UNIVERSIDADE FEDERAL DO MARANHÃO
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
DOUTORADO

ASSOCIAÇÃO MEDIADA POR TGF-β E IL-10 ENTRE PERIODONTOPATÓGENOS EM GESTANTES E NASCIMENTO PRÉ-TERMO

SÃO LUÍS
2019

UNIVERSIDADE FEDERAL DO MARANHÃO
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
DOUTORADO

ASSOCIAÇÃO MEDIADA POR TGF-β E IL-10 ENTRE PERIODONTOPATÓGENOS EM GESTANTES E NASCIMENTO PRÉ-TERMO

SÃO LUÍS
2019

CAMILLA SILVA DE ARAUJO FIGUEIREDO

ASSOCIAÇÃO MEDIADA POR TGF-β E IL-10 ENTRE PERIODONTOPATÓGENOS EM GESTANTES E NASCIMENTO PRÉ-TERMO

Tese apresentada ao Programa de Pós-Graduação em Odontologia como parte dos requisitos para a obtenção do título de Doutor em Odontologia.

Orientador: Profª Drª Erika Bárbara Abreu Fonseca Thomaz

Co-orientador: Profª Drª Cláudia Maria Coelho Alves

SÃO LUÍS
2019

Figueiredo, Camilla Silva de Araujo.
Associação mediada por TGF-β e IL-10 entre periodontopatógenos em gestantes e nascimento pré-termo / Camilla Silva de Araujo Figueiredo. - 2019.
49 f.

Coorientador(a): Cláudia Maria Coelho Alves.
Orientador(a): Erika Bárbara Abreu Fonseca Thomaz.
Tese (Doutorado) - Programa de Pós-graduação em Odontologia/ccbs, Universidade Federal do Maranhão, São Luís, 2020.
1. Bactérias anaeróbias. 2. Gravidez. 3. IL-10. 4. Nascimento prematuro. 5. TGF-Beta. I. Alves, Cláudia Maria Coelho. II. Thomaz, Erika Bárbara Abreu Fonseca. III. Título.

CAMILLA SILVA DE ARAUJO FIGUEIREDO

ASSOCIAÇÃO MEDIADA POR TGF-β E IL-10 ENTRE PERIODONTOPATÓGENOS EM GESTANTES E NASCIMENTO PRÉ-TERMO

A Comissão julgadora da Defesa do Trabalho Final de Doutorado em Odontologia, em sessão pública realizada no dia ___/___/____, considerou a candidata

( ) APROVADA ( ) REPROVADA

1) Examinador: Prof. Dr. Isaac Suzart Gomes Filho

2) Examinador: Profª Drª Ana Regina Oliveira Moreira

3) Examinador: Profª Drª Arlene de Jesus Mendes Caldas

4) Examinador: Prof. Dr. Bruno Braga Benatti

5) Presidente (Orientadora): Profª Drª Erika Bárbara Abreu Fonseca Thomaz

A Deus, sempre

Ao meu marido Guilherme

Às minhas filhas Beatriz e Isabella

Aos meus pais, Wilton e Priscimar.

AGRADECIMENTOS

Ao meu Deus, obrigada por permanecer comigo.

À minha orientadora, Profª Drª Erika Thomaz. Tive a honra de ter sua presença em minha banca de Mestrado e agora fui mais honrada ainda com sua orientação. Muito obrigada por tudo! Sua dedicação e profissionalismo são exemplos que busco seguir!

À minha co-orientadora, profª Drª Cláudia Alves, muito obrigada pelo carinho desde a graduação e pelas contribuições para esta tese!

À Coordenação do Programa de Pós-graduação em Odontologia da UFMA.

Aos professores do PPGO.

À Josinéia Costa Coelho, a querida Josi, secretária do PPGO, por toda a atenção e carinho!

À Ana Basília dos Reis Oliveira, a doce Aninha, sempre disposta a ajudar!

À meiga e competente Elisa, pela ajuda nos artigos!

Ao Programa de PGSC, por acolher-me sempre!

A todos os participantes da coorte BRISA.

Aos professores da banca de qualificação, Drº Bruno Benatti e Drª Arlene Caldas. Obrigada pelas valiosas contribuições!

Ao meu grande amor Guilherme, pelo companheirismo e dedicação à nossa família!

Às minhas filhas, por todo o amor com que me olham, me incentivando a ser uma pessoa melhor!

Aos meus pais, pela formação do meu caráter e pelo apoio incondicional!

À minha irmã Priscilla, ao meu cunhado Breno e sobrinhos Gui, Anna, Amandinha e Chande, pelo amor e torcida!

À minha amiga-irmã e comadre Érica Valois, pela ajuda na etapa final e incentivo sempre!

À Cíntia Rosalem, pela parceria e amizade!

“Dê ao mundo o melhor de você. Isso pode não ser o bastante, mas dê o melhor de você assim mesmo. Veja que, no final das contas, é tudo entre você e Deus. Nunca foi entre você e os outros.”

(Santa Teresa de Calcutá)

RESUMO

O organismo da mulher sofre diversas alterações fisiológicas, imunológicas e hormonais no período gestacional. Na cavidade oral parece haver um aumento na proporção de bactérias anaeróbias. Alguns estudos têm pesquisado a associação entre problemas bucais na gestação e o nascimento pré-termo (NPT). Entretanto, os resultados são ainda controversos e os mecanismos biológicos envolvidos são pouco conhecidos. São apresentados dois artigos neste estudo. O primeiro, já publicado, foi uma revisão de literatura sobre alterações bucais na gestação com o objetivo de discutir alterações bucais relacionadas à gestação, seus mecanismos sistêmicos e o tratamento odontológico na gestação, identificou que a inflamação dos tecidos periodontais aumenta em extensão e gravidade durante o curso de uma gravidez normal, mesmo sem alteração na quantidade de biofilme presente. Além disso, o tratamento odontológico durante a gravidez é seguro, indicado e pertinente, constituindo-se em importante conduta preventiva para o binômio mãe/filho. O segundo artigo, um estudo de caso-controle aninhado a uma coorte prospectiva de gestantes na cidade de São Luís-MA, Brasil (BRISA), tem o objetivo de avaliar a associação entre periodontopatógenos em gestantes e NPT. Neste estudo, utilizando análise de equações estruturais, não foi identificada associação entre a presença de periodontopatógenos e NPT. Conclui-se que o tratamento odontológico durante a gestação promove benefícios para a saúde da paciente, oferecendo uma condição oral de conforto, função e estética. Além disso, a presença de periodontopatógenos isoladamente não representa um fator de risco independente para o NPT, mas apenas ao considerar conjuntamente todas as infecções ocorridas durante a gestação.

Palavras-Chave: Infecção. Doenças Periodontais. Bactérias Anaeróbias. Nascimento Prematuro. Gravidez. IL-10. TGF-β.

ABSTRACT

The woman's body undergoes various physiological, immunological and hormonal changes during pregnancy. In the oral cavity appears to be an increase in the proportion of anaerobic bacteria. Some studies have investigated the association between oral health problems during pregnancy and preterm birth. However, the results are still controversial and the biological mechanisms involved are poorly understood. Two articles are presented. The first one, already published, was a review of the literature on oral changes during pregnancy. The objective was to discuss the major oral changes related to pregnancy, their possible systemic mechanisms and dental treatment during pregnancy, found that the inflammation of the periodontal tissues increases in extent and severity during the course of normal pregnancy, even without change in the amount of biofilm present. In addition, dental treatment during pregnancy is safe, suitable and relevant, constituting an important preventive procedure for both (mother/child). The second article, a case-control study nested in a prospective cohort of pregnant women in the city of São Luís, Brazil (BRISA), aims to evaluate the association between periodontal pathogens in pregnant women and preterm birth. In this study, using structural equation modeling, it was found that the presence of periodontal pathogens was not associated with preterm birth. We conclude that dental treatment during pregnancy promotes the health benefits of the patient, providing an oral condition of comfort, function and aesthetics. Moreover, the presence of periodontal pathogens alone does not represent an independent risk factor for preterm birth, but only when considering all infections during pregnancy.

Keywords: Infection. Periodontal Diseases. Anaerobic bacteria. Preterm birth. Pregnancy. IL-10. TGF-β.

SUMÁRIO

1 INTRODUÇÃO

A formação do biofilme oral é um fenômeno normal que contribui para as defesas do hospedeiro. Dessa forma, os problemas bucais são o resultado de um desequilíbrio da homeostase existente entre as comunidades microbianas no biofilme. Dois exemplos comuns são a cárie dentária e a doença periodontal (DP). Um iniciador desse desequilíbrio pode ser o excesso de carboidratos, que altera o biofilme supragengival, levando a uma tendência ao crescimento de bactérias mais acidúricas e acidogênicas (como *Streptococcus mutans*) e, consequentemente, à cárie. Posteriormente, o acúmulo deste biofilme, devido a uma pobre higiene oral, pode resultar em bolsas periodontais, criando meios anaeróbicos e favorecendo a proliferação de bactérias periodontopatogênicas, como *Fusobacterium spp* e *Prevotella spp*, dentre outras (PATIL et al., 2013).

Na gestação, alterações nos níveis hormonais maternos levam a uma mudança na resposta imunológica da gestante com o objetivo de fazer com que o organismo materno não rejeite o embrião (MICHELON et al., 2006; DAHER e MATTAR, 2009; WATANABE et al., 2014). Entretanto, essa mudança torna o organismo da mulher mais susceptível a infecção, inclusive orais (LÖE e SILNESS, 1963; SILNESS e LÖE, 1964; BARAK et al., 2003; LEAL, 2006; MINOZZI et al., 2008; ARMITAGE, 2013; FIGUEIREDO et al., 2017).

Ocorre ainda diminuição do pH e, consequentemente, da capacidade tampão da saliva durante a gravidez, o que, junto com a mudança de hábitos, contribui para o crescimento bacteriano e aumenta o risco à cárie (LAINE, 2002; MARTÍNEZ-PABÓN et al., 2014) e à doença periodontal (CARRILLO-DE-ALBORNOZ et al., 2010; REIS et al., 2010).

Além disso, alterações no estrogênio e progesterona associadas com a gravidez também têm um efeito sobre a composição da microbiota subgengival (CARRILLO-DE-ALBORNOZ et al., 2010), tornando-se mais anaeróbia com o progresso da gravidez (REIS et al., 2010).

Entre as alterações orais que acometem mulheres grávidas mais citadas na literatura estão: granuloma piogênico, gengivite e periodontite (GURSOY et al. 2008; RUSSELL e MAYBERRY 2008; SILK et al. 2008; FIGUEIREDO et al., 2017). Infecções periodontais são causadas por bactérias anaeróbicas gram-negativas e promovem um aumento no nível de prostaglandina, que é um importante indutor fisiológico do parto (LEAL, 2006). Estudos de meta-análise evidenciam que a doença periodontal pode ser considerada um fator

de risco independente para o nascimento pré-termo (NPT) ou o baixo peso ao nascer (BPN) (VERGNES e SIXOU, 2007; POLYZOS et al., 2009). No entanto, os mecanismos biológicos envolvidos nesse processo são pouco claros (AZARPAZHOOH e TENENBAUM, 2012; ARMITAGE, 2013).

O NPT é uma intercorrência mundialmente relevante por elevar as taxas de morbimortalidade neonatal (THOMAZ et al., 2015). Percebe-se que, apesar de mudanças favoráveis na situação sociodemográfica, no acesso a serviços de saúde e até mesmo na diminuição das taxas de restrição de crescimento intrauterino (RCIU), as taxas de BPN e NPT têm aumentado no decorrer dos anos em várias regiões do mundo (BLENCOWE et al., 2012; BLENCOWE et al., 2013; SILVA et al., 2015).

