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MATERNAL-FETAL MEDICINE

Periodontopathogenic microbiota, infectious mechanisms and preterm birth: analysis with structural equations (cohort—BRISA)

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Abstract

Purpose The association between periodontopathogenic microbiota and preterm birth (PTB) has been overly studied. However, the biological mechanisms involved are little known. The objective is to evaluate the effect of periodontopathogenic bacteria burden (PBB), periodontal disease and other infections during pregnancy on preterm birth (PTB), through Structural Equation Modeling.

Methods This was a case–control study nested in a prospective cohort called BRISA, including 330 pregnant women, 110 cases and 220 controls. This study included the following variables: cytokines interleukin-10 (IL-10) and transforming growth factor beta (TGF-β), periodontal disease, PBB, age, socioeconomic status (SES), systemic infections and PTB. The correlations between variables were analyzed using Standardized Coefficient (SC).

Results Greater PBB interfered positively with the occurrence of periodontal disease (SC: 0.027; *p*: 0.011), but these were not associated with the cytokines studied, nor with PTB. The lower serum levels of IL-10 (SC −0.330; *p* 0.022) and TGF-β (SC −0.612; $p < 0.001$), and the presence of other systemic infections during pregnancy (SC 0.159; 0.049) explained the higher occurrence of PTB.

Conclusion It is possible that only the more severe periodontal disease and other systemic infections are capable of altering the cascade of cytokines regulating the inflammatory process and have an effect on the occurrence of PTB.

Keywords Infection · Periodontal diseases · Anaerobic bacteria · Premature birth · Epidemiological studies

Introduction

Preterm birth (PTB) is the second leading cause of death in children under five and is one of the major risk factors for neonatal infections and growth disorders [1, 2]. The etiology of PTB has not yet been fully elucidated. However, socioeconomic conditions, maternal systemic, and oral infections have been identified as important risk factors for this outcome [3, 4].

Maternal infections may trigger inflammatory responses in the mother and fetal tissues that may lead to prostaglandin production, increased myometrial contractility, rupture of fetal membranes, and consequent PTB [4–6]. However, no study has simultaneously evaluated whether these systemic infections—bacterial vaginosis, urinary tract infection, measles, chickenpox, rubella, toxoplasmosis, and syphilis—and periodontal disease may interfere with PTB.

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Systemic infections may interfere with the inflammatory process and alter the number of regulatory cytokines, such as interleukin-10 and TGF. However, the pathophysiological mechanism is not yet known, nor is it known whether the inflammatory response of systemic infections differs from oral infections, such as periodontal disease [7, 8], for the PTB outcome.

The relationship between periodontal disease during pregnancy and the occurrence of adverse outcomes at birth, such as PTB, is still controversial [9–11]. The severity of periodontal disease in pregnant women has been considered a potential risk factor for the occurrence of PTB [9, 10]. The mechanism involved has not been fully elucidated, but there are two proposed pathways in which periodontal disease can be associated with PTB: (1) directly, when the periopathogens invade the fetal-placental unit, subsequently stimulating the inflammation; or (2) indirectly, when systemic inflammatory mediators act simultaneously with inflammation in the periodontal tissues [12, 13].

There are systematic reviews and meta-analyses [9, 11, 14] in which women with periodontitis were at increased risk of having babies with PTB. However, in a meta-analysis that included articles evaluating the effect of periodontal treatment during pregnancy on adverse outcomes at birth, no effect reversal was observed [15].

The distinct criteria for the diagnosis of periodontal disease may contribute to the under- or overestimation of this disease effect on the PTB [11]. How the PTB is classified, and the variability of settings used for confounding adjustment can also be responsible for differences in the results [15]. Therefore, the infectious mechanisms involved in the PTB are not fully understood.

Periodontal disease is an oral condition whose diagnosis is difficult to ascertain. In the present study, "Periodontopathogenic Burden" was a continuous latent variable, deduced from the observed correlations [16] among four bacteria: *Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum*, *Porphyromonas gingivalis,* and *Prevotella intermedia.* This strategy reduces measurement error [16]. Nevertheless, the presence of these bacteria is not enough by itself to measure periodontal disease. Therefore, we also considered other indicators as part of the theoretical model, like periodontal probing depth, clinical attachment level, and bleeding on probing. Furthermore, we are going to test direct and indirect associations between the risk factors and PTB.

