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ORIGINAL ARTICLE

Relationship between periodontal parameters and plasma cytokine profiles in pregnant woman with preterm birth or low birth weight

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Abstract
Objectives The aim was to determine whether clinical periodontal parameters are associated with plasma anti- and/or pro-inflammatory cytokines in pregnant woman with preterm birth (PB) or low birth weight (LBW) neonates.
Materials and methods An observational case-control study was performed in 131 puerperal women: mothers of PB/LBW neonates (cases, $n=67$) and mothers of full-term normal-weight neonates (controls, $n=64$). Sociodemographic and periodontal data was gathered from all participants, and interleukin (IL)-1β, IL-6, IL-8, IL-10, IL-17, IL-23, and tumor necrosis factor alpha (TNF-α) were determined in plasma.
Results In multiple linear regression models, clinical attachment loss was associated with TNF-α (0.28±0.14; 95 % confidence interval (CI) [0.006, 0.553]) and IL-1β (0.43±0.21; 95 %CI [0.018, 0.842]), independent of group membership. IL-1β (−1.67±0.27, 95 %CI [−2.199, −1.141]), IL-6 (−0.86±0.27; 95 %CI [−1.389, −0.331]), and IL-8 (−3.84±0.50, 95 %CI [−4.820, −2.860]) were lower, and IL-10 (0.86±0.26; 95 %CI [0.350, 1.370]) was higher in cases versus controls after adjusting for potential confounders.
Conclusions Clinical attachment loss was associated with plasma TNF-α and IL-1β levels. No plasma cytokine profiles suggestive of systemic inflammatory response were observed in the pregnant women with PB/LBW neonates.
Clinical relevance Clinical attachment loss, as the main periodontal measure, is associated with TNF-α and IL-1β plasma levels in pregnant women. No relationship was found between PB/LBW and the markers of systemic inflammatory response assessed in this study.

Keywords Premature birth · Chronic periodontitis · Enzyme-linked immunosorbent assay · Cytokines

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Introduction

Preterm birth (PB) (<37 weeks) and low birth weight (LBW) (<2500 g) neonates are the second leading cause of death in children under 5 years and constitute the main risk factor for neonatal infections and growth disorders [1]. Periodontitis has been suggested as a risk factor for PB/LBW either directly, through uterine infection with periodontal pathogens, or via an indirect mechanism in which an infected periodontium can act as a reservoir of pro-inflammatory cytokines, which have been shown to cause adverse pregnancy outcomes [2].

Published systematic reviews and meta-analyses on the association between periodontal disease and adverse pregnancy outcomes conclude that comparisons between the two pathologies should be interpreted with caution, due to the wide variations in study design, diagnostic method, and definition of periodontal disease [3, 4]. Different studies suggest that patients with gingivitis or periodontitis produce high gingival crevicular fluid (GCF) levels of pro-inflammatory mediators, mainly interleukin (IL)-1β, IL-6, and tumor necrosis factor alpha (TNF-α), which can lead to the loss of connective tissue

attachment and alveolar bone [5]. Periodontal treatment has been associated with a decrease in local [6] levels of these cytokines and recently showed little correlation with the serum levels of these mediators [7]. However, data on inflammatory mediators in the serum of patients with periodontitis and PB/LBW are scant and controversial [7–9]. Although elevated serum and amniotic fluid levels of these mediators have been associated with various adverse pregnancy outcomes, there is limited and mostly negative evidence of an association between the elevation of these mediators in GCF, serum, or amniotic fluid and pregnancy complications in periodontitis patients [10]. One of the recommendations of the Joint EFP/AAP Workshop of Periodontal Medicine 2013 [2] was the development of serum inflammatory markers to improve knowledge of the possible association between pregnancy complications and periodontitis,

The aim of this study was to determine whether periodontal clinical parameters are associated with plasma levels of anti- and/or pro-inflammatory cytokines in pregnant woman who give birth to PB/LBW neonates.

