le sérum sanguin par rapport au groupe de contrôle.

ABSTRACT

Prematurity is of one of the main causes of neonatal morbidity and mortality. Clinical observations show, that periodontitis in pregnant women can be a direct risk factor for preterm labor, with a greater influence rate compared to other risk factors. The aim of the study was to asses the relationship between periodontal diseases and PLBW in the population of women from the Lower Silesian Region (Poland), and the evaluation of prostaglandin E_2 (PGE_2), interleukin -1 beta (IL-1β) levels in gingival cervicular (GCF) and blood serum in women with PLBW and women giving birth on time as well as secretion of these proinflammatory mediators in whole blood after bacterial lipopolysaccharide stimulation. The study group consisted of 84 women with PLBW (39.2% primiparous), aged 17-41 (mean 27.57). The controls were 44 women (47.7% primiparous) aged 16-38 (mean 26.36) who gave birth on time to a normal birthweight baby. PGE_2 and IL-1β concentrations in serum and GCF were determined by means of immunoenzymatic method (EIA). In the studied population women over 28 years and exposed to medical risk factors had more frequent PLBW occurrence probability. In primiparous over 28 there is 4 times greater probability of preterm labor, and in case of the severe and generalized periodontitis presence there is 3.9 times higher possibility of PLBW compared to women with healthy periodontium. In all women with PLBW there is a significantly higher PGE_2 and IL-1β concentration in GCF, and in primiparous also PGE_2 level in blood serum, compared to controls.

INTRODUCTION

The term preterm labor (PLT) refers to the pregnancy outcome at less than 37 weeks' and after 22 weeks' gestation. Prematurity is strictly connected with preterm labor. Its obstetric criteria state as follows: infant's weight under 2500 g (LBW-low birth weight) and labor at less than 37 weeks' gestation (Breborowicz et al. 1999, Klimek et al. 1994). If the two criteria meet together a term preterm low birth weight (PLBW) is used.

The percentage of preterm births in the world varies between 3% in the Netherlands and Finland, 4% in France and Switzerland, 6-7% in Great Britain, in Poland 7.2-8.4%, 7.9-11% in the USA and 34.7% in India (Reroń 2001, Savitz et al. 1991, Wasiela et al. 2001). Apart from the infant mortality index, the percentage of prematurity and of low birth weight are the indices of national health status.

Prematurity is one of the leading causes of morbidity and mortality among neonates in the developing countries. It is said, that over 60% of the mortality occurring among infants is due to the complications of preterm labor, excluding the anatomic and chromosomal congenital defects (McCormick 1985). This percentage is still maintaining at such high level in spite of undoubted progress in neonatology, which can be expressed in 80% life maintenance possibility for neonates with 500-1000 g birth weight. Among diseases more often affecting neonates with PLBW compared to babies delivered on time (Breborowicz et al. 1999, McCormick 1985) are: lower-respiratory-tract diseases and respiratory-distress syndrome (RDS), bronchopulmonary dysplasia, cerebral palsy (25 times more in infants weighing under 1500g), cerebral intraventricular hemorrhage, epilepsy, retinopathy leading to blindness, patent ductus arteriosus, hypoglycemia, anemia, necrotizing enterocolitis. The costs associated with the care of preterm infants are very high, for example life maintenance of 1000-1500g infant costs about 74000$ (citation according to Breborowicz et al. 1999). Emotional, psychological and financial problems of families affected with PLBW have serious and long-term social consequences.

Despite the intensive research on the etiology of preterm birth is has been assessed that in over 50% of clinical cases its cause remains unknown (Breborowicz et al. 1999). Risk factors for this pathology are usually divided into two groups medical and social (Reron 2001). The first group includes (Breborowicz et al. 1999, Reroń 2001, Wasiela et al. 2001, Offenbacher et al. 1998): woman's obstetrical past - miscarriages and PLBW in medical history; multifetal pregnancy; placental pathologies e.g. placenta previa and congenital or acquired defects of uterus e.g. isthmus uteri failure, myomata; mother's diseases - lower genital tract hemorrhage, anemia, hypertension, diabetes, immunological diseases e.g. lupus erythematosus, presence of anticardiolipin and lymphocytotoxic antibodies.