Studies evaluating factors associated with PTB used a traditional multiple regression approach [13, 17–19], without analyzing the direct and indirect effects of a set of independent variables on this outcome. The classic analytical methods cannot deal with this kind of complexity. Therefore, we advocate the use of the Structural Equation Modeling—SEM—to analyze different pathways. SEM may be a better tool for studying complex phenomena [16].

The present study aimed to evaluate the effect of PBB, periodontal disease (PD), and other infections during pregnancy on PTB, as well as the possible explanatory pathways (direct and indirect) of these associations, through SEM.

Methods

Study design and sample characterization

We developed a case–control study nested to the prospective *BRISA* [20] cohort. The reference population consisted of pregnant women who received prenatal care in public and private health services and were referred to the University Hospital of the Federal University of Maranhão, where they underwent ultrasonography between 22 and 25 months of gestational age (GA) and were included in the study.

The recruitment took place between 02/2010 and 11/2011, involving 1.447 pregnant women (*baseline* or T1). Of these, 66 did not show up to follow-up visits or did not answer the questionnaires. A total of 1381 (93.94%) were followed up on at the time of the baby's birth (T2). For this study, all pregnant women whose babies were born preterm ($n = 110$) and a control sample, 2:1 ($n = 220$), selected by simple random draw without replacement, were included in the study, of which pregnant women whose babies were born full-term, totalizing 330 dyads (pregnant women and children).

Sample power calculation

We estimated that this sample size would have an 87% probability to identify significant odds ratios of 2.5, considering a 5% probability of type I error and prevalence of 12.0% of exposure among controls.

Data collection

In the *baseline*, the following variables were collected: age (in years), economic classification according to the Brazilian Association of Study and Research (ABEP in Portuguese) [21] criteria, income, PBB measured from the gingival crevicular fluid, periodontal clinical parameters and serum IL-10 and TGF-β.

Duly trained examiners (Kappa ≥ 0.80) collected the gingival crevicular fluid and gauged the periodontal clinical parameters.

We collected the gingival crevicular fluid of the pregnant women through an absorbent paper cone, which was filled into tubes containing 5 mM of EDTA solution. Subsequently, the saliva was clarified through centrifugation at 13.000 rpm in a cooled microcentrifuge (at 4 °C) and then frozen at − 70 °C. We performed laboratory tests for

determining the PBB in the gingival crevicular fluid using checkerboard DNA–DNA hybridization technology [22]. We evaluated 13 different types of bacteria, but, for this manuscript, we selected four periodontopathogenic bacteria: *Aggregatibacter actinomycetemcomitans* (ATCC 29,523), *Fusobacterium nucleatum* (ATCC 25,586), *Porphyromonas gingivalis* (ATCC 33,277) and *Prevotella intermedia* (ATCC 25,611) [22].

The following periodontal clinical parameters were measured: Periodontal Probing Depth (PPD), Clinical Attachment Level (CAL), and Bleeding on Probing (BOP) in all teeth, except third molars, in six sites per tooth [23] with the aid of millimeter periodontal probe (North Carolina no 15 / WHO # 11.5, Hu-Friedy, Chicago, USA). We measured periodontal disease from the gingival margin to the most apical extension of the sulcus or pocket. We estimated the CAL from cement–enamel junction to the most apical extension of the sulcus or pocket. We verified the BOP by the presence or absence of bleeding after periodontal probing.

During the *baseline*, blood samples from pregnant women were collected through venipuncture by a nursing technician. The blood was submitted to the ELISA test to determine the presence and quantity of cytokines interleukin-10 (IL-10) and transforming growth factor beta (TGF-β). All used reagents were from the cytokine kit Th1/Th2/Th17 purchased by Becton Dickinson Biosciences (San Jose, CA, USA) [24].

In the second stage (T2), we collected data regarding GA and the following systemic infections during pregnancy: bacterial vaginosis, urinary tract infection, measles, chickenpox, rubella, toxoplasmosis, and syphilis. The women underwent a gynecological examination with the use of a disposable speculum. The criteria for the diagnosis of bacterial vaginosis were Nugent score and/or the presence of indicator cells [25]. We considered the presence of a ≥ 7 score as indicative of bacterial vaginosis. For the other infections, we interviewed women using a structured questionnaire. We asked about the presence of one or more of the systemic infections (listed above) diagnosed by a doctor, nurse, or dentist.