Algumas hipóteses – neuroendócrina, de intervenção médica e imunoinflamatória – para explicar as taxas de NPT têm sido levantadas (SILVA et al., 2014). Segundo a hipótese neuroendócrina, fatores como discriminação, pouco suporte social, violência, estresse e depressão, ou mesmo alterações genéticas, podem ativar o eixo hipotálamo-hipofisário, iniciando uma cascata de eventos neuroendócrinos, mediados pela ação de ocitocinas e prostaglandinas, dentre outros, e culminando com o NPT. Uma segunda hipótese, a da intervenção médica, defende que o aumento das tecnologias médicas têm reduzido as taxas de natimortalidade por meio de intervenção cirúrgica cesariana, aumentando o NPT (JOSEPH et al., 2005; BETTIOL et al., 2000; BARROS et al., 2005; SILVA et al., 2004). Uma terceira hipótese, a imunoinflamatória, refere que infecções maternas durante a gestação – inclusive a doença periodontal – seriam capazes de iniciar uma resposta imunoinflamatória mediada pela ação de citocinas proinflamatórias, induzindo inflamação intra-amniótica e trabalho de parto prematuro (ROMERO et al., 1989; XIONG et al., 2006; MACONES et al., 2004; MOORE et al., 2004; HUCK et al., 2011; FOXMAN et al., 2014). No entanto, essas hipóteses ainda não foram adequadamente testadas.

Especificamente sobre o papel da doença periodontal e das bactérias periodontopatogênicas sobre a ocorrência de NPT, não identificamos estudos que avaliassem os possíveis caminhos causais envolvidos nessa relação. Além disso, os resultados de revisões sistemáticas e de meta-análises são controversos; alguns identificam associação (KHADER YS, TA'ANI, 2005; GEORGE et al., 2011; MATEVOSYAN, 2011; CORBELLA et al., 2012; STADELMANN et al., 2013) e outros não (POLYZOS et al., 2010; UPPAL et al., 2010; BACCAGLINI et al., 2011; CHAMBRONE et al., 2011; ROSA et al., 2012; BOUTIN et al., 2013); e alguns alertam para problemas na qualidade dos estudos (XIONG et al., 2007;

GEORGE et al., 2011; RAMCHANDANI et al., 2011; CORBELLA et al., 2012; STADELMANN et al., 2013; LÓPEZ et al., 2015).

2 OBJETIVOS

- Discutir alterações bucais relacionadas à gestação, seus mecanismos sistêmicos e o tratamento odontológico na gestação;
- Analisar a associação mediada por IL-10 e TGF-β entre periodontopatógenos em gestantes e nascimento pré-termo.

3 REFERENCIAL TEÓRICO

3.1 Gestação e alterações microbiológicas na cavidade oral

Desde 1980, Kornman e Loesche descobriram que, durante a gravidez, há um aumento da proporção de bactérias anaeróbias e aeróbias, como *Prevotella intermedia* e *Porphyromonas gingivalis*. Em 1981, Jensen et al. estudaram o efeito de níveis hormonais sobre o estado gengival de 54 mulheres grávidas, 23 mulheres não grávidas que utilizavam contraceptivos orais e 27 controles não gestantes. Eles descobriram que as mulheres grávidas tinham um nível de *Bacteroides spp* 55 vezes maior do que a do grupo de controle não grávidas. Além disso, as mulheres que receberam a terapia de contraceptivos orais tinham um nível 16 vezes maior de *Bacteroides spp* em comparação com as mulheres do grupo controle. No entanto, de acordo com os autores, os referidos aumentos de bactérias específicas parecem ser cíclicos por natureza, pois seguem as alterações fisiológicas normais e geralmente não apresentam nenhuma consequência. Os autores não estudaram a relação entre as bactérias e desfechos gestacionais.

Os hormônios esteróides específicos da gravidez parecem capazes de influenciar a microbiota normal e induzir alterações na ecologia subgengival (RODRIGUES et al., 2004). No segundo trimestre de gestação, ocorre um aumento significante de gengivite e na proporção de bactérias anaeróbias/aeróbias. Também foram encontradas correlações entre os níveis de hormônios maternos e *Prevotella intermedia* (LINDHE, 1997; ARMITAGE, 2013; CARRILLO-DE-ALBORNOZ et al., 2010) e *Porphyromonas gingivalis* (CARRILLO-DE-ALBORNOZ et al., 2010).

A sobrevivência das bactérias localmente invasivas, como *Prevotella intermedia* e *Aggregatibacter actinomycetemcomitans* (previamente denominada *Actinobacillus actinomycetemcomitans*) na gestação pode também ser explicada por alterações imunológicas que aumentam a susceptibilidade para os agentes patogénicos intracelulares (ARMITAGE, 2013). Este autor sugere que, na gestação, há redução da atividade antimicrobiana dos neutrófilos periféricos, componentes essenciais das defesas imunitárias inatas dos tecidos periodontais. Isso poderia estar relacionado ao bem documentado aumento da inflamação gengival observado durante a gestação (LÖE e SILNESS, 1963; SILNESS e LÖE, 1964;

BARAK et al., 2003; LEAL, 2006; TSUKIMORI et al., 2006; MINOZZI et al., 2008; ARMITAGE, 2013).

Diante do exposto, pode-se verificar que as doenças periodontais induzidas por biofilme, como gengivite e periodontite, são infecções multifatoriais envolvendo interações complexas de biofilmes microbianos com respostas imunes inatas e adaptativas do hospedeiro. As alterações fisiológicas associadas com a gravidez têm efeitos profundos sobre as interações parasita-hospedeiro encontradas nestas doenças (ARMITAGE, 2013; FIGUEIREDO et al., 2017). Embora os mecanismos responsáveis pelo aumento da inflamação gengival observado durante a gravidez ainda não estejam completamente compreendidos, está claro que vários fatores, como desordens da função dos neutrófilos, mudanças na fisiologia celular induzidas por hormônios e efeitos locais sobre a ecologia microbiana desempenham, conjuntamente, papeis importantes na doença periodontal.

Os profissionais da saúde devem ter conhecimento acerca do aumento na predisposição a tais intercorrências para esclarecer às mulheres sobre a necessidade de procurar atendimento odontológico na gestação. Entretanto, a saúde bucal, parte importante e indissociável da saúde geral, vem sendo pouco abordada e priorizada nas políticas de atenção à saúde das gestantes no Brasil e no mundo (MOIMAZ et al., 2006; RESSLER-MAERLENDER et al., 2010).

Assim, a atenção odontológica à mulher no período gravídico é indicada e pertinente, seja como uma prevenção das doenças bucais e suas possíveis consequências, seja para tratar doenças já instaladas (FIGUEIREDO et al., 2017). Além disso, com certas precauções, o tratamento é seguro. Entretanto, crenças, mitos, receios e resistência dificultaram por muito tempo esse cuidado e, ainda hoje, confundem pacientes e profissionais (LEAL, 2006; RESSLER-MAERLENDER et al., 2010).

3.2 Imunomodulação na gravidez

Para o sucesso de uma gestação, são necessários diversos mecanismos e alterações no organismo da mãe, principalmente em seus sistemas endócrino e imunológico. Caso não ocorra essa modulação o feto pode ser rejeitado (WATANABE et al., 2014).

O sistema imunológico apresenta dois tipos de resposta: a inata e a adaptativa. A resposta inata é responsável por uma reação não específica aos antígenos, enquanto a resposta

adaptativa é extremamente específica aos antígenos (AAGAARD-TILLERY et al., 2006). Durante a gestação, há mudanças nas citocinas mediadoras dessas respostas para que o feto não seja rejeitado e o equilíbrio imunológico seja mantido (WATANABE et al., 2014). Porém, esse processo é mais difícil diante de infecções sistêmicas e morbidades como a hipertensão (MATSUOKA, 2001).

A resposta imunológica da gestante sofre, portanto, um abrandamento, diminuindo a atividade inflamatória, com alteração nas citocinas *T helper* (Th). Essa imunomodulação é induzida pelos hormônios progesterona e estrogênio, induzindo uma resposta Th2 do organismo, o que permite a não rejeição do feto e a manutenção da gravidez. Entretanto, as citocinas Th1 têm um papel essencial para a implantação e o desenvolvimento placentário. Dessa forma, é fundamental o equilíbrio entre citocinas Th1 e Th2 (WATANABE et al., 2014).

As citocinas do tipo Th1 podem produzir um meio pró-inflamatório. O excesso de citocinas do tipo Th1 (IL-2, TNF-α e IFN-γ) tem sido relacionado a prejuízos para o feto e associado com a pré-eclâmpsia (SZARKA et al, 2010). As citocinas Th2 estão envolvidas na regulação do desenvolvimento de citocinas Th1 e na manutenção de um ambiente anti-inflamatório. As citocinas Th2 têm capacidade para proteger contra a rejeição, portanto, desempenham um papel fundamental durante a gravidez (SYKES et al., 2012).

O balanceamento da resposta imunológica da gestante, com controle dos mecanismos inflamatórios, depende essencialmente da Interleucina 10 (IL-10) e do *Transforming Growth Factor Beta* – TGF-β (DAHER e MATTAR, 2009).

A progesterona influencia o balanço Th1/Th2 através do aumento da produção de IL-10 e da diminuição da produção de citocinas Th1 (DAHER e MATTAR, 2009).

A IL-10 desempenha um papel fundamental na manutenção e desenvolvimento da gravidez, contribuindo para a manutenção do corpo lúteo e placenta (BROGIN MORELI et al., 2012).

A diminuição dos níveis de IL-10 pode inibir a resposta Th2, levando à pré-eclâmpsia, NPT e RCIU, eventos frequentemente associados à infecção durante a gravidez (THAXTON e SHARMA, 2010; FERGUSON et al., 2014). É provável que as infecções bacterianas, virais e parasitárias induzam a resposta imune Th1, promovendo a propagação do IFN-γ, a regulação da IL-12 e a inibição das respostas Th2 (THAXTON e SHARMA, 2010).

Existem, entretanto, muitas divergências quanto à associação entre o nível de IL-10 e NPT, pois níveis mais baixos já foram apontados como fatores de risco para desfechos

perinatais adversos, assim como níveis mais elevados (WEISSENBACHER et al., 2013). Portanto, a relação entre os níveis de citocinas anti-inflamatórias e pró-inflamatórias, doenças sistêmicas, desfechos gestacionais adversos ao nascimento apresenta resultados conflitantes.

O tipo celular Th2 estimula a produção de TGF-β, outra citocina imunossupressora que exerce papel fundamental na imunomodulação e no estabelecimento da gravidez, auxiliando na não rejeição do feto (GORCZYNSKI et al., 2002).

O TGF-β direciona todo o processo de fecundação e implantação, sendo encontrado no líquido seminal e produzido precocemente na interface materno-fetal pelo embrião e pela decídua. Influencia, portanto, na implantação e no desenvolvimento placentário e fetal (DAHER e MATTAR, 2009).

Pela diminuição da resposta das citocinas Th-1, o TGF-β cumpre um papel relevante no sistema imune periférico, mediando a aquisição da tolerância imunológica (SCHMIDT-WEBER e BLASER, 2004). A tolerância é essencial para a prevenção de respostas imunitárias inapropriadas, sendo um componente essencial para o ambiente uterino, permitindo a implantação do embrião e consequente gestação (JONES et al., 2006).