The most essential risk factor for preterm delivery is genitourinary tract infection (vaginosis and intrauterine inflammation). Studies of Gratacos et al. show, that in the case of bacterial vaginosis the PLT relative risk was 3.1 (citation according to Wasiela et al. 2001). Multiprofile inflammatory effect (direct placenta destruction, the increase of prostaglandin, cytokine, chemokine, and metalloproteinase secretion) may lead to uterus constrictions, premature rupture of membranes (PROM) and preterm labor.

Social risk factors for preterm gestation are (Breborowicz et al. 1999, Reroń 2001,Gibbs et al. 1992): mother's age (under 17 years of age and over 35), black race, low socioeconomic status e.g. low education, which can be expressed in shorter than 9 years the period of education, smoking, excessive alcohol consumption and drug use during pregnancy, inadequate prenatal care, some types of mother's work during pregnancy and high psychological stress.

In 1996 Offenbacher et al. performed studies on periodontal status in 124 women with PLBW from North Carolina 3 days after labor. The control group consisted of mothers, who delivered on time normal birth weight babies. In the examined groups the occurrence of known risk factors for PLBW was taken into consideration. The risks were: age, race, number of pregnancies, smoking, use of alcohol, the level of prenatal care, genitourinary tract infections in past medical history. The logistic regression has revealed, that the probability of preterm labor was 7.5 (in primiparous 7.9) times higher in female patients with attachment loss $\geq$ 3mm. Significantly higher attachment loss was observed in women with PLBW compared to the control group. These observations showed that periodontitis in pregnant women, can be a direct risk factor for preterm gestation, with a much greater influence than other risk factor such as: age, race, previous pregnancies, bacterial vaginosis and past bacterial urinary tract infections.

The aim of the study was to estimate the association between periodontitis and preterm birth of an infant with low birth weight, in a population of Polish women, and the evaluation of prostaglandin E_2 (PGE_2) and interleukin 1-beta (IL-1β) levels in gingival cervicular fluid (GCF) and blood serum in patients with PLBW compared to the control group, and the secretion of these proinflammatory mediators in whole blood, after bacterial lipopolysaccharide (LPS) stimulation in those two groups.

MATERIAL AND METHODS

The case-control study was conducted on 128 women aged 16-41 (mean 27.15). The examination of periodontal status took place on the third day after labor. Each participant received an appropriate description on the study and signed a consent form, approved by the Review Board at Wroclaw Medical University.

The information about present and past pregnancy was collected in a very detailed interview. This data was verified by using a perinatal history record. Special interest was paid to any known risk factor of prematurity, which affected past pregnancies (number of PTL, PROM, number of live births, miscarriages), present pregnancy (mother's age, multifetal pregnancy, placenta and uterus pathologies, co-existing systemic diseases and genitourinary tract infections, smoking). The study group consisted of women, who gave birth to an infant weighing less than 2500g at less than 37 weeks' gestation or who had PLBW or PROM in the past, what is in accordance with the conditions accepted by Offenbacher and co-workers (1996). From the study group the following patients were excluded: women who delivered a baby with multiple developmental defects, patients who were treated for infertility, women after in vitro fertilization and in whom the labor was provoked, and also women who during pregnancy had systemic infections (apart from genitourinary tract infection). The study group consisted of 84 women aged 17-41 (mean 27.57), in which 33 (39.2%) were primiparous cases. The criteria of being primiparous was accepted, when the present labor was the first one, which ended delivering a live infant. The controls were women with normal pregnancy outcomes at this delivery and who had no prior abnormal pregnancy. This group counted 44 women aged 16-38 (mean 26.36), in which 21 (47.7%) were primiparous. In each case periodontal diagnosis was made, according to the criteria proposed in the periodontal classification from *International Workshop for Classification* of Periodontal Diseases and Conditions in Illinois in 1999 (Armitage 1999).