Materials and methods

An observational case/control study was conducted in pregnant women hospitalized in the "Virgen de las Nieves" University Maternity Hospital in Granada (Spain) between January 2012 and November 2012. Inclusion criteria for cases were age ≥18 years with PB (<37 weeks of gestation) and/or LBW (<2500 g) newborn. Inclusion criteria for controls were age ≥18 years with full-term (≥37 weeks of gestation) and normal-weight (>2500 g) newborn. Sample Power 2.0 (IBM Inc., Chicago, IL) was used for post hoc estimation of the statistical power. Using the *t* test for independent groups and a sample size of 53, with a significance level of alpha= 0.05, a moderate standardized difference (0.5 according to Cohen's scale [11]) could be detected in the analyzed variables (inflammatory mediators) with a power of 76 %. Cases and controls were recruited consecutively. Exclusion criteria for both cases and controls were as follows: age <18 years, presence of diabetes or systemic disease not related to the pregnancy, a history of multiple pregnancies, interruption of the pregnancy for medical reasons, and receipt of periodontal or anti-microbial therapy in the previous 6 months. The study was approved by the hospital ethics committee. Written informed consent was obtained from all study participants.

Data on the mothers were gathered from their clinical records, and the estimation of gestational age was based on an ultrasound examination in the first trimester. Information on the newborn weight was obtained from the Neonatology Department records.

Sociodemographic and periodontal variables:

A single researcher (E.P.) interviewed the mothers to gather sociodemographic data on the following: age; ethnicity; marital status at the delivery (married, single, separated, widow); employment (yes/no); socioeconomic level, according to the modified classification of J. Goldthorpe cited by E. Regidor [12]; years of schooling; and consumption during pregnancy of tobacco (cigarettes/day), alcohol (grams/day), and coffee (cups/day).

Periodontal variables were measured at a single time point during the 24 h after delivery (48–72 h after a caesarean delivery). The periodontal examination was performed by a single, blinded examiner (E.P.) in the hospital room, following World Health Organization recommendations for oral examinations and using a PCPUNC15 calibrated periodontal probe (University of North Carolina No. 15 probe; Hu-Friedy, Chicago, IL). Beforehand, intra-examiner and inter-examiner reliability was assessed using kappa statistics, obtaining similar high values (0.77 and 0.81, respectively) according to the Landis and Koch's scale [13] and demonstrating a high degree of conformity in the observations.

Periodontal status was assessed by determining: the gingival bleeding index of Ainamo and Bay [14]; the gingival recession for all teeth present, except for third molars, and the probing depth (PD) and clinical attachment (CA) loss at four sites per tooth (mesial, distal, vestibular, and palatine/lingual). Periodontitis was diagnosed when four or more teeth showed one or more sites with PD ≥4 mm and CA loss ≥3 mm, based on the definition by López et al. [15]. Patients who were not diagnosed with periodontitis but showed bleeding on probing were diagnosed with gingivitis.

Biochemical variables

Blood samples were collected from all women in ethylenediaminetetraacetic acid (EDTA)-treated tubes at the time of delivery. The blood samples were centrifuged at 3000 rpm for 10 min and frozen at −80 °C. Plasma cytokine levels were measured by commercially available high-sensitivity enzyme-linked immunosorbent assay (ELISA) (Human Immunoassay Quantikine kits, R&D, Inc, Minneapolis, USA), adding 100 μL plasma to each well according to the manufacturer's instructions. Quantification of the studied cytokines (IL-1β, IL-6, IL-8, IL-10, IL-17, IL-23, TNF-α) was in picograms per milliliter, and the lowest cytokine level detected was 0.03 pg/mL. According to the kit manufacturer, there is no cross-detection of other cytokines. The intra-assay variation was <10 %. All samples, standards, and controls were assayed in duplicate.

Statistical analysis

SPSS v.20.0 for Windows (IBM, Chicago, IL, USA) was used for the statistical analysis. The normality of the distribution of variables was examined with the one-dimensional Kolmogorov-Smirnov test. Seven multiple linear regression models were constructed, i.e., one for each cytokine as dependent variable with logarithmic transformation (LN cytokine +1). Given our interest in estimating the adjusted effect of group (case/control) and periodontitis (CA loss) on cytokine levels, we developed complete regression models, following recommendations [16], forcing not only group and periodontitis but also some well-known confounding factors (age, diabetes, AHT, GU infections, coffee, and tobacco), whether significant or not. The statistical tests used are reported in the table footnotes.