O TGF-β está expresso em três isoformas distintas no endométrio, o TGF-β2 predominantemente localizado no estroma, enquanto TGF-β1 e TGF-β3 estão presentes no epitélio e células do estroma (JONES et al., 2006). Já foi observado que níveis séricos mais baixos de TGF-β1 estão associados à ocorrência de RCIU (LI, 2014). Além disso, mutações no TGF-β2 podem interferir na formação e crescimento do feto (SHAARAWY, EL MELEIGY, RASHEED, 2001). Níveis mais elevados de endoglina solúvel em gestações com RCIU podem estar relacionados com a hipóxia placentária, envolvendo o TGF-β3 (YINON et al., 2008).

O subtipo TGF-β1 atua no desenvolvimento embrionário e na regulação imunológica da gravidez, na inibição da produção de citocinas do tipo Th1 e promoção da diferenciação Th2 (BRIDOUX et al., 1997).

Somente com o equilíbrio e a coordenação imunológica e hormonal é possível ocorrer a tolerância da mãe ao feto (WATANABE et al., 2014). Como essas alterações se fazem sistemicamente, é impossível dissociar a condição oral da gestante ao resto do corpo, portanto a resposta imunológica abrandada interferirá também na saúde oral da mulher grávida, tornando-a mais susceptível a infecções orais (FIGUEIREDO et al., 2017).

4 CAPÍTULO I

THE JOURNAL OF
Obstetrics and Gynaecology Research

doi:10.1111/jog.13150 J. Obstet. Gynaecol. Res. Vol. 43, No. 1: 16–22, January 2017

Systemic alterations and their oral manifestations in pregnant women

Camilla Silva de Araujo Figueiredo, Cíntia Gonçalves Carvalho Rosalem, Andre Luis Costa Cantanhede, Érika Bárbara Abreu Fonseca Thomaz and Maria Carmen Fontoura Nogueira da Cruz

Clinica Odontologica, Federal University of Maranhao, São Luís, Brazil

Abstract

The aims of this literature review are: to depict the main oral diseases that are related to pregnancy; to clarify some of the possible systemic mechanisms that are associated with these changes; and to address issues about oral care during pregnancy. A woman's organs undergo various physiological, neurological, and hormonal changes during pregnancy. Such changes occur gradually and are essential for the development of the fetus, providing what is needed for tissue formation and establishment of reserves for uterine and fetal life. In turn, the oral cavity shows some events during this period. Among the changes most frequently cited in the literature are pyogenic granuloma, gingivitis, and periodontitis. The inflammation of the periodontal tissues due to the formation of the biofilm increases dramatically in size and severity during the course of a normal pregnancy, even without changes in the amount of biofilm present. In addition, a decrease in salivary pH is observed in pregnant women and may lead to an increased incidence of dental caries in this period.

Key words: dental caries, endocrine, periodontal diseases, prematurity – risk assessment and prevention, prenatal care.

Introduction

During the course of a normal pregnancy, several deep and dynamic physiological changes occur in both the mother and the developing baby. Some of the endocrine and immune changes induced by pregnancy increase the susceptibility of the mother to various infections, including those of the oral cavity.[1–6]

Researches have shown that pregnant women are exposed to a higher risk of gingival alterations. The increased susceptibility to infections in the oral cavity can occur due to pH decrease and, consequently, the salivary buffer capacity during pregnancy, which, along with the change of dietary and oral hygiene habits, contributes to bacterial growth and increases the risk of caries.[7,8]

Among the changes most frequently cited in the literature are: pyogenic granuloma (Fig. 1), gingivitis (Fig. 2), and periodontitis (Fig. 3).[9–11] It is important to consider that there is evidence of the association between oral health during pregnancy and adverse problems, including low birthweight, preterm birth, and pre-eclampsia.[6,12,13]

Dental care for women during pregnancy is indicated and appropriate, either as prevention of oral diseases and their possible consequences, or to treat existing diseases. With certain precautions, the treatment is safe. Nonetheless, dental care during pregnancy has long been hindered by various beliefs, myths, fears, and resistance and many patients and professionals are still confused about the topic.[4,14]

Methods

We carried out a literature review using electronic retrieval systems and databases (PubMed [MEDLINE], ScienceDirect, and Scielo), accessing published work

Received: March 3 2016.
Accepted: August 7 2016.
Correspondence: Dr Camilla Silva de Araujo Figueiredo, DDS, MD, PhD in Dentistry, Clinica Odontologica, UFMA, Av. dos Portugueses, 1966 - Bacanga, São Luís - MA, 65080-805, Brazil. Email: camilla_araujo@hotmail.com

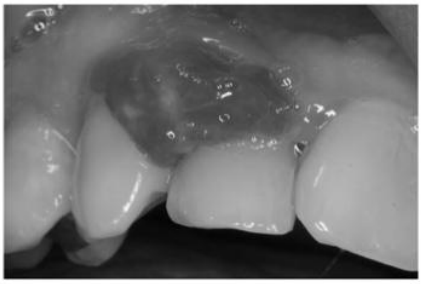

Figure 1 Pyogenic granuloma. Note the presence of a lobulated, usually pedunculated lesion, with color ranging from pink to red or purple.

Figure 2 Gingivitis. Note the increased tissue volume and more red gum.

from 1960 to 2015. We aimed: to depict the major systemic manifestations that can interfere with oral manifestations during pregnancy; to highlight the main oral changes related to pregnancy; and to address important aspects about dental treatment during pregnancy. We used the following search terms: 'prenatal care,' 'endocrine,' 'prematurity – risk assessment and prevention,' 'immunological factors,' 'periodontal disease,' 'pregnant,' and 'dental caries.'

Results and Discussion

Systemic alterations

A woman's body undergoes several changes during gestation. These changes occur gradually and are essential for the development of the fetus, providing what is needed for tissue formation and the establishment of reserves for uterine fetal life.[15] The fundamental physiological changes that occur in the body during pregnancy as well as the immune and endocrine changes that are related to the most prevalent oral manifestations during pregnancy are depicted below.

Physiological changes during pregnancy

- Increased heart rate around 10 b.p.m. from 14 to 30 gestational weeks;
- Increased respiratory rate;
- Increased oxygen consumption and a decrease in residual respiratory volume to about 15–20%;

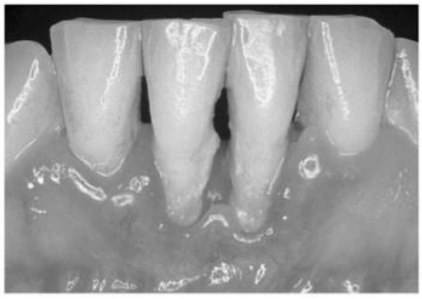

Figure 3 Periodontitis. Note the loss of bone tissue.

- Increased blood volume by about 1500 mL (normal volume in women is 4–4.5 L), setting a clinical framework for physiological anemia, which can cause gingival bleeding;
- Fatigue can occur in the metabolism of carbohydrates, requiring an increase in levels of insulin and can thus convert asymptomatic subclinical diabetes into clinical diabetes mellitus, which in turn is called gestational diabetes. This increases the risk of gingival and periodontal diseases.[16]

Immunological changes and their oral implications during pregnancy

- During pregnancy, there is a reduction of antimicrobial activity of peripheral neutrophils, essential components of the innate immune defenses of the periodontal tissues. This could be related to the well-documented increase in gingival inflammation observed during pregnancy;
- Predilection for inflammatory gingival and periodontal diseases during the imbalance of sexual hormones;
- The prevalence and/or worsening of an existing gum disease is higher during pregnancy due to the sharp response to the biofilm;
- The effects of pregnancy on pre-existing gingival inflammation can be felt from the second month because it is at exactly this point that an elevation on plasma levels of estrogen and progesterone occurs. This level reaches its peak in the 8th month, when the gravidarum gingivitis reaches its maximum severity.[4,17]

Hormonal changes and their oral implications during pregnancy

- Microcirculatory system alterations produced by the hormones estrogen, progesterone, and chorionic gonadotropin lead to the following changes: swelling of the endothelial cells, increase of platelets and

leukocyte adhesion to vessel walls, formation of microthrombi, disruption of perivascular mast cells, increased vascular permeability, and vascular proliferation.[18]

- The estrogen affects the salivary peroxidases, operating against various types of bacteria by changing the oxidation–reduction potential.[17]
- Both estrogen and progesterone, associated with inflammatory mediators, can promote changes in vascular responses and connective tissue turnover in the periodontium. This association may explain the higher prevalence of inflammation during periods of hormonal fluctuation.[17]
- Well-documented changes in the microbiota occur during these hormonal changes with an increase in the proportion of anaerobic and aerobic bacteria, such as *Bacteroides melaninogenicus*, *Prevotella intermedia*, and *Porphyromonas gingivalis*.[19]

One of the main changes in the immune system during pregnancy is the partial mitigation of the immune responses that are mediated by the mother's cells, as 50% of the antigens in the cells of the fetus are derived from the father, and these cells are chronically exposed to the mother's immune system. Several complex physiological changes are therefore induced during pregnancy in order to prevent immune rejection in the mother's body of her fetus.[6] The adjustment of the mother's immune system occurs soon after birth. Linked to this post-partum recovery of inflammatory responses, some latent infections that had been suppressed during pregnancy might be activated. This phenomenon has been called 'immune reconstitution syndrome.'[20]

Although the biofilm has long been established as an etiological factor *sine qua non* for the development of periodontal disease,[21] it has also been shown that the biofilm is not likely the sole cause, as merely the presence of periodontal pathogens is not sufficient for disease to occur.[22] Hence, there must be an understanding on how systemic factors may influence the development, progression, and severity of periodontal disease. This aspect has been addressed in the classification of periodontal disease proposed by Armitage in 1999, when he included those diseases related to sexual hormones in the following categories: gingivitis associated with puberty, gingivitis associated with the menstrual cycle, and pregnancy-associated gingivitis.[6]

During pregnancy, women can produce large amounts of sexual steroidal hormones, such as estrogen and progesterone. According to Lindhe *et al.*,[23] gingival inflammation triggered by biofilm can be accentuated by these hormonal changes, especially in the second and third trimesters of pregnancy. In 2000, Tilakaratne *et al.*[24] conducted a controlled study in a rural community in Sri Lanka to evaluate periodontal disease during pregnancy as well as at 3 months post-partum. The authors found that the incidence of gingival bleeding during pregnancy increased significantly compared with the control group, being more significant in the third trimester, despite similar levels of biofilm between the groups. Furthermore, the bleeding index had decreased at 3 months post-partum. Markou *et al.*[25] agreed with these results, stating that changes in the gingival tissues are not only caused by hormonal changes, and that these reactions may also enhance the periodontal tissues' reaction to the biofilm, thus contributing to the installation of periodontal disease.

Main oral manifestations in pregnant women

It is well known that due to some systemic changes, the situation of pregnancy exhibits a greater predisposition for some oral problems. Salivary disorders, such as reduction of pH and buffer capacity, may lead to an increased risk of oral diseases, including caries, gingivitis, and periodontal disease.[8] The following section will discuss the conditions most commonly addressed in the published work.