We assessed GA through an algorithm obtained from the ultrasound performed in the first trimester of gestation and by the date of the last menstrual period (LMP).

Observed variables

(1) Maternal age (in years); (2) periodontal disease (yes, if PD ≥ 4 mm, presence of BOP and CAL ≥ 4 mm, or not); (3) systemic infections in pregnancy (yes, if you have at least one of the above infections, or not); (4) PTB (yes if GA < 37 weeks, or not).

Latent variables

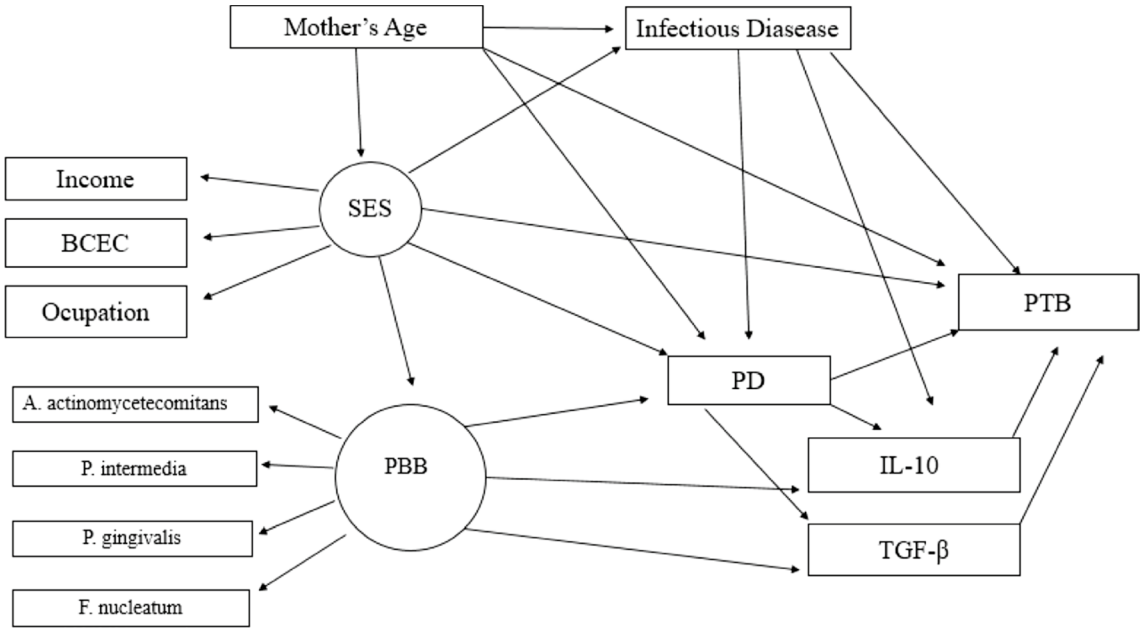
1. Socioeconomic status (SES)—it consisted of the following variables: (a) monthly family income based on the Brazilian minimum wage (R$ 510.00 / US$ 296.51 in 2010), categorized as: less than 1 salary, from 1 to 3 salaries, 3–5 salaries and higher/equal to 5 salaries; (b) occupation of the head of household (unskilled labor, semi-skilled labor, skilled labor, office roles, professional of higher level and administrators/managers/directors/owners); and (c) economic class according to the Brazilian Criteria of Economic Classification—BCEC (In Portuguese: Critério de Classificação Econômica Brasil—CCEB) [21], categorized as A/B (best), C and D/E (worst).
2. PBB—it consisted of four types of periodontopathogenic bacteria, identified in the gingival crevicular fluid of pregnant women: *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, and *Prevotella intermedia.*

Statistical analysis

In the analysis, we estimated the absolute and percentage frequencies and 95% confidence intervals (95% CI) for categorical variables, as well as means (± standard deviations) or medians (± interquartile deviations), respectively, for numerical variables with the symmetric or asymmetric distribution. We used Stata 14.0 (Stata Corp., College Station, United States) for this purpose.

To investigate the effects (direct, indirect and total) of PBB, Periodontal disease and other infections in pregnant women on PTB, adjusted for confounders, a theoretical model was initially proposed (Fig. 1), which were tested by SEM, using the software Mplus 7.0 (Muthen & Muthen, Philadelphia, Pennsylvania, United States).