Results

After application of the study eligibility criteria, the final study sample included 131 puerperal women: 64 mothers with full-term normal-weight newborn (controls) and 67 with PB/LBW (cases). In the control group, the mean age was 29.4±4.4 years, mean gestation period 39.6±1.1 weeks, and the mean newborn weight was 3340±384 g. In the case group, the mean age was 29.9±7.1 years, mean gestation 35.2±2.1 weeks, and mean newborn weight 2290±400 g; in this group, 10 children had normal weight (≥2500 g) but <37 weeks of gestation, 10 children weighed<2500 g but were full-term deliveries, while 47 children weighed <2500 g and were preterm deliveries.

Table 1 compares sociodemographic and periodontal data between cases and controls, with periodontal data showing worse values for all variables in the case group. Although the difference in CA loss between cases and controls was significant in the unadjusted analysis, statistical significance was lost after adjustment for socioeconomic level and tobacco use (p=0.249).

Out of the total of 131 samples, 14 (11 controls and three cases) were lost due to problems during their cold storage. Therefore, IL levels were determined (by ELISA) in 53 controls and 64 cases. There was no significant difference in the characteristics listed in Table 1 between the women whose samples were and were not analyzed (data not shown). Higher levels of three pro-inflammatory ILs (IL-1β, IL-6, IL-8) were found in controls versus cases, while a higher level of anti-inflammatory IL-10 was found in cases versus controls, although this level never exceeded the normal range as reported by Szarka et al. [17] (Table 2). Table 3 exhibits the seven multiple linear regression models constructed for each IL (dependent variables) to obtain their adjusted effect. CA loss was associated with TNF-α (0.28±0.14; 95 % confidence interval (CI) [0.006, 0.553]) and IL-1β (0.43±0.21; 95 %CI [0.018, 0.842]) levels, independently of group membership. The case group showed lower IL-1β (−1.67±0.27, 95 %CI [−2.199, −1.141]), IL-6 (−0.86±0.27; 95 %CI [−1.389, −0.331]), and IL-8 (−3.84±0.50, 95 %CI [−4.820 −2.860]) levels and higher IL−10 (0.86±0.26; 95 %CI [0.350, 1.370]) levels versus controls after adjusting for potential confounders.

Discussion

In this study, pregnant women with PB/LBW newborn showed a worse periodontal health versus those with full-delivery normal-weight newborn in the crude analysis, but the difference lost significance after adjustment for socioeconomic level and tobacco use. The women with PB/LBW also evidenced an absence of elevated plasma cytokine levels, which are indicators of a systemic inflammatory condition. Higher plasma TNF-α and IL-1β levels were found in women with greater CA loss, regardless of their group membership (case or control).

The association between periodontal disease and adverse pregnancy outcomes, primarily PB, remains controversial. In our study, more coffee was consumed by the pregnant women with PB/LBW newborn, but there was no difference between the groups in CA loss, the main periodontal variable, in the adjusted analysis. Only 11 women had periodontitis in the case group and 5 in the control group. This low prevalence of periodontitis may have two explanations: the age of the women (mean age of ~30 years in both groups) and criteria used for the definition of periodontitis, following López et al., i.e., presence of at least four teeth with at least one site with PD >4 mm [15] compared with the presence of at least two teeth with periodontal lesions in the definition used by other authors [18, 19]. As recommended by Manau et al. and Guimaraes et al., this type of study requires an objective periodontal examination using a properly calibrated manual probe with adequate examiner calibration and a clear and strict definition of periodontitis [20, 21].

Elevated amounts of pro-inflammatory cytokines, chemokines, and adhesion molecules in the maternal circulation might play a central role in the excessive systemic inflammatory response (Szarka et al.) [17]. It has been suggested that an increase in pro-inflammatory cytokines due to periodontal inflammation can facilitate or accelerate development of the mechanisms that initiate childbirth, constituting the indirect pathway in the relationship between periodontitis and PB/LBW [2]. This hypothesis is further supported by studies suggesting that PGE2, IL-1β, IL-6, and TNF-α play an important role in normal physiological childbirth as well as in pathologic prematurity [22, 23]. IL-1β is a strong stimulator of the prostaglandin synthesis in the decidua and amnion and is the first IL involved at the beginning of labor in the presence of

Table 1 Description and comparison of the sociodemographic and periodontal variables of the pregnant women (n=131)