Pregnancy and high susceptibility to pyogenic granuloma

Gingival pyogenic granuloma is a nonspecific inflammatory lesion that can occur in both sexes. However, it is closely related to the gestational period. When this lesion is found during pregnancy, it is called 'granuloma or pregnancy tumor.' Characteristically, it presents as a lobulated, usually pedunculated lesion, with color ranging from pink to red or purple; it has a fast-growing and varied size, and can bleed profusely when touched[6] Furthermore, it is more common in the anterior and buccal aspect of maxilla, beginning at 23 gestational weeks.[17] Gingival pyogenic granuloma occurs in 0.5–5% of pregnant women and regresses spontaneously after childbirth, which makes it associated with hormonal changes.[17,26]

Notwithstanding the fact that the causes of pyogenic granuloma development have not yet been clarified, most of the injuries are associated with the presence of local irritants.[27,28] However, some authors have reported that the pathogenesis of the lesion is linked to female sexual hormones, which stimulate an increase of the local production of angiogenic factors, such as vascular endothelial growth factor.[29,30] This was confirmed by the study of Cardoso *et al.*,[27] who observed an association

between pyogenic granuloma and pregnant women aged older than 25 years in the first trimester (50%, $P = 0.05$) and between granuloma and pregnant women aged 26–35 years in the third trimester (78.9%, $P = 0.001$). According to Armitage,[6] clinical symptoms associated with pyogenic granuloma during pregnancy are relatively small, and usually include bleeding gums, sensitivity, and aesthetic problems.

Treatment for this condition may include surgical removal, especially if the lesion is large and symptomatic. Nevertheless, in many cases, pyogenic granuloma decreases partially or completely after childbirth, particularly if local irritants are removed.[6]

Effects of pregnancy on periodontal diseases

Taking into account the profound disturbances in the maternal immune system during pregnancy and in the post-partum period, it is not surprising that clinical and biological characteristics of periodontal infections are affected.[6] The percentage of pregnant women who need periodontal treatment can reach 100%.[31]

According to Rodrigues *et al*.,[32] pregnancy and specific steroid hormones seem to be able to influence the normal microflora and induce changes in the subgingival ecology. In the second trimester, there is a significant increase of gingivitis, and in the ratio of anaerobic and aerobic bacteria; also, there is a correlation between plasma levels of estrogen and progesterone and the presence of *B. melaninogenicus* sp (singular species) intermedius.

On the other hand, studies conducted by Figuero *et al*.[33] and Carrillo-de-Albornoz *et al*.,[34] despite having found an increase in gingival index during pregnancy, have found no relation between gingivitis and increase in levels of hormones in saliva during pregnancy.

Despite several results of epidemiological studies on the causes, there are abundant data and a general consensus that the severity and extent of gingival inflammation tends to increase during pregnancy.[9,35–38] In addition, cohort studies that compared pregnant and non-pregnant women without periodontal disease showed that there was more gingival inflammation during pregnancy, even though there were no significant differences in the plaque scores.[33,34]

In some cases, the gingival inflammation is severe and may be accompanied by intense bleeding and gingival sensitivity.[6] Longitudinal studies have shown that during pregnancy, the probing depth increases as the gingival inflammation worsens.[9,36] The increase in the probing depth was attributed to the movement of the gingival margin coronally due to swelling induced by inflammation. In turn, Gürsoy *et al*.[9] found that, generally, there is no permanent loss of clinical attachment. Nonetheless, in some pregnant women, especially those who acquired chronic periodontitis before pregnancy, progression with periodontitis occurs afterwards.[38,39] In fact, during pregnancy, there are some changes in the interactions between periodontal microbiota and the host, which can be favorable for periodontal damage.[6] Regarding the role of cytokines, expression levels of interleukins (IL-1β and IL-6), tumor necrosis factor-α, and inducible nitric oxide synthesis were recently evaluated in pregnant women with and without periodontal disease compared to non-pregnant women with and without periodontal disease, and it was found that this disease is not influenced by pregnancy.[40]

Previous analyses of microbiological culture have demonstrated that changes in estrogen and progesterone associated with pregnancy have an effect on the composition of the subgingival microbiota,[19,41] which was also confirmed by more recent studies.[32,34] Reis *et al*. corroborate this assertion by reporting that the subgingival microbiota becomes, with the progress of pregnancy, more anaerobic.[42]

Correlations were found between the levels of maternal hormones and *P. intermedia*,[19,34] *P. gingivalis*,[34] and species of *Bacteroides*[41] and *Campylobacter retus*.[43]

Among the immunological changes associated with pregnancy is an increase in susceptibility to intracellular pathogens, and it is not surprising that the survival of locally invasive bacteria, such as P. intermedia and *Aggregatibacter actinomycetemcomitans*, increases during pregnancy.[6] It is very likely that the reduction in bactericidal activity of peripheral neutrophils in pregnancy is related to the well-documented increase in gingiva inflammation seen during pregnancy.[44]

Given the assertions above, it can be seen that biofilm-induced periodontal diseases, such as gingivitis and periodontitis, are multifactorial infections, which involve complex interactions of microbial biofilms with innate and adaptive immune responses of the host. The physiological changes associated with pregnancy have profound effects on host–parasite interactions found in those diseases.[6] Although the mechanisms responsible for the increase in gingival inflammation observed during pregnancy have not been fully elucidated, it is clear that several factors, such as disorders in the neutrophil function, changes in cell physiology induced by hormones, and local effects on the microbial ecology, play an important role in the process of periodontal disease as a whole.

Salivary changes and risk of tooth decay

Higher incidence of dental caries in pregnant women than in non-pregnant women has been observed in different studies.[45–47] During pregnancy, dietary changes are common, such as regular consumption of snacks, sugary drinks, and citrus foods, to satisfy desires or prevent nausea. Such habits may lead to a drop in salivary pH, thus favoring an increase in the number of acidophilic microorganisms.[8,47] Jain and Kaur[47] observed an increase of *Streptococcus mutans* and *Lactobacillus* levels, especially in late pregnancy, comparing the saliva of pregnant with non-pregnant women.[47]

However, a poor oral hygiene practice is often observed during gestation, which may explain an increase in bacterial growth in the oral cavity, leading to an increased incidence of dental decay, as well as the gingival and periodontal changes.

It can be deduced that most of these effects on the oral tissues during pregnancy could be avoided by practicing proper oral hygiene and clinical dental follow-up.[7,47]

Dental management during pregnancy

Many authors[4,6,8,15,31,48] state that pregnant women should be considered as a priority population group for dental care because:

1 Pregnant women can have some oral changes specific to this period;
2 They may have accumulated necessities that can jeopardize their own health and that of their children;
3 They should be targeted by health education programs because they have a core role in the attitudes in the family network, influencing eating and hygiene habits of all family members;
4 They are a group that is easy to access during gestation, consistently attending health services, which are an important facilitator; furthermore, they can be enrolled in programs with scheduled intervals and a failure to address them would be a missed opportunity.

The simple incidence of oral problems in pregnant women has already justified the need for treatment in this group.[49] Furthermore, the vertical transmission of pathogenic oral microorganisms has been a concern, when pathogens are transmitted between members of the same family, and the first oral colonization is established between 5 and 7 years old. Children of mothers with periodontal disease are at high risk of developing destructive periodontal disease according to Asikanen *et al.*[50]

Another very important aspect is that studies have indicated associations between periodontal disease and preterm birth as well as low-birthweight babies.[51–53] Periodontal infections are caused by Gram-negative anaerobic bacteria that promote an increase in the level of prostaglandin, which is an important physiological inducer of early childbirth.[4]

Therefore, oral treatment during pregnancy is also necessary in order to minimize the risk of complications and the transmissibility of microorganisms; it has become an important preventive procedure for the mother and child. Hence, diagnosing and treating oral diseases that can compromise a mother's oral health and her baby are key steps to ensuring a safe prenatal period.[4,47]

Conclusion

During pregnancy there are changes in the adaptive immunity that results in an impact on the clinical course of various infectious diseases. Due to the formation of the biofilm, the inflammation of the periodontal tissues increases dramatically in size and severity during the course of a normal pregnancy, so it is extremely important to include visits to the dentist during the pregnancy period.

Moreover, it should not be forgotten that controlling and treating oral infections, alone, are promoters of health benefits for these patients, offering them an oral condition of comfort, function and aesthetics.

Acknowledgments

The authors wish to thank the Graduation Pro-Rectory in the Department of Dentistry, Federal University of Maranhao for the encouragement and collaboration with this research.

Disclosure

None declared.

References

1. Löe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand* 1963; **21**: 533–551.
2. Silness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* 1964; **22**: 121–135.