The SEM is a technique to deal with multiple dependency relations simultaneously and to be able to represent concepts not observed in these relations, reducing the measurement error in the estimation process. This statistical analysis estimates a series of multiple regression equations. The model is a supposed pattern of direct and indirect linear relations between a set of observed variables and constructs [16]. It consists of two sub-models: the measurement model, which establishes how the constructs are measured; and the structural model, which analyzes the theoretical model as a whole, where the associations among the variables are estimated by Standardized Coefficient (SC). We interpreted the SC of the structural model as follows: coefficients with values close to 0.10 indicate a small effect, around 0.30 indicate minor effect and above 0.50 indicate a major effect. Negative SCs indicate inverse association and positive SC indicates a direct association [16].



IL-10: Interleukin-10

PBB: Periodontopathogenic Bacteria Burden

PD: Periodontal Disease

PTB: Preterm Birth

SES: socioeconomic status

TGF-β: Transforming Growth Factor Beta

Fig. 1 Theoretical model considering periodontogenic microbiota, infectious mechanisms and preterm birth. *IL-10* interleukin-10. *PBB* periodontopathogenic bacteria burden, *PD* periodontal disease, *PTB* preterm birth, *SES* socioeconomic status, *TGF-β* transforming growth factor beta

The elaboration of a latent variable is carried out in the measurement model, where latent variable indicators are specified. A good latent variable has convergent validity, verified by standardized factor loadings with high values (greater than 0.50) [16].

We evaluated the study model by the adjustment index, including RMSEA (Root Mean Square Error of Approximation), CFI (Comparative Fit Index), TLI (Tucker–Lewis Index) and WRMR (Weighted Root Mean Square Residual). Acceptable index values were considered for the models: RMSE < 0.05; the upper limits of 90% CI of RMSEA < 0.08; the values of CFI and TLI > 0.95; WRMR < 1.00. We also evaluated the Chi square, degrees of freedom and *p* value, but we did not adopt them as parameters for the model adjustment, given its sensitivity to the sample size (19). We considered the WLSMV estimator (Mean- and Variance-Adjusted Weighted Least Squares) which incorporates categorical variables [16]. The associations were estimated using the SFL, considering a 5% alpha.

Proposed theoretical model

For the construction of the theoretical model, we inferred that high PBB and the presence of PD activity during pregnancy might increase the probability of occurrence of PTB [17]. We also considered that the presence of systemic infections [26] during pregnancy might interfere with the progression of PD in PBB as well as in the PTB outcome [27]. We also believed that these infectious pathways could be explained directly or mediated by the action of cytokines IL-10 or TGF-β [27] (Fig. 1).

Results

We included 330 women: 110 with children born preterm and 220 full-term. The average age of women was 25.9 (± 5.7) years, ranging from 15 to 45 years. A higher proportion of women with a family income of 4.7 minimum

Table 1 Characteristics of the study population—BRISA (2011–2013)

Categoric variables	*n* (330)	%	
Systemic infections*			
Yes	121	36.7	
No	209	63.3	
A. actinomycetemcomitans			
Yes	34	10.3	
No	296	89.7	
Prevotella intermedia			
Yes	24	7.3	
No	306	92.7	
Porphyromonas gingivalis			
Yes	36	10.9	
No	294	89.1	
Fusobacterium nucleatum			
Yes	68	20.6	
No	262	79.4	
Preterm birth			
Yes	110	36.7	
No	220	63.3	
BCEC		%	CI 95%
A–B	64	19.4	15.3–24.1
C	222	67.3	61.9–72.3
D–E	44	13.3	9.8–17.5
Numeric variable	x (dp)	Med	(Q1–Q3)
Age of pregnant woman	25.9 (5.7)	25.00	22.00–29.00
Income (minimum wages)	4.7 (2.9)	4.00	2.00–7.00
IL-10 (pg/mL)	0.43 (1.06)	0.001	0.0001–0.25
TGF-β (pg/mL)	927,187,1 (3,544,191,0)	174,690,0	46,715,0–428,499,5
Gestational age (weeks)	37.91 (3.44)	39.00	37.00–40.00

N absolute frequency, *%* percentual, *x* mean, *SD* standard deviation, *Md* median, *Q1–Q3* 1st quartile–3rd quartile

*Measles, chickenpox, rubella, toxoplasmosis, syphilis and bacterial vaginosis

wages was observed, with 17.9% in the labor market, belonging to social class C (67.3%) (Table 1).

Table 2 shows the adjustment index of the models tested. All adjustment indicators were satisfactory for the model.