Periodontal Disease Index (PDI) proposed by Wroclaw center (Konopka 1998/1999) was used to make an overall evaluation of the severity of gingivitis and the destruction of periodontium and the extensity of these processes in periodontal tissues. This index is the sum of: Periodontal Bleeding Index (PBI) – according to Saxer and Mühlemann, the mean gingival pocket depth measured in 4 sites/tooth (GK1) and the number of periodontal pockets over 5 mm (GK2) in each sextant (sextant is present if there are at least 2 teeth). The PDI states as follows:

PDI=PBI+GK1+GK2/number of sextants

The IL-1β and PGE_2 levels in blood serum and gingival cervicular fluid were determined by immunoenzymatic method (EIA), using R&D Systems kits Quantikine, Minneapolis, USA. Assay procedures were carried out according to manufacturer's directions.

The evaluation of IL-1β and PGE_2 production, after bacterial lipopolysaccharide stimulation was done by the means of Schytte Blix et al. method (1999). 10 pg/ml of phenol-water-extracted lipopolysaccharide 026:B6 *Escherichia coli* rod (Sigma), was added to 2 ml of venous blood, which was collected into a tube containing sodium citrate. It was then incubated at the temperature of 37°C and occasionally gentle shaking was performed. After 6 hours whole blood was centrifuged for 15 minutes at the speed of 1000xG. Serum until determined for IL-1β and PGE_2 levels was kept at a temperature of –70°C. In the frequency analysis of variables in the nominal scales in both groups, V-squared test and chi-squared test with Yates correction was used. In all examined women and in primiparous odds ratio was calculated for PLBW occurrence with the influence of potential risk factors for this pathology. The rejectable quality level for null hypothesis was $\alpha=0.05$. The evaluation of normal distribution of evaluated variables was performed using normality Shapiro-wilk test. At the same time the hypothesis without normal distribution with $p\leq0.05$ was rejected. In the case of normal distribution to test the significance of differences between means in the study and control group Student's t-test was used in unrelated variables, in other cases Mann-Whitney's test was used. The accepted significance level was $\alpha=0.05$. To determine the relationship between chosen variables Spearman's rank correlation coefficient was used, with the accepted significance level $\alpha=0.01$.

RESULTS

Tables 1 and 2 describe the frequency of periodontitis in patients with PLBW and in the controls. Significantly higher frequency (p=0.038) of periodontitis was noted in women with PLBW. In primiparous cases this relationship was even more impressive (p=0.014).

Tables 3 and 4 present odds ratio for PLBW in women who were exposed to potential risk factors of this pathology in all examined groups. The following factors

Tab. 1: Frequency of periodontitis in all examined women

	Healthy periodontium and periodontitis	Periodontitis (chronic and aggressive)	All
PLBW +	61 (47.6%)	23 (17.9%)	84 (65.6%)
PLBW -	39 (30.4%)	5 (3.9%)	44 (34.4%)
All	100 (78.1%)	28 (21.9%)	128 (100%)

V-squared test, test's value = 4.3 **p=0.038**

Tab. 2: Frequency of periodontitis in primiparous

	Healthy periodontium and periodontitis	Periodontitis (chronic and aggressive)	All
PLBW +	23 (42.6%)	10 (18.5%)	33 (61.1%)
PLBW -	21 (38.9%)	0	21 (38.9%)
All	44 (81.5%)	10 (18.8%)	54 (100%)

Chi - squared test with Yates correction, test's value = 5.93 **p=0.014**

Variable		PLBW group (n= 84)	Control group (n = 44)	Odds ratio	95% confidence interval
age	¤ 28	38 (45%)	14 (31%)	1.77	0.77-4.1
	<28	46 (55%)	30 (69%)		
Number of live births	1	33 (39%)	21 (48%)	0.70	0.3- 1.05
	¤ 2	51 (61%)	23 (52%)		
Medical risk factors for PLBW	yes	37 (44%)	12 (27%)	2.09	0.89- 5.00
	no	47 (56%)	32 (73%)		
PDI	¤ 4	27 (32%)	12 (27%)	1.26	0.53-3.06
	<4	57 (68%)	32 (73%)		