Variable	Controls (n=64)	Cases (PB/LBW) (n=67)	P values
Ethnicity (% Caucasian)	82.8	83.5	0.793[a]
Marital status (% married)	68.0	58.2	<0.001[a]
Employed (% yes)	53.1	55.2	0.337[a]
Social level (range, I–VII), median	VI	VII	0.219[b]
Schooling[e] (years)	11.5±4.5	10.2±4.2	0.106[c]
Coffee (% no-one cup/week-one cup daily)	60-4-0	42-23-2	0.016[b]
Tobacco (% never/ex-smoker-≤10/day->10/day)	77-3-6-14	49-24-8-19	0.793[b]
Alcohol (% none-≤5 g/day->5 g/day)	63-1-0	66-0-1	0.328[b]
Gestational diabetes (%)	3.1	7.5	0.441[d]
Arterial hypertension (%)	9.4	4.5	0.317[d]
Genitourinary infections (%)	21.9	32.8	0.227[a]
Chorioamnionitis (%)	0.0	1.5	≈1[d]
Premature membrane rupture (%)	7.8	49.3	<0.001[a]
Gingival bleeding index[e] (%)	18.2±24.7	24.6±30.6	0.188[c]
Gingival recession[e] (mm)	0.04±0.09	0.10±0.23	0.097[c]
Probing depth[e] (mm)	1.59±0.34	1.76±0.62	0.050[c]
Clinical attachment loss[e] (mm).	1.64±0.38	1.86±0.81	0.049[c]
Patients with periodontitis (%)	7.81	16.41	0.216[a]

[a] Chi-square test with Yates' correction

[b] Mann-Whitney U test

[c] Student's t test

[d] Bilateral Fisher's exact test

[e] Values are expressed as mean±standard deviation

infection [24]. Elevated amniotic fluid TNF-α and IL-6 levels have been related to neurological problems in PBs [24]. In a recent systemic review of 17 observational studies on inflammatory ILs in asymptomatic women with spontaneous PBs (total of 6270 participants), Wei et al. reported an association between PB and elevated IL-6 levels in vaginal and amniotic fluids but not in serum, suggesting that an etiopathogenic role is played in prematurity by local inflammation at the maternal-fetal interface rather than by systemic inflammation [25].

Table 2 Comparison of plasma cytokine levels between the two groups of pregnant women (n=117).

Variable	Controls (n=53)		Cases (PB/LBW) (n=64)		
	n	Mean±SD[a]	n	Mean±SD	P values[b]
Inflammation mediators (pg/ml)					
TNF-α	53	0.085±0.434	64	0.066±0.370	0.835
IL-1β	53	0.913±0.766	63	0.249±0.456	<0.001
IL-6	53	1.453±0.505	64	1.172±0.689	0.033
IL-8	53	2.140±1.232	64	0.481±0.987	<0.001
IL-10	53	0.108±0.381	63	0.568±0.712	<0.001
IL-17	53	0.030±0.220	63	0	0.276
IL-23	52	0.173±0.496	64	0.127±0.400	0.543

[a] Values are expressed as mean±standard deviation of decimal logarithm (x+1)

[b] Mann-Whitney U test

Various authors have observed a linear relationship between GCF IL levels and periodontal lesions [26, 27] and an inverse association between GCF IL levels and birth weight [7, 28, 29]. A recent systematic review concluded that there may be a positive association between GCF inflammatory mediator levels (IL-1β, PGE2, and TNF-α) and PB/LBW but warned that their findings should be interpreted with caution due to the wide variations among studies. Further research in wide patient samples is required to allow definitive conclusions to be drawn on this relationship [30]. Moreover, Fiorini et al. found that GCF IL levels were not strongly associated with serum IL levels in pregnant women with widespread periodontal inflammation [7].

To our best knowledge, only three articles have addressed serum cytokine levels in pregnant women with periodontitis [7–9]. In a follow-up study of 823 pregnant women with periodontal disease, Michalowicz et al. analyzed serum samples drawn before and after treatment and concluded that periodontal treatment before week 21 of pregnancy did not reduce inflammation markers in serum; they reported that levels of these markers between 13 and 17 weeks and between 29 and 32 weeks of gestation were not associated with an increased risk of PB and/or LBW [9]. In contrast, a cross-sectional study

Table 3 Multiple linear regression models for cytokine levels in the pregnant women (n=117) adjusting for potential confounders