The variables that formed the latent SSE and PBB presented SFL greater than 0.5, indicating good convergent validity (Table 3).

The higher PBB interfered positively in the occurrence of PD (SC 0.027; *p* 0.011). We observed that lower serum levels of IL-10 explain the presence of systemic infections (SC −0.317; *p* 0.005). The lower serum levels of IL-10 (SC −0.330; *p* 0.022) and TGF-β (SC −0.612; $p < 0.001$) and the presence of other systemic infections during pregnancy (SC 0.159; 0.049) explain the higher occurrence of PTB (Table 4).

Discussion

Higher PBB had a direct and positive effect on periodontal disease activity in pregnancy, but these factors did not increase the risk of PTB. The lower serum levels of IL-10 were explained by the presence of systemic infections during pregnancy. The presence of systemic infections and the lower serum levels of IL-10 and TGF-β contributed to the increase in PTB.

The pathogenesis of the periodontal disease is a result of the accumulation of bacterial species in subgingival biofilm, particularly by Gram-negative anaerobic and microaerophilic bacteria, such as *Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens, Tannerella forsythia, Treponema denticola, Fusobacterium*

Table 2 Adjustment indices for modeling of structural equations—BRISA (2011–2013)

Index	Model
X^{2a}	60.716
Degrees of freedom	54
p value X^2	0.246
RMSEA	0.020
90% CI	0.000–0.041
Probability RMSEA < = .05[c]	0.994
CFI[d]	0.995
TLI[e]	0.993
WRSM[f]	0.706

[a]Certifying Chi square—$\chi 2$ (reference: lowest value)

[b]Interval 90% confidence (reference: IC 90% upper bound less than 0.08)

[c]Root Mean Square Error of Approximation—RMSEA (reference: less than 0.05)

[d]Comparative Fit Index—CFI (reference: greater than 0.90)

[e]Tucker–Lewis Index—TFI (reference: greater than 0.90)

[f]Weighted Root Mean Square Residual—WRMR (reference: less than 1.00)

Table 3 Factor loadings, standard errors, and *P* values for indicators of the latent variables—BRISA (2011–2013)

Latent variable	Factor loading	Standard error	*P* value
SES			
Income	0.660	0.078	< 0.001
BCEC	0.647	0.073	< 0.001
Ocupation	0.588	0.069	< 0.001
PBB			
Aggregatibacter actinomycetemcomitans	0.952	0.033	< 0.001
Prevotella intermedia	0.977	0.032	< 0.001
Porphyromonas gingivalis	0.922	0.038	< 0.001
Fusobacterium nucleatum	0.872	0.040	< 0.001

BCEC Brazilian economic classification criteria, *PBB* periodontopathogenic bacteria burden, *SES* socieconomic status

nucleatum, Aggregatibacter actinomycetemcomitans, and *Campylobacter rectus* [28–30]. The association between PBB and periodontal disease corroborates the idea that oral microbiota becomes more periodontopathogenic in pregnancy [17]. However, the evidence from previous studies is limited, since it is difficult to measure both periodontal disease and the dynamics of this microbiota [31, 32].

Various types of analysis and models have been helpful in understanding the multifactorial causes of periodontal disease [28–30]. Although the PBB has consisted of four types of periodontopathogenic bacteria, we also evaluated some other bacteria—*Morax catarrhalis, Pseudomonas aeruginosa, Haemophilus influenzae, Streptococcus gordonii, Streptococcus mutans, Streptococcus sobrinus, Streptococcus intermedius, Streptococcus constellatus,* and *Streptococcus mitis*. However, we selected for our study those most associated with periodontal disease [28–30], as well as those that produced a good construct (latent variable—PBB), with the best fit indices of the model. Although the restriction to only four bacteria is a limitation of our study, the formation of a latent PBB variable reproduces more accurately the specific coaggregation interactions between the periodontopathogenic microorganisms that occur during periodontal disease.

PBB and periodontal disease did not affect PTB. Studies on the relationship between PD and adverse perinatal outcomes present conflicting results [8, 18, 33–35]. Meta-analysis studies have yielded controversial results [9, 11, 15]. Some showed an association between periodontal infection and PTB [9, 11], while others did not observe any association [15]. Therefore, there are still doubts regarding these inconclusive results. These different results may be partially explained by the heterogeneity of the diagnostic criteria and the classification of periodontal disease. In this study, we measured the clinical parameters for periodontal disease in all teeth, which reduces the issues regarding the under- or overestimation of this exposure. Furthermore, differences in study design, sample size, and selection can also be responsible for differences in the results found [15]. In addition, the variability of settings for confounding may be a source of bias, to the point where infectious mechanisms involved in the PTB are not fully understood [12, 13]. Furthermore, it is possible that the association between periodontal disease and PTB is influenced by other systemic infections and inflammatory mechanisms [36].