3. Barak S, Oettinger-Barak O, Oettinger M, Machtei EE, Peled M, Ohel G. Common oral manifestations during pregnancy: A review. *Obstet Gynecol Surv* 2003; **58**: 624–628.
4. Leal NP. *Saúde bucal da gestante: Conhecimentos, práticas e representações do médico, do dentista e da paciente*. (Dissertation), Oswaldo Cruz Foundation. Instituto Fernandes Figueira. Department of Education. Postgraduate Program in Child and Women's Health, Rio de Janeiro, 2006.
5. Minozzi F, Chipaila N, Unfer V, Minozzi M. Odontostomatological approach to the pregnant patient. *Eur Rev Med Pharmacol Sci* 2008; **12**: 397–409.
6. Armitage GC. Bi-directional relationship between pregnancy and periodontal disease. *Periodontol 2000* 2013; **61**: 160–176.
7. Laine MA. Effect of pregnancy on periodontal and dental health. *Acta Odontol Scand* 2002; **60**: 257–264.
8. Martínez-Pabón MC, Delgado CMM, López-Palacio AM, Patiño-Gomes LM, Arango-Pérez EA. Características fisicoquímicas y microbiológicas de la saliva durante y después del embarazo. *Rev Salud Pública* 2014; **16**: 128–138.
9. Gürsoy M, Pajukanta R, Sorsa T, Könönen E. Clinical changes in periodontium during pregnancy and postpartum. *J Clin Periodontol* 2008; **35**: 576–583.
10. Russell SL, Mayberry LJ. Pregnancy and oral health: A review and recommendations to reduce gaps in practice and research. *MCN Am J Mat Child Nurs* 2008; **33**: 32–37.
11. Silk H, Douglass AB, Douglass JM, Silk L. Oral health during pregnancy. *Am Fam Physician* 2008; **77**: 1139–1144.
12. Vettore MV, Lamarca GA, Leão ATT, Thomaz FB, Sheiham A, Leal MC. Periodontal infection and adverse pregnancy outcomes: A systematic review of epidemiological studies. *Cad Saude Publica* 2006; **22**: 2041–2053.
13. Boggess KA. Maternal oral health in pregnancy. *Obstet Gynecol* 2008; **111**: 976–986.
14. Ressler-Maerlender J, Krishna R, Robison V. Oral health during pregnancy: Current research. *J Womens Health* 2010; **14**: 880–882.
15. Moimaz SAS, Garbin CAS, Saliba NA, Zina LG. Periodontal status during pregnancy in a group of Brazilian women. *Cienc Odontol Bras* 2006; **9**: 59–66.
16. Xavier HS, Xavier VBC. Alterações decorrentes da gravidez. In: *Cuidados Odontológicos com a gestante*, 1st edn. São Paulo: Editora Santos, 2010; 3–7.
17. Lindhe J. *Clinical Periodontology and Implant Dentistry*, 3rd edn. Copenhagen: Blackell Munksgard, 1997; 1–72.
18. Zachariasen R. Ovarian hormones and oral health: Pregnancy gingivitis. *Compend Cont Educ Dent* 1989; **10**: 508–512.
19. Kornman KS, Loesche WJ. The subgingival microbial flora during pregnancy. *J Periodontal Res* 1980; **15**: 111–122.
20. Singh N, Perfect JR. Immune reconstitution syndrome and exacerbation of infections after pregnancy. *Clin Infect Dis* 2007; **45**: 1191–1199.
21. Bjorby A, Löe H. The relative significance of different local factors in the initiation and development of periodontal inflammation. *J Periodontal Res* 1967; **2**: 76–77.
22. Lang NP, Liel RA, Anderhalden K. Clinical and microbiological effects of subgingival restorations with overhanging or clinically perfect margins. *J Clin Periodontol* 1983; **10**: 563–578.
23. Lindhe J, Karring T, Lang NP. Fatores modificadores. In: Koogan G (ed). *Tratado de periodontia clínica e implantologia oral*, 5th edn. Rio de Janeiro: Guanabara Koogan, 2010; 292–311.
24. Tilakaratne A, Sory M, Ranasinghe AW, Corea SMX, Ekanayake SL, de Silva M. Periodontal disease status during pregnancy and 3 months post-partum, in a rural population of Sri-Lankan women. *J Clin Periodontol* 2000; **27**: 787–792.
25. Markou E, Eleana B, Lazaros T, Antonios K. The influence of sex steroid hormones on gingiva of women. *Open Dent J* 2009; **3**: 114–119.
26. Demir Y, Demir S, Aktepe F. Cutaneous lobular capillary hemangioma induced by pregnancy. *J Cutan Pathol* 2004; **31**: 77–80.
27. Cardoso JA, Spanemberg JC, Salum FG. Oral granuloma gravidarum: A retrospective study of 41 cases in Southern Brazil. *J Appl Oral Sci* 2013; **21**: 215–218.
28. Krishnapillai R, Punnoose K, Angadi PV, Koneru A. Oral pyogenic granuloma – a review of 215 cases in a South Indian Teaching Hospital, Karnataka, over a period of 20 years. *Oral Maxillofac Surg* 2012; **16**: 305–309.
29. Yuan K, Wing LYC, Lin MT. Pathogenic roles of angiogenic factors in pyogenic granulomas in pregnancy are modulated by female sex hormones. *J Periodontol* 2002; **73**: 701–708.
30. Yuan K, Lin MT. The roles of vascular endothelial growth factor and angiopoietin-2 in the regression of pregnancy pyogenic granuloma. *Oral Dis* 2004; **10**: 179–185.
31. Jeremias F, Silva SRC, Valsecki A Jr, Tagliaferro EPS, Rosell FL. Self-perception and oral health status in pregnant women. *Odontol Clin Cient* 2010; **9**: 359–363.
32. Rodrigues AS, Ariana S, Lima DBGO *et al.* Preterm and low birth weight and the relationship with periodontal disease: Clinical, microbiological and immunological aspects. *Rev Odontol UNICID* 2004; **16**: 55–61.
33. Figuero E, Carrillo-de-Albornoz A, Herrera D, Bascones A. Gingival changes during pregnancy: I. Influence of hormonal variations on clinical and immunological parameters. *J Clin Periodontol* 2010; **37**: 220–229.
34. Carrillo-de-Albornoz A, Figuero E, Herrera D, Cuesta P, Bascones-Martinez A. Gingival changes during pregnancy: III. Impact of clinical, microbiological, immunological and socio-demographic factors on gingival inflammation. *J Clin Periodontol* 2012; **39**: 272–283.
35. Taani DQ, Habashneh R, Hammad MM, Batieha A. The periodontal status of pregnant women and its relationship with socio-demographic and clinical variables. *J Oral Rehabil* 2003; **30**: 440–445.
36. Lieff S, Boggess KA, Murtha AP *et al.* The oral conditions and pregnancy study: Periodontal status of a cohort of pregnant women. *J Periodontol* 2004; **75**: 116–126.
37. Tatakis DN, Trombelli L. Modulation of clinical expression of plaque-induced gingivitis. I. Background review and rationale. *J Clin Periodontol* 2004; **31**: 229–238.
38. Moss KL, Ruvo AT, Offenbacher S, Beck JD, Mauriello SM, White RP Jr. Third molars and progression of periodontal pathology during pregnancy. *J Oral Maxillofac Surg* 2007; **65**: 1065–1069.
39. Offenbacher S, Boggess KA, Murtha AP *et al.* Progressive periodontal disease and risk of very preterm delivery. *Obstet Gynecol* 2006; **107**: 29–36.
40. Otenio CCM, Fonseca I, Martins MF *et al.* Expression of IL-1β, IL-6, TNF-α and iNOS in pregnant women with periodontal disease. *Genet Mol Res* 2012; **11**: 4468–4478.
41. Jensen J, Liljemark W, Bloomquist C. The effect of female sex hormones on subgingival plaque. *J Periodontol* 1981; **52**: 599–602.
42. Reis DM, Pitta DR, Ferreira HMB, Jesus MCP, Moraes M, Soares MG. Health education as a strategy for the promotion

of oral health in the pregnancy period. *Ciênc Saúde Colet* 2010; **15**: 269–276.
43. Yokoyama M, Hinode D, Yoshioka M *et al.* Relationship between Campylobacter rectus and periodontal status during pregnancy. *Oral Microbiol Immunol* 2008; **23**: 55–59.
44. Tsukimori K, Fukushima K, Komatsu H, Nakano H. Neutrophil function during pregnancy: Is nitric acid production correlated with superoxide production? *Am J Reprod Immunol* 2006; **55**: 99–105.
45. Vasiliauskiene I. Oral health status of pregnant women. *Stomatologija* 2003; **5**: 57–61.
46. Tonello AS, Zuchieri MABO, Pardi V. Assessment of oral health status of pregnant women participating in a family health program in the city of Lucas do Rio Verde – MT – Brazil. *Braz J Oral Sci* 2007; **6**: 1265–1268.
47. Jain K, Kaur H. Prevalence of oral lesions and measurement of salivary pH in the different trimesters of pregnancy. *Singapore Med J* 2015; **56**: 53–57.
48. Lachat MF, Solnik AL, Nana AD, Citron TL. Periodontal disease in pregnancy – review of the evidence and prevention strategies. *J Perinat Neonatal Nurs* 2011; **25**: 312–319.
49. Lopes MWF, Gusmão ES, Alves RV, Cimões R. Impact of periodontal diseases on quality of life. *Rev Gaúcha Odontol* 2011; **59**: 39–44.
50. Asikanen S, Chen C, Alaluusa S, Slots J. Can one acquire periodontal bacteria and periodontitis from a family member? *J Am Dent Assoc* 1997; **128**: 1263–1271.
51. Alves RT, Ribeiro RA. Relationship between maternal periodontal disease and birth of preterm low weight babies. *Braz Oral Res* 2006; **20**: 318–323.
52. Agueda A, Ramón JM, Manau C, Guerrero A, Echeverría JJ. Periodontal disease as a risk factor for adverse pregnancy outcomes: A prospective cohort study. *J Clin Periodontol* 2008; **35**: 16–22.
53. Kumar J, Samelson R. Oral health care during pregnancy recommendations for oral health professionals. *N Y State Dent J* 2009; **75**: 55–66.

5 CAPÍTULO II

Archives of Gynecology and Obstetrics (2019) 300:1521–1530
https://doi.org/10.1007/s00404-019-05355-x

MATERNAL-FETAL MEDICINE

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

Elisa Miranda Costa[1] · Camilla Silva de Araujo Figueiredo[2] · Rafiza Félix Marão Martins[1] · Cecília Claudia Costa Ribeiro[1,2] · Claudia Maria Coelho Alves[1,2] · Maria Lúcia Talarico Sesso[3] · Ruchele Dias Nogueira[4] · Maria da Conceição Saraiva[5] · Marco Antônio Barbieri[6] · Heloísa Bettiol[6] · Antônio Augusto Moura da Silva[1] · Erika Bárbara Abreu Fonseca Thomaz[1,2]

Received: 12 May 2019 / Accepted: 18 October 2019 / Published online: 1 November 2019

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

✉ Elisa Miranda Costa
elisamirandac@hotmail.com

[1] Department of Public Health, Federal University of Maranhão, Rua Barão de Itapary, 155 - Centro, São Luís, Maranhão CEP 65020-070, Brazil

[2] Department of Dentistry II, Federal University of Maranhão, São Luís, Maranhão, Brazil

[3] Department of Pediatrics, Medical School of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

[4] Department of Dentistry, University of Uberaba, Uberaba, Minas Gerais, Brazil

[5] Department of Pediatrics and Social Dentistry, Ribeirão Preto School of Dentistry, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

[6] Department of Puericulture and Pediatrics, Graduate Program in Child and Adolescent Health, São Paulo University, Ribeirão Preto, São Paulo, Brazil

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.

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].

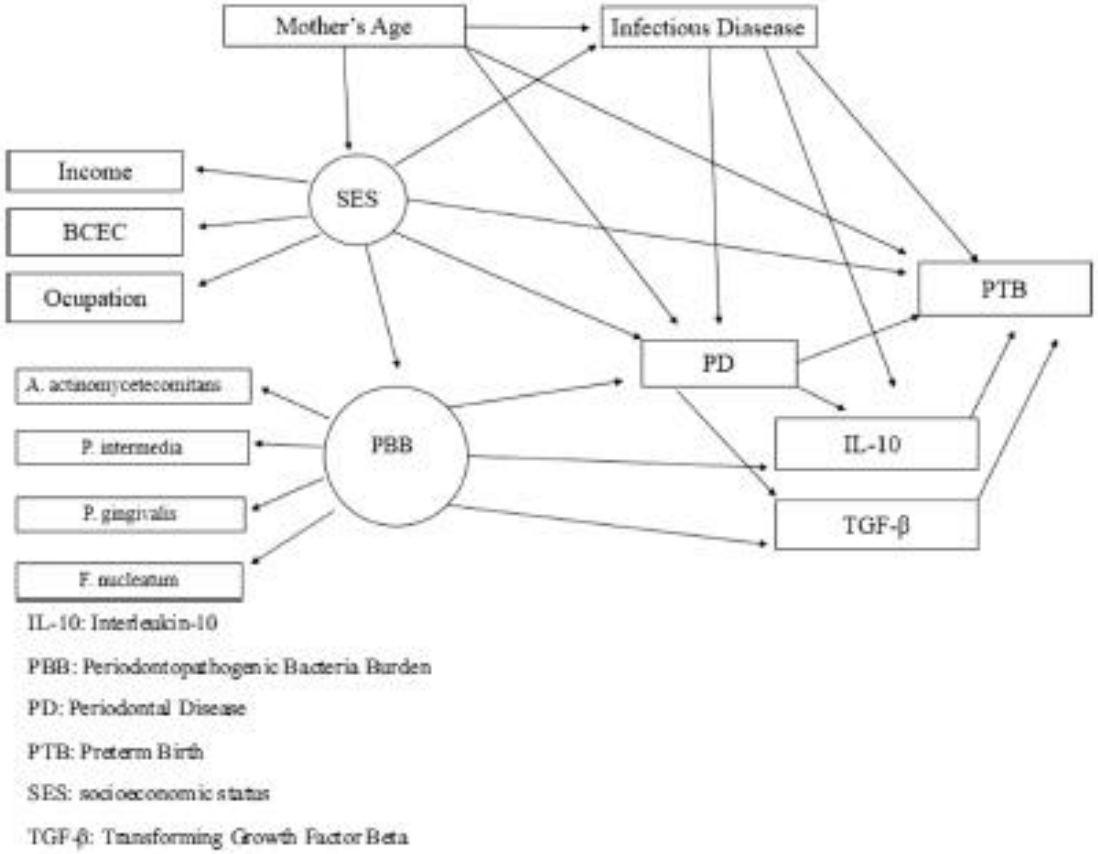

Fig. 1 Theoretical model considering periodontogenic microbiota, infectious mechanisms and preterm birth. *IL-10* interleukin-10. *PBB* periodontopathogenic bacteria burden, *PD* periodontal disease, *PTB* preterm birth, *SES* socioeconomic status, *TGF-β* transforming growth factor beta

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.