Tab. 3: Odds ratios for PLBW risk factors using all cases and controls

Tab. 4: Odds ratios for PLBW risk factors using primiparous cases and controls

Variable		PLBW group (n= 33)	Control group (n = 21)	Odds ratio	95% confidence interval
age	¤ 28	6 (18%)	1 (5%)	4.44	0.46-79.00
	<28	27 (82%)	20 (95%)		
Medical risk factors for PLBW	yes	12 (36%)	9 (42%)	0.76	0.21- 2.69
	no	21 (64%)	12 (58%)		
PDI	¤ 4	13 (39%)	3 (14%)	3.90*	0.93-19.14
	<4	20 (61%)	18 (86%)		

* **p=0.04**

were taken into consideration: age (over and under 28 years), the presence of medical risk factors for PLBW, the value of PDI (over and under 4), and the number of live births. The occurrence of medical risk factors was observed in 37 women with PLBW (44%). In case of the control group these conditions were observed in 12 subjects (27%). In all women there was no variable, which significantly influenced PLBW, although patients suffering from a disease considered to be a risk factor of this pathology had twice the possibility to develop preterm labor. In the group of primiparous with PLBW, 12 subjects (36%) were subjected to the influence of medical risk factors of this pathology, while in the controls only 9 women (42%) were subjected. In primiparous women there was 4 times greater probability of PLBW if the woman's age was over 28, and about 4 times greater probability of this pathology, when the intensity of pathologic periodontal processes reflected in the PDI value >4. In the second case, the difference was statistically significant (p=0.048).

Detectable IL-1β levels in blood serum were noted in only 3 patients with PLBW (3.5%). The two-way table test did not confirm, that the IL-1β presence in blood serum over detectable value in the used test was significantly associated with PLBW. In all women with PLBW, as well as in primiparous with PLBW, there was a statistically higher IL-1β concentration in gingival cervicular fluid than in the controls (Tab. 5). Significantly higher IL-1β secretion was observed in whole blood after *E. coli* LPS stimulation, in women with periodontitis compared to patients with gingivitis and with no periodontal changes (Tab. 5). No marked differences in IL-1β secretion was seen after stimulation in women with PLBW and in the controls.

No significant difference was seen in PGE_2 concentration in serum of women with PLBW compared to the control group. But in primiparous cases with PLBW this level was significantly higher. Significantly higher PGE_2 concentrations in gingival cervicular fluid were in patients with periodontitis compared to women with gingivitis (Tab. 5). Both women with PLBW and primiparous with PLBW had significantly higher GCF-PGE_2 concentration compared to women, who gave birth on time to an infant weighing over 2500g (Tab. 5). No significant difference in PGE_2 secretion after bacterial stimulation was found in both the study and the control group.

The PLBW group showed significant correlations between the GCF-IL-1β concentration level and its secretion after LPS stimulation, and also between the PGE_2 concentration in GCF (Tab. 6). In the control group there was a significant correlation between the GCF- IL-1β concentration and IL-1β secretion after stimulation. In women with PLBW there were significant correlations between IL-1β secretion level after LPS stimulation and GCF-PGE_2 concentration, concentration of PGE_2 after LPS stimulation (Tab. 6). In this group there was also a significant correlation between PGE_2 concentration in blood serum with its concentration after LPS stimulation. Both in PLBW and control group a significant correlation between GCF-PGE_2 concentration and PGE_2 concentration after LPS stimulation was observed. In all primiparous cases there was a significant negative correlation between infant's birth weight and GCF-PGE concentration (Tab. 6).