T lymphocytes play an important role in pregnancy maintenance, especially in the balance between the T helper 1 (Th1) response, predominantly proinflammatory, and the T helper 2 (Th2), predominantly anti-inflammatory [37]. Activation of regulatory T cells (Treg) [38] leads to the production of interleukin-10 (IL-10) and transforming growth factor β (TGF-β), which act by inhibiting the inflammatory response [39]. In front of systemic infections, the Th2 response is suppressed, thus compromising this immune balance [40].

The lower levels of IL-10 were explained by the occurrence of systemic infections during pregnancy. This result corroborates the literature, since the deficiency of this cytokine is associated with hypoxia and viral and/or bacterial infections. IL-10 stimulates the production of tolerogenic dendritic cells, essential for the mechanism of maternal immunological tolerance. However, in the presence of

Table 4 Association with periodontopathogenic bacteria burden, periodontal disease and systemic infections during pregnancy on preterm birth—BRISA (2011–2013)

Explanatory variables	Outcomes	Standardized coefficient	Standard error	*P* value
SES	DP	0.109	0.102	0.288
PBB		0.027	0.122	0.011*
Systemic infectious	IL-10	−0.317	0.112	0.005*
PD		0.021	0.088	0.812
Systemic infectious	TGF-β	−0.088	0.075	0.238
PD		−0.117	0.089	0.188
PD	PTB	−0.143	0.110	0.192
IL-10		−0.330	0.144	0.022*
TGF-β		−0.612	0.061	< 0.001*
Systemic infectious		0.159	0.081	0.049*

IL-10 interleukin-10, *PBB* periodontopathogenic bacteria burden, *SES* socioeconomic status, *PD* periodontal disease, *PTB* preterm birth, *TGF-β* transforming growth factor beta

*$p < 0.05$

systemic infections, there is a decrease in levels, which compromises this immune balance [41–43].

The decrease in IL-10 levels and the lower serum TGF-β levels explained the higher occurrence of PTB. These results are following the studies of Ruiz et al. (2012) [44] and Harper et al. (2013) [44], in which PTB was associated with low serum levels of IL-10. However, a positive correlation between IL-10 and PTB has already been observed, suggesting that IL-10 can also be considered a biomarker of inflammation [45]. Therefore, the results are also underlined by major discrepancies. We speculate that there may be different subtypes of IL-10, as well as different subtypes of TGF-β [46, 47].

TGF-β is essential in maternal immune responses. It acts by decreasing the response of Th-1 cells and is essential for embryo implantation, growth, and maturation of the fetus [48]. Decreased levels of this parameter may contribute to the development of PTB [48]. However, higher levels of TGF-β were associated with a greater chance of PTB < 35 weeks [37], whereas in another study this association was not detected [49].

The divergence of the results regarding the role of these cytokines in the occurrence of PTB can be partially explained by methodological differences in the study design, size, and period to collect the biological samples for determining the serum levels of these cytokines, in the criteria for the GA classification and in the variables taken into account for the model adjustment.

The presence of systemic infections during pregnancy explained the higher occurrence of PTB. This result is in line with the current literature, since the intra-amniotic inflammation can occur due to the presence of microorganisms (bacteria, parasites or viruses) or other disease mechanisms, in which the necrosis or the cellular stress induce the release of important inflammatory mediators in the induction of PTB [50, 51].

The acting mechanism of this possible association is not fully elucidated. It may be mediated through proinflammatory cytokines with the release of prostaglandin, increased uterine contractility, favoring premature rupture of fetal membranes [52]. Another possibility is that systemic infections may unbalance the expression of regulatory cytokines, such as IL-10 and TGF-β, increasing the risk for PTB [24].