	Model (dependent variable)						
Variable	TNF-α	IL-1β	IL-6	IL-8	IL-10	IL-17	IL-23
Case	−0.08±0.18	−1.67±0.27*	−0.86±0.27*	−3.84±0.50*	0.86±0.26*	−0.02±0.06	−0.14±0.21
CA loss (mm)	0.28±0.14*	0.43±0.21*	0.18±0.22	0.15±0.40	0.29±0.21	−0.03±0.05	−0.04±0.16
Age (years)	−0.02±0.02	−0.05±0.03*	−0.04±0.03	−0.05±0.05	−0.04±0.03	0.00±0.01	−0.00±0.02
Diabetes	−0.38±0.48	0.25±0.73	−0.42±0.74	−0.52±1.37	0.20±0.70	0.06±0.17	−0.48±0.56
AHT	−0.47±0.42	−1.25±0.62*	−1.38±0.63*	−0.52±1.17	−1.11±0.60	0.72±0.14*	−0.43±0.48
GU infections	0.39±0.22	−0.09±0.33	0.30±0.33	1.00±0.61	0.52±0.32	−0.16±0.08*	0.10±0.25
Coffee	−0.08±0.21	0.41±0.32	0.56±0.32	−0.19±0.60	0.25±0.31	0.01±0.07	0.23±0.25
Tobacco	−0.08±0.08	−0.12±0.12	−0.01±0.12	−0.20±0.23	0.07±0.12	−0.02±0.03	−0.06±0.09

CA clinical attachment, *AHT* arterial hypertension, *GU* genitourinary

Beta±standard error (β±SE), with *P<0.05

by Sert et al. found higher serum IL-1β and IL-1β/IL-10 levels in pregnant women with PB/LBW neonates and periodontitis, supporting the hypothesis that periodontitis, as a chronic inflammatory disease, may be a risk factor for PB/LBW via placental dysfunction [8]. In a mouse study, Lin et al. observed that infection with *Porphyromonas gingivalis* during gestation increased TNF-α and suppressed IL-10 levels, associating these results with fetal growth restriction [31]. Other cytokines, such as IL-6, IL-8, and TNF-α, may be relevant in the pathophysiology of periodontitis, and their measurement may be useful to identify patients with periodontitis [32].

Our cross-sectional study found no association between plasma levels of pro-inflammatory cytokines and an increased risk of adverse pregnancy outcome. In fact, plasma IL-1β, IL-6, and IL-8 levels were significantly higher in the control group. These results contradict the "indirect" pathogenic mechanism of increased systemic inflammatory cytokines as a possible link of the association between periodontitis and PB/LBW. We found an association between two inflammatory cytokines (TNF-α and IL-1β) and CA loss, but this was independent of group membership (case or control). The absence of a systemic inflammatory state in our cases is further supported by a significant increase in their IL-10 levels. The anti-inflammatory IL-10 plays a role in preventing exaggerated inflammatory and immune responses and protecting the host from immune-mediated damage [33]. Sert et al. also found higher levels of IL-10 in mothers with periodontitis and PB/LBW neonates, although the difference with the control group was not statistically significant [8]. Further research is warranted to verify these unexpected findings.

Although the analysis of cytokines in serum was not possible in 14 of the women in our study, this was due to storage problems and there were no significant differences in characteristics between the included and excluded women, suggesting that no selection bias was produced.

To our best knowledge, the present study is the first to quantify IL-17 and IL-23 levels, which have recently been linked to periodontitis [34]. No plasma IL-17 was detected in the cases, and very low levels were found in the controls, while low and similar IL-23 levels were observed in the two groups. Although some authors have proposed a local role for these ILs in the periodontal destruction process, their low plasma levels in the present study suggest that they would have no relevant effect on birth outcomes. IL-23 is an essential mediator in expansion of the Th17 lineage, which is associated with numerous immune-related destructive tissue diseases. IL-17 expression was significantly higher in periodontal lesions than in control sites, especially in the tissue adjacent to bone destruction, suggesting that the IL-23-induced Th17 pathway is stimulated in inflammatory periodontal lesions [35].

In conclusion, the present findings indicate that CA loss is associated with plasma TNF-α and IL-1β levels. No plasma cytokine profiles suggestive of systemic inflammatory response were observed in pregnant women with PB/LBW neonates.

Conflict of interest The authors declare no financial interests, either directly or indirectly, in the products or information listed in the paper.

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