Inflammatory mediator	Compared groups	Mean concentrations	p
GCF - IL-1 (pg/ml)	PLBW vs control	107.9⌖ 89.9 vs. 56.,6⌖ 47.9	p=0.05 **
	Priniparous PLBW vs. control	134.3⌖ 79.8 vs. 67.4⌖ 66.1	p=0.032 **
	gingivitis vs periodontitis	58.3⌖ 52.2 vs. 128.4⌖ 93.5	p=0.001 **
IL-1β after *E. coli* LPS stimulation (pg/ml)	PLBW vs. control	123.9⌖112,6 vs 132.7⌖125.8	ns **
	priniparous PLBW vs. control	161.8⌖111.9 vs 131.2⌖139.4	ns **
	gingivitis vs. periodontitis	107.5⌖103 vs. 182.6⌖128.5	p=0.022 **
	healthy periodontium. vs. periodontitis.	106.5⌖114.3 vs 182.6⌖128.5	p=0.02 **
PGE_2 in blood serum (ng/ml)	PLBW vs. control	0.33⌖ 0.25 vs. 0.22⌖ 0.24	ns**
	priniparous PLBW vs. control	0.36⌖ 0.25 vs. 0.18⌖ 0.25	p=0.032 **
	healthy periodontium vs gingivitis	0.18⌖ 0.23 vs. 0.39⌖ 0.26	p=0.004 **
PGE_2 in GCF (ng/ml)	PLBW vs. control	34.48⌖ 20.5 vs. 17.44⌖ 20	p=0.0008 **
	Priniparous PLBW vs. control	41⌖ 13.1 vs. 18.56⌖ 17.6	p=0.008 **
	gingivitis vs. periodontitis	21⌖ 20.6 vs. 37.22⌖ 20.6	p=0.003 **
PGE_2 after *E.coli* LPS stimulation (ng/ml)	PLBW vs. control	2.15⌖ 1.22 vs. 1.79⌖ 0.99	ns *
	Priniparous PLBW vs. control	2.2⌖ 1.27 vs. 1.78⌖ 1.2	ns *
	gingivitis vs. periodontitis	1.92⌖ 1.02 vs. 3⌖ 1.17	p=0.0006*
	healthy periodontium vs. periodontitis	1.47⌖ 0.85 vs. 3⌖ 1.17	p<0.0001 *

Tab. 5: IL-1β and PGE_2 concentrations in examined groups

* Student's t-test,
** Mann-Whitney's test, ns-no significance

inflammatory mediator	variable	group	Spearman's rank correlation coefficient R	p
IL-1β in GCF	IL-1β after LPS stimulation	PLBW	0.86	p < 0.0001
	PGE_2 in GCF	PLBW	0.61	p= 0.0003
	PGE_2 after LPS stimulation	PLBW	0.43	ns
	IL-1β after LPS stimulation	control	0.59	p= 0.002
	PGE_2 in GCF	control	0.39	ns
IL-1β after *E. coli* LPS stimulation	PGE_2 in blood serum	PLBW	0.3	ns
	PGE_2 in GCF	PLBW	0.59	p= 0.0006
	PGE_2 after LPS stimulation	PLBW	0.47	p= 0.0003
	PGE_2 in GCF	control	0.47	ns
	PGE_2 after LPS stimulation	control	0.23	ns
PGE_2 in blood serum	PGE_2 in GCF	PLBW	0.11	ns
	PGE_2 after LPS stimulation	PLBW	0.38	p= 0.005
	PGE_2 in GCF	control	0.42	ns
	PGE_2 after LPS stimulation	control	0.43	p= 0.01
PGE_2 in GCF	PGE_2 after LPS stimulation	PLBW	0.54	p= 0.001
	PGE_2 after LPS stimulation	control	0.57	p= 0.003
	Infant's birth weigh	all	-0.21	ns
	Infant's birth weigh	priniparous	-0.61	p= 0.006

Tab. 6: Correlations analysis between IL-1β and PGE_2 levels and chosen variables