A limitation of our study was that infectious diseases, during pregnancy, except bacterial vaginosis, were self-reported, thus being subjected to memory bias and misestimation. However, we asked for infections occurring during pregnancy, thus, at a time still very close to the interview, reducing the possibility of memory bias. Furthermore, we only consider the presence of disease when diagnosed by a healthcare professional, which reduces misclassification. Another important issue is the possibility of disease treatment interference in the study outcome. Systematic reviews indicate that antibiotic treatment of bacterial vaginosis in pregnant women with abnormal vaginal flora might reduce PTB [53, 54]. For ethical reasons, we referred all women diagnosed with some infection to a medical or dental appointment. It may have reduced the strength of the association. However, some meta-analyses showed no evidence that periodontal treatment during pregnancy prevents adverse pregnancy outcomes, such as PTB [15, 35, 55], nor emphasize the low quality of the evidence [56–58]. Nevertheless, when analyzed together, infections remained associated with the occurrence of PTB.

This study underlines strengths such as the data collection in two moments; periodontal examination in all teeth at six different sites each, in the second trimester of gestation; the objective diagnosis for bacterial vaginosis; the

way we collected the GA variable through LMP, combined with the ultrasound analysis, reduced a possible memory and measurement bias for the outcome. Adjustment for systemic infections is another important aspect of this study, since much of the studies evaluating the association between periodontal disease, and adverse gestational outcomes, do not consider other infections in confounding adjustment. We were unable to identify other studies that had conducted analyses to qualify the oral bacteria involved in this association in theoretical models, including periodontal disease as well.

There is little evidence-based on case–control studies nested in a cohort with adequate sampling power. We were unable to identify other studies that had performed analyses to qualify the oral bacteria involved in this association in theoretical models, including periodontal disease as well. In addition, we did not identify studies that had used SEM. One of the strengths of this study is the statistical method used to simultaneously test the association of SES, PBB, periodontal disease, systemic infections, and cytokines, with PTB, using SEM. By being able to estimate a series of separate and interdependent multiple regression equations, this method tends to yield more reliable results. Moreover, it allows the estimate of the total, direct, and indirect effects between variables, presenting the ones that are mediating the total effect [16]. In addition, this method yields results that are easy to interpret and allows us to work with initial losses of variables that can be imputed by the method of estimation.

Thus, although the research question is not unprecedented, the controversial results in the literature, indicating the need for studies with more robust methodological design [9, 11–13, 15], motivated us to conduct this investigation.

This study sample was nested to the Brazilian birth cohorts (BRISA). The main purpose of BRISA was to identify risk factors for preterm birth (PTB), using more accurate methods to have greater predictive power than other classic studies [20, 59]. Some of these methodological strategies were blood cytokine dosage, bacterial identification by DNA analysis, periodontal examinations at six sites per tooth in all teeth, calculation of gestational age by algorithm considering date of last menstruation and ultrasound, among others procedures. We also included in our theoretical model some of the risk factors already identified in previous studies using the BRISA cohort data as socioeconomic factors [60], maternal age [61], and regulatory cytokine expression [24]. Studies have revealed a possible association between periodontal disease during pregnancy and PTB [9, 11, 14]. These findings indicate the importance of including oral health variables for mother and child in cohort studies to understand how these factors are associated with different outcomes. In the current literature, most of the risk factors have been tested individually, while the BRISA produces studies in which the factors are investigated using an integrated multidisciplinary approach to propose more effective strategies for the reduction of PTB.

The presence of PBB explains the higher occurrence of periodontal disease during pregnancy, but these factors do not interfere in the increase in PTB. The presence of systemic infections explains lower serum levels of IL-10. The lower serum levels of IL-10 and TGF-β and the presence of systemic infections explain the occurrence of PTB. Therefore, it is possible that only the more severe periodontal disease and other systemic infections are capable of altering the cascade of cytokines regulating the inflammatory process and have an effect on the occurrence of PTB.

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Author contributions EBAFT, CCCR, AAMS, HB, and MCS designed the study. EMC, CMSAF, RFMM, and CMCA reviewed the literature. EMC and EBAFT performed the statical analyses. EMC, MLTS, RFMM, and RDS performed the laboratorian analyses. EMC, RFMM, and CSAF wrote the draft. EBAFT, EMC, and RFMM critically revised the manuscript. All the authors approved the final version.

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Compliance with ethical standards

Conflict of interest There is no conflict of interest in this paper.

Ethical standards The study was approved by the Research Ethics of the University Hospital of the Federal University of Maranhão under the no 223/2009, protocol: 4771 / 2008–30. All participants have signed the free and informed consent form.

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