Acknowledgements Thanks to all the pregnant women who participated in the study.

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.

Funding This work was supported by the Maranhão Research and Scientific and Technological Development Foundation (FAPEMA); National Council of Scientific and Technological Development (CNPq); Program of Support to Cores of Excellence (PRONEX); and Foundation of Support to Research of the State of São Paulo (FAPESP).

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.

References

1. Chawanpaiboon S, Vogel JP, Moller AB, Lumbiganon P, Petzold M, Hogan D et al (2019) Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. Lancet Glob Health. 7:37–46
2. Silveira MF, Victora CG, Horta BL, da Silva BGC, Matijasevich A, Barros FC (2019) Low birthweight and preterm birth: trends and inequalities in four population-based birth cohorts in Pelotas, Brazil, 1982–2015. Int J Epidemiol 48:46–53
3. Stylianou-Riga P, Kouis P, Kinni P, Rigas A, Papadouri T, Yiallouros PK et al (2018) Maternal socioeconomic factors and the risk of premature birth and low birth weight in Cyprus: a case-control study. Reprod Health. 15:157
4. Pizzo G, La Cara M, Conti Nibali M, Guiglia R (2005) Periodontitis and preterm delivery. A review of the literature. Minerva Stomatol. 54:1–14
5. Xiong X, Buekens P, Fraser WD, Beck J, Offenbacher S (2006) Periodontal disease and adverse pregnancy outcomes: a systematic review. BJOG 113:135–143

6. Pretorius C, Jagatt A, Lamont R F (2007) The relationship between periodontal disease, bacterial vaginosis, and preterm birth. J Perinat Med 35:93–99
7. HEC da Silva CM Stefani MN Santos de Almeida de Lima A Rosing CK Porporatti AL et al 2017 Effect of intra-pregnancy nonsurgical periodontal therapy on inflammatory biomarkers and adverse pregnancy outcomes: a systematic review with meta-analysis Syst Rev. 6 197
8. Cobb CM, Kelly PJ, Williams KB, Babbar S, Angolkar M, Derman RJ (2017) The oral microbiome and adverse pregnancy outcomes. Int J Womens Health. 9:551–559
9. Corbella S, Taschieri S, Francetti L, De Siena F, Del Fabbro M (2012) Periodontal disease as a risk factor for adverse pregnancy outcomes: a systematic review and meta-analysis of case–control studies. Odontology. 100:232–240
10. Rosa MI, Pires PD, Medeiros LR, Edelweiss MI, Martinez-Mesa J (2012) Periodontal disease treatment and risk of preterm birth: a systematic review and meta-analysis. Cad Saude Publica. 28:1823–1833
11. Corbella S, Taschieri S, Del Fabbro M, Francetti L, Weinstein R, Ferrazzi E (2016) Adverse pregnancy outcomes and periodontitis: A systematic review and meta-analysis exploring potential association. Quintessence Int. 47:193–204
12. Perunovic ND, Rakic MM, Nikolic LI, Jankovic SM, Aleksic ZM, Plecas DV et al (2016) The Association Between Periodontal Inflammation and Labor Triggers (Elevated Cytokine Levels) in Preterm Birth: A Cross-Sectional Study. J. Periodontol. 87:248–256
13. Tellapragada C, Eshwara VK, Bhat P, Acharya S, Kamath A, Bhat S et al (2016) Risk Factors for Preterm Birth and Low Birth Weight Among Pregnant Indian Women: A Hospital-based Prospective Study. J Prev Med Public Health 49:165–175
14. Mesa F, Pozo E, Blanc V, Puertas A, Bravo M, O'Valle F (2013) Are periodontal bacterial profiles and placental inflammatory infiltrate in pregnancy related to birth outcomes? J Periodontol 84:1327–1336
15. Iheozor-Ejiofor Z, Middleton P, Esposito M, Glenny AM (2017). Treating periodontal disease for preventing adverse birth outcomes in pregnant women. Cochrane Database Syst Rev. 6:CD005297.
16. Kline RB (2015). Principles and practice of structural equation modeling: Guilford publications.
17. Kruse AB, Kuerschner AC, Kunze M, Woelber JP, Al-Ahmad A, Wittmer A et al (2018) Association between high risk for preterm birth and changes in gingiva parameters during pregnancy—a prospective cohort study. Clinical Oral Investig. 22:1263–1271
18. Lohana MH, Suragimath G, Patange RP, Varma S, Zope SA (2017) A Prospective Cohort Study to Assess and Correlate the Maternal Periodontal Status with Their Pregnancy Outcome. J Obstet Gynaecol India. 67:27–32
19. Basha S, Swamy HS, Mohamed RN, Shivalinga Swamy H, Noor Mohamed R (2015) Maternal Periodontitis as a Possible Risk Factor for Preterm Birth and Low Birth Weight–A Prospective Study. Oral health Prev Dent. 13:537–544
20. da Silva AA, Simoes VM, Barbieri MA, Cardoso VC, Alves CM, Thomaz EB et al (2014) A protocol to identify non-classical risk factors for preterm births: the Brazilian Ribeirao Preto and Sao Luis prenatal cohort (BRISA). Reprod Health. 11:79
21. Pesquisa ABdEd. Códigos e guias: CCEB-Critério de Classificação Econômica Brasil.: ABEP; 2009 [cited 2018].
22. Adriaens LM, Alessandri R, Spörri S, Lang NP, Persson GR (2009) Does pregnancy have an impact on the subgingival microbiota? J. Periodontol. 80:72–81
23. Carmo CDS, Ribeiro MRC, Teixeira JXP, Alves CMC, Franco MM, Franca A et al (2018) Added Sugar Consumption and Chronic Oral Disease Burden among Adolescents in Brazil. J Dent Res. 97:508–514
24. Pereira TB, Thomaz EB, Nascimento PR, Santos AP, Batista RL, Bettiol H et al (2016) Regulatory Cytokine Expression and Preterm Birth: Case-Control Study Nested in a Cohort. PLoS ONE 11:e0158380
25. Nugent RP, Krohn MA, Hillier SL (1991) Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. J Clin Microbiol. 29:297–301
26. Kemp MW (2014) Preterm birth, intrauterine infection, and fetal inflammation. Front Immunol. 5:574
27. Sood G, Argani C, Ghanem KG, Perl TM, Sheffield JS (2018) Infections complicating cesarean delivery. Curr Opin Infect Dis. 31:368–376
28. Barak S, Oettinger-Barak O, Machtei EE, Sprecher H, Ohel G (2007) Evidence of periodonpathogenic microorganisms in placentas of women with preeclampsia. J Periodontol. 78:670–676
29. Borgo PV, Rodrigues VA, Feitosa AC, Xavier KC, Avila-Campos MJ (2014) Association between periodontal condition and subgingival microbiota in women during pregnancy: a longitudinal study. J Appl Oral Sci. 22:528–533
30. Machado FC, Cesar DE, Apolônio ACM, Ribeiro LC, Ribeiro RA (2016) Longitudinal study on clinical and microbial analysis of periodontal status in pregnancy. Braz. Oral Res. 30:1–8
31. Armitage GC (2013) Bi-directional relationship between pregnancy and periodontal disease. Periodontol 2000(61):160–176
32. Shao H, Demuth DR (2010) Quorum sensing regulation of biofilm growth and gene expression by oral bacteria and periodontal pathogens. Periodontol 2000(52):53–67
33. Scannapieco FA, Bush RB, Paju S (2003) Periodontal disease as a risk factor for adverse pregnancy outcomes. A systematic review. Annals of Periodontology. 8:70–78
34. Nugent JL, Baker PN (2006) Periodontal disease and adverse pregnancy outcomes: a systematic review. BJOG 113:135–143
35. Chambrone L, Pannuti CM, Guglielmetti MR, Chambrone LA (2011) Evidence grade associating periodontitis with preterm birth and/or low birth weight: II: a systematic review of randomized trials evaluating the effects of periodontal treatment. J Clin Periodontol. 38:902–914
36. Martínez-Martínez RE, Moreno-Castillo DF, Loyola-Rodríguez JP, Sánchez-Medrano AG, San Miguel-Hernández JH, Olvera-Delgado JH et al (2016) Association between periodontitis, periodontopathogens and preterm birth: is it real? Arch Gynecol Obstet 294:47–54
37. Gargano JW, Holzman C, Senagore P, Thorsen P, Skogstrand K, Hougaard DM et al (2008) Mid-pregnancy circulating cytokine levels, histologic chorioamnionitis and spontaneous preterm birth. J Reprod Immunol. 79:100–110
38. Denney JM, Nelson EL, Wadhwa PD, Waters TP, Mathew L, Chung EK et al (2011) Longitudinal modulation of immune system cytokine profile during pregnancy. Cytokine 53:170–177
39. Shigeru S, Akitoshi N, Subaru M-H, Shiozaki A (2008) The balance between cytotoxic NK cells and regulatory NK cells in human pregnancy. J Reprod Immunol 77:14–22
40. Challis JR, Lockwood CJ, Myatt L, Norman JE, Strauss JF 3rd, Petraglia F (2009) Inflammation and pregnancy. Reprod Sci. 16:206–215
41. Alijotas-Reig J, Lliurba E, Gris JM (2014) Potentiating maternal immune tolerance in pregnancy: a new challenging role for regulatory T cells. Placenta 35:241–248
42. Schumacher A, Costa SD, Zenclussen AC (2014) Endocrine factors modulating immune responses in pregnancy. Front Immunol 5:196
43. Polliotti BM, Fry AG, Saller DN Jr, Mooney RA, Cox C, Miller RK (2003) Second-trimester maternal serum placental growth