DISCUSSION

Although, in the present study the percentage of women with healthy periodontium, in the study group was 26.1 and 34 in the control group, no significant difference was noted. Dasanayake (1998) observed statistically significant higher number of healthy sextants in CPITN, in patients giving birth to infants weighing over 2500g compared to LBW. Offenbacher et al. (2001) noticed successively decreasing number of patients with healthy periodontium (the lack of pockets over 3 mm and surfaces with attachment loss over 2 mm) along with tightened prematurity conditions - for full term delivery 25.4%, for delivery before 37th week of gestation 22.3%, before 35th week 14.8%, before 32nd week 11.4%, before 28th week 11.1%. In our study group no such trend was observed. The differences can derive from a much bigger group of examined women (812, in which 188 with PTL) and from race diversity in the American research.

In the present study, all of the examined women with PLBW had significantly higher frequency of periodontitis (chronic and aggressive) compared to the control group.

Similar observation revealed Offenbacher et al. (2001) in their studies, in which they have proved the significantly higher frequency of moderate and severe periodontitis for all stated above prematurity ranges, compared to women who gave birth on time. Among women with periodontitis in our study, only 17.8% gave birth on time to an infant weighing over 2500g. Periodontitis was observed in 32.1% labors between 35th and 37th week and in about 25% labors between 28th-35th week and under 28th week. In case of LBW periodontitis occurred in 35.7% births with weight from 2000 to 2499g, in 25% labors with infant's birth weight from 1000-1999g and in 21.4% labors with weigh under 1000g. This proves the relationship between the occurrence of periodontitis and preterm labor with low birth weight. Our observation show the increase of this relationship with gestational age, what can depend on the accumulation of risk factors of this pathology. This relationship does not seem to derive from bad oral hygiene and that is why the occurrence of periodontitis should not be treated as social risk factor for preterm birth.

In our analysis we have taken into consideration the influence of age, the number of live births, the occurrence of medical risk factors for PLBW and the severe and generalized periodontitis. The value of 4 of the periodontal disease index (PDI) was chosen as the periodontal, dichotomic, independent variable. In all women at the age over 28 and exposed to medical risk factors (PROM, miscarriage or past PLBW, placental or uterus abnormalities, multifetal pregnancy, anemia, urinary tract infections, hypertension, genital tract hemorrhage, diabetes) there was a slightly higher probability for PLBW. In fact these correlations were not statistically significant. In primiparous over 28 years of age there was over 4 times greater probability of preterm labor, but this difference was not significant. In primiparous with generalized periodontitis (PDI>4) had significant, 3.9 times greater probability of PLBW compared to women without periodontitis or with mild periodontal disease.

A very high significance in the increase of GCF-PGE_2 level was noted in all women with PLBW and in primiparous with this pathology compared to the controls. In all primiparous the inverse correlation was seen, between the infant's birth weight and the GCF-PGE_2 level. These observation fully correlate with the results of Offenbacher et al. (1998), who stated 2 times greater level of PGE_2 in this environment in women with PLBW compared to women who gave birth on time to a normal birth weight infant. Their study shows that in primiparous the significant inverse difference between birth weight and the concentration of this mediator in gingival cervicular fluid. These results point out, that this is not only a relationship between the increasing concentration of GCF-PGE_2 level and the severity of periodontitis, but also the decreasing birth weight. Damaré et al. (1998) showed the positive correlation between the PGE_2 concentration in gingival cervicular fluid and in amniotic fluids and significantly higher concentration of this mediator in these fluids compared to serum. The synchrony of PGE_2 expression in GCF and amniotic fluid can point out to the similarity of specific patient response mainly to macrophages on bacterial endotoxins. In this context further study should be established whether non invasive evaluation of the PGE_2 concentration in GCF may be used as a predictive diagnostic test for LBW. In adequate sensitivity and specification this test could serve as a screening examination, preceding amniocentesis.

The evaluation of PGE_2 concentrations in serum has bounded validity, for the period of its half-existence in this environment is very short (citation according to Saji et al. 2000). The observed concentration were about 100 times lower than in gingival cervicular fluid. Also in

serum there was doubled difference in the prostaglandin concentration in women with PLBW and in the controls. In primiparous it was on the level of statistical significance.