factor and vascular endothelial growth factor for predicting severe, early-onset preeclampsia. Obstet Gynecol 101:1266–1274
44. Harper M, Li L, Zhao Y, Klebanoff MA, Thorp JM Jr, Sorokin Y et al (2013) Change in mononuclear leukocyte responsiveness in midpregnancy and subsequent preterm birth. Obstet Gynecol. 121:805–811
45. Ferguson KK, McElrath TF, Chen YH, Mukherjee B, Meeker JD (2014) Longitudinal profiling of inflammatory cytokines and C-reactive protein during uncomplicated and preterm pregnancy. Am J Reprod Immunol. 72:326–336
46. Jones RL, Stoikos C, Findlay JK, Salamonsen LA (2006) TGF-beta superfamily expression and actions in the endometrium and placenta. Reproduction 132:217–232
47. Li Q (2014) Transforming growth factor beta signaling in uterine development and function. J Anim Sci Biotechnol 5:52
48. Shaarawy M, El Meleigy M, Rasheed K (2001) Maternal serum transforming growth factor beta-2 in preeclampsia and eclampsia, a potential biomarker for the assessment of disease severity and fetal outcome. J Soc Gynecol Investig. 8:27–31
49. Tsiartas P, Holst RM, Wennerholm UB, Hagberg H, Hougaard DM, Skogstrand K et al (2012) Prediction of spontaneous preterm delivery in women with threatened preterm labour: a prospective cohort study of multiple proteins in maternal serum. BJOG 119:866–873
50. Miralles R, Hodge R, McParland PC, Field DJ, Bell SC, Taylor DJ, Grant WD, Kotecha S (2005) Relationship between antenatal inflammation and antenatal infection identified by detection of microbial genes by polymerase chain reaction. Pediatr Res. 57:473–474
51. Vrachnis N, Vitoratos N, Iliodromiti Z, Sifakis S, Deligeoroglou E, Creatsas G (2010) Intrauterine inflammation and preterm delivery. Ann N Y Acad Sci. 1205:118–122
52. Morris M, Nicoll A, Simms I, Wilson J, Catchpole M (2001) Bacterial vaginosis: a public health review. BJOG 108:439–450
53. Sangkomkamhang US, Lumbiganon P, Prasertcharoensuk W, Laopaiboon M (2015) Antenatal lower genital tract infection screening and treatment programs for preventing preterm delivery (Review). Cochrane Database Syst Rev 2:1–27
54. Brocklehurst P, Gordon A, Heatley E, Milan SJ (2013). Antibiotics for treating bacterial vaginosis in pregnancy. Cochrane Database Syst Rev. 31:CD000262
55. Baccaglini L (2011) A meta-analysis of randomized controlled trials shows no evidence that periodontal treatment during pregnancy prevents adverse pregnancy outcomes. J Am Dent Assoc. 142:1192–1193
56. Rosa MI, Pires PD, Medeiros LR, Edelweiss MI, Martínez-Mesa J (2012) Periodontal disease treatment and risk of preterm birth: a systematic review and meta-analysis. Cad Saude Publica. 28:1823–1833
57. Polyzos NP, Polyzos IP, Zavos A, Valachis A, Mauri D, Papanikolaou EG, Tzioras S, Weber D, Messinis IE (2010) Obstetric outcomes after treatment of periodontal disease during pregnancy: systematic review and meta-analysis. BMJ 341:c7017
58. López NJ, Uribe S (2000) Martinez B (2015) Effect of periodontal treatment on preterm birth rate: a systematic review of meta-analyses. Periodontol 67:87–130
59. Thomaz ÉB, Alves CM, Ribeiro CC, Batista RF, Simões VM, Cavalli R, Saraiva Mda C, Cardoso WC, Bettiol H, Barbieri MA, da Silva AA (2015) Perinatal outcomes and changes in the oral cavity: Brazilian cohorts of Ribeirão Preto and São Luís. Rev Bras Epidemiol. 18:966–970
60. Cavalcante NCN, Simões VMF, Ribeiro MRC, Lamy-Filho F, Barbieri MA, Bettiol H, Silva AAMD (2017) Maternal socioeconomic factors and adverse perinatal outcomes in two birth cohorts, 1997/98 and 2010, in São Luís. Brazil. Rev Bras Epidemiol. 20:676–687
61. Figueredo ED, Lamy F, Lamy ZC, Silva AAM (2014) Maternal age and adverse perinatal outcomes in a birth cohort (BRISA) from a Northeastern Brazilian city. Rev. Bras. Ginecol. Obstet. 6:562–568

6 CONSIDERAÇÕES FINAIS

Durante a gravidez há modificações na imunidade adaptativa que resultam em um impacto sobre o curso clínico de várias doenças infecciosas, incluindo o granuloma piogênico e as doenças periodontais. A inflamação dos tecidos periodontais aumenta dramaticamente em extensão e gravidade durante o curso de uma gravidez normal, mesmo sem alteração na quantidade de biofilme presente – é mister enfatizar. Este aumento da incidência de inflamação gengival durante o período gestacional é um fenômeno bem documentado e universalmente aceito pela comunidade científica.

A presença de bactérias periodontopatogênicas isoladamente parece não representar um fator de risco independente para o NPT. É possível que essa associação não apareça porque não estão sendo medidas todas as infecções ocorridas na gestação. Quando vistas em conjunto, as infecções sistêmicas explicam parte deste desfecho, via TGF-β.

De modo que a farmacogenética baseia-se no fato de que existem razões genéticas para que algumas pessoas respondam favoravelmente a um medicamento e outras experimentem reações negativas para o mesmo agente, há razões provavelmente genéticas para que as mulheres grávidas respondam diferentemente a encargos inflamatórios ou infecciosos causados pelo biofilme.

Além disso, não se deve esquecer que o controle e tratamento de infecções periodontais, por si só, são promotores de benefícios para a saúde da gestante, oferecendo uma condição oral de conforto, função e estética.

REFERÊNCIAS

AAGAARD-TILLER, K.M; SILVER, Robert; DALTON, Jess. Immunology of normal pregnancy. In: Seminars in Fetal and Neonatal Medicine. **WB Saunders**, p. 279-95, 2016.

ABEP. Associação Brasileira de Empresas de Pesquisa. Critério de Classificação Econômica do Brasil / 2010. Disponível em:<file:///C:/Users/USUARIO/Downloads/03_cceb_2012_base_lse_2010.pdf>. Acesso em 23 Jan. 2017.

ADANE, Akilew Awoke et al. Adverse birth outcomes among deliveries at Gondar University Hospital, Northwest Ethiopia. **BMC Pregnancy Childbirth**, London, v. 14, n. 1, p. 1-8, 2014.

ÀGUEDA, Anna; ECHEVERRÍA, Ana; MANAU, Carolina. Association between periodontitis in pregnancy and preterm or low birth weight: Review of the literature. **Medicina Oral, Patología Oral y Cirurgía Bucal**, Valencia, Suppl. 1, v. 13, n. 9, E609-15, 2008a.

ÀGUEDA, Ana et al. Periodontal disease as a risk factor for adverse pregnancy outcomes: a prospective cohort study. **Journal of Clinical Periodontology**, v. 35, n. 1, p. 16-22, 2008b.

ALVES, Renata Tolêdo; RIBEIRO, Rosângela Almeida. Relationship between maternal periodontal disease and birth of preterm low weight babies. **Brazilian Oral Research**, São Paulo, v. 20, n. 1, p. 318-23, 2006.

ARAFAT, Amira H. Periodontal status during pregnancy. **Journal of Periodontology**, Chicago, v. 45, n.1, p. 641-3, 1974.

ARAÚJO, Breno Fauth de; TANAKA, Ana Cristina d' Andretta. Fatores de risco associados ao nascimento de recém-nascidos de muito baixo peso em uma população de baixa renda. **Cadernos de Saúde Pública**, Rio de Janeiro, v. 23, n. 12, p. 2869-77, 2007.

ARMITAGE, Gary C. Bi-directional relationship between pregnancy and periodontal disease. **Periodontology 2000**, USA, v. 61, n.1, p. 160-76, 2013.

ARMITAGE, Gary C. Development of a classification system for periodontal diseases and conditions. **Annals of Periodontology**, Chicago, v. 80, n.1, p. 457-67, 1999.

ASIKANEN, Sirkka. Can one acquire periodontal bacteria and periodontitis from a family member? **Journal American Dental Association,** Canadá, v. 128, n. 9, p. 1263-71, 1997.

AZARPAZHOOH, Amir; TENENBAUM, Howard C. Separating fact from fiction: use of high-level evidence from research syntheses to identify diseases and disorders associated with periodontal disease. **Journal Canadian Dental Association**, Canadá, v. 78, n. 1, p. 25, 2012.

BACCAGLINI, Lorena. A meta-analysis of randomized controlled trials shows no evidence that periodontal treatment during pregnancy prevents adverse pregnancy outcomes. **Journal American Dental Association**, Canadá, v. 142, n. 10, p. 1192-3, 2011.

BARAK S. Common oral manifestations during pregnancy: a review. **Obstetrical y Gynecological Survey**, US, v. 58, n. 9, p. 624-8, 2003.

BARBIERE, Marco Antonio et al. Fatores etiológicos do nascimento pré-termo e conseqüências dos fatores perinatais na saúde da criança: coortes de nascimentos em duas cidades brasileiras. Projeto Temático. Ribeirão Preto/São Luís; 2007.

BARRETT, Paul T. Structural equation modeling: adjudging model fit. **Personality and Individual Differences**, v. 42, n. 5, p. 815-24, 2007.

BARROS, Fernando C. The challenge of reducing neonatal mortality in middle-income countries: findings from three Brazilian birth cohorts in 1982, 1993, and 2004. **The Lancet**, London, v. 365, n. 1, p. 847-54, 2005, doi: 10.1016/S0140-6736(05)71042-4.

BETTIOL, Heloisa et al. Factors associated with preterm births in southeast Brazil: a comparison of two birth cohorts born 15 years apart. **Paediatric and Perinatal Epidemiology**, US, v. 14, n. 1, p. 30-8, 2000, doi: 10.1046/j.1365-3016.2000.00222.x.

BJORBY, A.; LÖE, H. The relative significance of different local factors in the initiation and development of periodontal inflammation. **Journal of Periodontal Research**, USA, v. 2, n. 20, p. 76-7, 1967.

BLENCOWE, Hannah. Born Too Soon Preterm Birth Action Group. Born too soon: the global epidemiology of 15 million preterm births. **Reprodructive Health**, London, v. 10, Suppl 1: S2, 2013. doi: 10.1186/1742-4755-10-S1-S2.

BLENCOWE, Hannah. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. **The Lancet**, London, v. 379, n. 1, p. 2162-72, 2012, doi: 10.1016/S0140-6736(12)60820-4.

BOGGESS, Kim A. Maternal oral health in pregnancy. **Obstetrics & Gynecology**, USA, 2008; v. 111, n. 1, p. 976-86, 2008.

BOUTIN, Amélie et al. Treatment of periodontal disease and prevention of preterm birth: systematic review and meta-analysis. **American Journal of Perinatology**, New York, v. 30. n. 7, p. 537-44, 2013.

BRIDOUX, Frank et al. Transforming growth factor ß (TGF-ß)-dependent inhibition of helper cell 2 (Th2)-induced autoimmunity by self-major histocompatibility complex (MHC) class II-specific, regulatory CD4+ T cell lines. **The Journal of Experimental Medicine**, New York, v. 185, n. 10, p. 1769-75, 1997.

BUDUNELI, Nurcan. Periodontal infections and pre-term low birth weight: a case-control study. **Journal of Clinical Periodontology**, USA, v. 32, n. 2, p. 174-81, 2005.

CARDOSO, Juliana Andrade et al. Oral granuloma gravidarum: a retrospective study of 41 cases in Southern Brazil. **Journal of Applied Oral Science**, Bauru (SP), v. 21, n. 3, p. 215-8, 2013.

CARRILLO-DE-ALBORNOZ, Ana et al. Gingival changes during pregnancy: II. Influence of hormonal variations on the subgingival biofilm. **Journal of Clinical Periodontology**, USA, v. 37, n. 1, p. 230-40, 2010.

CARRILLO-DE-ALBORNOZ, Ana et al. Gingival changes during pregnancy: III. Impact of clinical, microbiological, immunological and socio-demographic factors on gingival inflammation. **Journal of Clinical Periodontology**, USA, v. 39, n. 1, p. 272-83, 2012.

CHAMBRONE, Leandro et al. Evidence grade associating periodontitis with preterm birth and/or low birth weight: II: a systematic review of randomized trials evaluating the effects of periodontal treatment. **Journal of Clinical Periodontology**, USA, v. 38, n. 10, p. 902-14, 2011.