It could be alleged, that in patients with periodontitis and PLBW there is a population of hyperactive monocytes, which produce excessive amounts of PGE_2 after LPS stimulation, and the cause of this pathology could be genetically determined. In our study no significant differences in PGE_2 secretion after *E. coli* LPS stimulation was seen in the group of women with and without PLBW, although a higher secretion level was present in the study group. This slightly increased secretion was probably the result of greater prevalence of periodontitis in patients with PLBW. In periodontitis we have revealed a decisively higher PGE_2 secretion after stimulation compared to patients with gingivitis or with healthy periodontium. Against the presence of a subpopulation of hyperactive monocytes in periodontitis state observations of Hernichel-Gorbach et al. (1994). Using the *LPS of Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis* and *Salmonella Typhimurium*, they did not show significant differences in secretion of this mediator in patients with gingivitis and periodontitis.

Of the fact that the dynamics of PGE_2 secretion in peripheral blood is similar in the place where is the highest concentration of bacterial LPS (i.e. in periodontal tissues), states the positive significant correlations between PGE_2 concentration after stimulation and in gingival cervicular fluid in both the study and control group. In patients with PLBW (and in practice in control group – p=0.01043) also positive significant correlation between PGE_2 after stimulation and its concentration in peripheral blood was noted, which is obvious. The results of these studies concerning PGE_2 concentration in the place of periodontal infection and in peripheral blood in women with PLBW seem to point out that in these pathologies there is no systemic or local secretive hyperactivity of monocytes and macrophages, but periodontium similarly to amniotic membranes, thanks to residual cells, is an organ secreting prostaglandins. The dynamics of theses processes can be similar and the alternation with bacterial LPS stimulation during periodontal and membrane infection leads to pathology in both places.

In all women as well as in primiparous with PLBW there was a significantly higher GCF - IL-1β concentrations compared to control group. In similar studies Offenbacher et al. (1998) despite the higher level of this cytokine in GCF in PLBW women compared to those giving birth on time (1217.8 versus 720.2 ng/ml) did not state that this difference was statistically significant. This seemingly contradiction results from the difference in the number of examined women (44 in the studies of Offenbacher and 128 in our study). Stated difference in present study in all patients with PLBW was on the threshold of statistic significance (p=0.05). The results of both observations are in fact close to the trend which characterizes in greater IL-1β concentration level in GCF in patients with preterm labor, what is more spectacular in primiparous. During pregnancy (especially the first one) the activity of these processes can be that big, that the systemic exposition to LPS of pathogenic bacteria for periodontal tissues, which can play a role in the start of the immunological response cascade of in foetal membranes, may result in shortening of the pregnancy period. Such hypothesis assumes the influence of environmental factor (the exposition of pathogenic bacteria to periodontal tissues and/or their products) on PLBW pathomechanism.

The detectable IL-1β levels in blood serum (<1pg/ml) were seen only in 3.5% of patients with PLBW. In the study of Von Minkwitz et al. (2000), the detectable levels of IL-1β in blood serum were seen in 20.8% women with PLT, 17.5% with PROM and 17.4% in control group. Medium levels of this cytokine did not differ significantly from those three groups of women (Von Minkwitz et al. 2000). Generally, it is considered that the inflammation of amniotic membranes does not lead to marked increase of IL-1β concentration in blood serum (Romero et al. 1993). Also during chronic periodontitis there is no increase of proinflammatory cytokine concentration over detectable level (Prabhn et al. 1996). Even no significant increase in the expression of IL-1 mRNA on peripheral blood cells is seen. All these studies state, that blood serum is not a good environment to monitor subtle differences of proinflammatory cytokine concentrations in PLBW as well as in periodontal diseases.

In our patients no differences were observed in the secretion of IL-1β after *E. coli* LPS stimulation in patients with PLBW and in the controls. But significantly higher secretion of this cytokine was seen after stimulation in patients with gingivitis and periodontitis compared to subjects with no changes in periodontal tissues. It seems to point out that the IL-1β secretion is mainly the function of special cells exposition to bacterial products and in given population

it is hard to relate PLBW with frequent incidence of phenotype for hyperactive (allergised) cells synthesizing IL-1. In some patients with PLBW and with periodontitis we have observed specific low level of secretion of this cytokine, what is proved by very high standard deviation in the control and study group. This proves the interpersonal differences in host response on local infection, what was proposed by Takahaschi et al. (2001), while evaluating a group of peripheral neutrophils and lymphocytes functions of in patients with aggressive periodontitis. The evaluation of IL-1, IL-6, TNF-α secretion revealed "hyper-" and "hypo-secretors".

In women with PLBW we have showed significant correlations between the concentration of IL-1β after stimulation and PGE_2 concentration in gingival cervicular fluid and after stimulation. These correlations prove the similar mechanisms of secretion of inflammatory mediators locally and in peripheral blood. They have also proved the influence of IL-1β on prostaglandin synthesis. The question concerning the meaning of this synergism in secretion of pro-inflammatory mediators in PLBW still not answered. Perhaps it is because of the frequent occurrence of periodontitis in this group, what provides a special reservoir for bacterial LPS, which stimuli special cells to secrete inflammatory mediators.

The positive correlation between the IL-1β concentration in gingival cervicular fluid and the concentration of this cytokine after LPS stimulation in the study and control group that despite of the use of standardized LPS of normal bacteria for periodontal tissues the dynamics of secretion of this cytokine is similar as in periodontium under stimulation completely different microorganisms. The studies of Yoshimura et al. (1997) show that the E. coli and Actinobacillus actinomycetemcomitans LPS strongly stimuli monocytes and neutrophils to the production of IL-1β and TNF-α in great quantities compared to Porphyromonas gingivalis and Capnocytophaga ochracea LPS. Other research studies prove the ability of strengthening neutrophils by *E. coli* and A.a. LPS, what leads to hyperactivity of these cells (citation according to Takahaschi et al. 2001). Perhaps this is the important mechanism of "making allergic" the secretory cells, which take part in PLT influenced by LPS of bacteria pathogenic to periodontal tissues. Schytte Blix et al. (1999) showed, that A.a. LPS was the strongest stimulator of IL-1β, IL-6 and TNF-α in whole blood, and *E. coli* LPS had to be added in 100-1000 times higher concentration to give the similar effect to secrete these cytokines. This discrepancy result from different LPS concentrations, the virulence variety bacterial species as sources of LPS, the ways of extracting. Comparison of inflammatory mediator secretion after stimulation of bacterial products has to be very careful.

CONCLUSIONS

1. In the studied population, women over 28 years of age and exposed to medical risk factors had more frequent PLBW occurrence probability. In primiparous over 28 years of age there is 4 times more greater probability of preterm labor, and in case of the presence of severe and generalized periodontitis there is 3.9 times higher possibility of PLBW compared to women with healthy periodontium.

2. In all women with PLBW there is a significantly higher PGE_2 and IL-1b concentration in gingival cervicular fluid, and in primiparous also PGE_2 level in blood serum, compared to controls.

3. The inverse association between gingival cervicular fluid PGE_2 concentration and infant's low birth weight suggests the proinflammatory mediators level in gingival cervicular fluid may eventually be used in second trimester of pregnancy as a predictive diagnostic test for LBW.

4. The lack of significant differences in women with PLBW and the controls together with a significantly higher secretion of PGE_2 and IL-1β in whole blood after *E. coli* LPS stimulation in patients with periodontitis and gingivitis compared to subjects with healthy periodontal tissues seem to point out, that the synthesis of proinflammatory mediators is mainly the function of the specific cells exposition to bacterial products. Therefore more frequent occurrence of phenotype of hyperactive cells synthesizing these mediators is rather not responsible for PLBW.

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