CHATTERJEE, Piyali et al. Regulation of the anti-inflammatory cytokines interleukin-4 and interleukin-10 during pregnancy. **Immune Interactions during the Reproductive Cycle**, Switzerland, v. 5, n. 253, p. 124, 2015.

CHENG, S. B., & SHARMA de. Interleucina-10: um regulador pleiotrópicos na gravidez. **American Journal of Reproductive Immunology**, USA, v. 73, n. 6, p. 487-500, 2015.

CONDYLIS, B. et al. Interest of periodontitis screening and treatment in pregnancy: systematic review. **Journal de Gynecologie, Obstetrique et Biologie de la Reproduction (Paris)**, v. 42, n. 6, p. 511-7, 2013.

CORBELLA, Stefano. Periodontal disease as a risk factor for adverse pregnancy outcomes: a systematic review and meta-analysis of case-control studies. **Odontology**, Tokyo, v. 100, n. 2, p. 232-40, 2012.

CRUZ, Simone S. et al. Contribution of periodontal disease in pregnant women as a risk factor for low birth weight. **Community Dental Oral Epidemiology**, USA, v. 37, n. 6, p. 527-33, 2009.

DAHER, S.; MATTAR, R. Pregnancy: an immunological phenomenon? **Revista Brasileira de Alergia e Imunopatologia**, São Paulo, v. 32, n. 2, p. 63-7, 2009.

DATASUS. Departamento de Informática do Sistema único de Saúde. Definições. Disponível em: <http://www.datasus.gov.br/cid10/V2008/ WebHelp/definicoes.htm>. Acesso em: 21 março 2016.

DEMIR,Yavuz; DEMIR, Serap; AKTEPE, Fatma. Cutaneous lobular capillary hemangioma induced by pregnancy. **Journal of Cutaneous Pathology**, USA, v. 31, n. 1, p. 77-80, 2004.

DI RENZO, Gian Carlo et al. Maternal risk factors for preterm birth: a country-based population analysis. **European Journal of Obstetrics & Gynecology and Reproductive Biology**, Netherlands, v. 159, n. 2, p. 342-6, 2011.

DUARTE, P. M. et al. Do subjects with aggressive and chronic periodontitis exhibit a different cytokine/chemokine profile in the gingival crevicular fluid? A systematic review. **Journal of Periodontal Research**, USA, v. 50, n. 1, p. 18-27, 2015.

EBERSOLE, Jeffrey L. Periodontitis in pregnant baboons: systemic inflammation and adaptive immune responses and pregnancy outcomes in a baboon model. **Journal of Periodontal Research**, USA, v. 49, n. 2, p. 226-36, 2014.

ELIAS, R. et al. Atendimento estomatológico em gestantes: verdades e mitos. **Revista da Associação Brasileira de Odontologia**, Rio de Janeiro, v. 11, n. 6, p. 333-4, 2004.

FERGUSON, K K. et al. Longitudinal Profiling of Inflammatory Cytokines and C-reactive Protein during Uncomplicated and Preterm Pregnancy. **American Journal of Reproductive Immunology**, USA, v. 72, n. 3, p. 326-36, 2014.

FIGUEIREDO, Camilla Silva de Araújo et al. Systemic alterations and their oral manifestations in pregnant women. **The Journal of Obstetrics and Gynaecology Research**, USA, v. 43, n. 1, p. 16-22, Jan. 2017.

FIGUERO, Elena et al. Gingival changes during pregnancy: I. Influence of hormonal variations on clinical and immunological parameters. **Journal of Clinical Periodontolology**, USA, v. 37, n.1, p. 220-9, 2010.

FIGUERO, Elena et al. Effect of pregnancy on gingival inflammation in systemically healthy women: a systematic review. **Journal of Clinical Periodontology**, USA, v. 40, n.1, p. 457-73, 2013.

FOXMAN, Betsy et al. Mycoplasma, bacterial vaginosis-associated bacteria BVAB3, race, and risk of preterm birth in a high-risk cohort. **American Journal of Obstetrics & Gynecology**, Netherlands, v. 210, n.1, p. 226.el-7, 2014.

GARGANO, Julia Warner et al. Mid-pregnancy circulating cytokine levels, histologic chorioamnionitis and spontaneous preterm birth. **Journal of Reproductive Immunology**, Netherlands, v. 79, n.1, p. 100-10, 2008.

GEORGE, A. et al. Periodontal treatment during pregnancy and birth outcomes: a meta-analysis of randomised trials. **International Journal of Evidence-Based Healthcare**, USA, v. 9, n. 2, p. 122-47, 2011.

GOLDENBERG, Robert L. et al. Epidemiology and causes of preterm birth. **The Lancet**, London, v. 371, n. 9606, p. 75-84, 2008.

GORCZYNSKI, Reginald M. et al. The same immunoregulatory molecules contribute to successful pregnancy and transplantation. **American Journal of Reproductive Immunology**, USA, v. 48, n. 1, p. 18-26, 2002.

GÜRSOY, Mervi et al. Clinical changes in periodontium during pregnancy and postpartum. **Journal of Clinical Periodontology**, USA, v. 35, n. 1, p. 576-83, 2008.

HARPER, Margaret et al. Change in Mononuclear Leukocyte Responsiveness in Midpregnancy and Subsequent Preterm Birth. **Obstetrics & Gynecology**, USA, v. 121, n. 4, p. 1-13, 2013.

HUCK, O.; TENENBAUM, H.; DAVIDEAU, J. L. Relationship between periodontal diseases and preterm birth: recent epidemiological and biological data. **Journal of Pregnancy**, London, v. 2011, n. 164654, p. 1-8, 2011.

IDE, Mark; PAPAPANOU, Panos N. Epidemiology of association between maternal periodontal disease and adverse pregnancy outcomes: systematic review. **Journal of Periodontology**, USA, v. 84, Suppl. 4, p.181-94, 2013.

JAIN, Kanu; KAUR, Harshaminder. Prevalence of oral lesions and measurement of salivary ph in the different trimesters of pregnancy. **Singapore Medical Journal**, Singapore, v. 56, n.1, p. 53-7, 2015.

JENSEN, J.; LILJEMARK, W.; BLOOMQUIST, C. The effect of female sex hormones on subgingival plaque. **Journal of Periodontology**, USA, v. 52, n.1, p. 599-602, 1981.

JEREMIAS, Fabiano et al. Self-perception and oral health status in pregnant women. **Odontologia Clínico-científica**, Recife, v. 9, n. 4. p. 359-63, 2010.

JONES, Rebecca L. et al. TGF-β superfamily expression and actions in the endometrium and placenta. **Reproduction**, United Kingdom, v. 132, n. 2, p. 217-32, 2006.

JOSEPH, K. S.; DEMISSIE, K.; KRAMER, M. S. Obstetric intervention, stillbirth, and preterm birth. **Seminars in Perinatology**, Netherlands, v. 26, n.1, p. 250-9, 2002.

KALKUNTE, Satyan. Sera from preeclampsia patients elicit symptoms of human disease in mice and provide a basis for an in vitro predictive assay. **The American Journal of Pathology**, Netherlands, v. 177, n. 5, p. 2387-98, 2010.

KHADER, Yousef S.; TA'ANI, Quteish. Periodontal diseases and the risk of preterm birth and low birth weight: a meta-analysis. **Journal of Periodontolology**, USA, v. 76, n. 2, p. 161-5, 2005.

KORNMAN, Kenneth S.; LOESCHE, Walter J. The subgingival microbial flora during pregnancy. **Journal of Periodontal Research**, USA, v. 15, n. 1, p. 111-22, 1980.

KREJCI, Charlene B.; BISSADA, Nabil F. Women's health: periodontitis and its relation to hormonal changes, adverse pregnancy outcomes and osteoporosis. **Oral Health & Preventive Dentistry**, Berlin, v. 10, n. 1, p. 83-92, 2012.

KRISHNAPILLAI, Rekha et al. Oral pyogenic granuloma: a review of 215 cases in a South Indian Teaching Hospital, Karnataka, over a period of 20 years. **Journal of Oral and Maxillofacial Surgery**, Netherlands, v. 16, n. 3, p. 305-9, 2012.

KUMAR Jayanth, SAMELSON, Renee. Oral Health Care During Pregnancy Recommendations for Oral Health Professionals. **New York State Dental Journal**, New York, v. 75, n. 6, p. 55-66, 2009.

KUMAR, Praveen et al. Association of Antenatal Steriod Use with Cord Blood Immune Biomarkers in Preterm Births. **Early Humam Development**, London, v. 87, n. 1, p. 559-67, 2011.

LACHAT, Maryanne F. et al. Periodontal disease in pregnancy: review of the evidence and prevention strategies. **Journal of Perinatal & Neonatal Nursing**, USA, v. 25, n. 4, p. 312-9, 2011.

LAINE, Merja A. Effect of pregnancy on periodontal and dental health. **Acta Odontologica Scandinavica**, United States, v. 60, n. 5, p. 257-64, 2002.

LANG, Niklaus P.; LIEL, Robert A.; ANDERHALDEN, Katharina. Clinical and microbiological effects of subgingival restorations with overhanging or clinically perfect margins. **Journal of Clinical Periodontology**, USA, v. 10, n. 1, p. 563-78, 1983.

LEAL, Neide Pires. **Saúde bucal da gestante: conhecimentos, práticas e representações do médico, do dentista e da paciente**. 93f. Dissertação (Mestrado em Saúde da Criança e da Mulher) - Instituto Fernandes Figueira, Fundação Oswaldo Cruz, Rio de Janeiro, 2006.

LEVINE, Richard J, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. **The New England Journal of Medicine**, New England, v. 355, n. 1, p. 992-1005, 2006.

LI, Qinglei. Transforming growth factor β signaling in uterine development and function. **Journal of Animal Science and Biotechnology**, London, v. 5, n. 1, p. 1, 2014.

LIEFF, Susan et al. The oral conditions and pregnancy study: periodontal status of a cohort of pregnant women. **Journal of Periodontolology**, USA, v. 75, n. 1, 116-26, 2004.

LINDHE, Jan et al. Fatores modificadores. In: KARRING, Thorkild; LANG, Niklaus P. **Tratado de periodontia clínica e implantologia oral**. 5. ed. Rio de Janeiro: Guanabara Koogan, 2010, p. 292-311.

LINDHE, Jan. **Clinical Periodontology and Implant Dentistry**. 3. ed. Copenhagen: Blackell Munksgard; 1997, 72p.

LINDHE, Jan. **Tratado de periodontia clínica e implantologia oral**. Rio de Janeiro: Guanabara Koogan S.A., 1997.

LÖE, Harald; SILNESS, John. Periodontal disease in pregnancy. I. Prevalence and severity. **Acta Odontologica Scandinavica**, United States, v. 21, n. 1, p. 533-51, 1963.

LÖE, Harald. Periodontal changes in pregnancy. **Journal of Periodontology**, USA, v. 36, p. 209-17, 1965.

LOPES, Manuela Wanderley Ferreira. Impact of periodontal diseases on quality of life. **Revista Gaúcha de Odontologia (Online)**, Porto Alegre, v. 59, n. 1, p. 39-44, 2011.

LÓPEZ, Néstor J.; URIBE Sergio.; MARTINEZ, Benjamín. Effect of periodontal treatment on preterm birth rate: a systematic review of meta-analyses. **Periodontology 2000**, USA, v. 67, n. 1, p. 87-130, 2015.

LUNARDELLI, Aberlardo Nunes; PERES, Marco Aurélio. Is there an association between periodontal disease, prematurity and low birth weight? A population-based study. **Journal of Clinical Periodontology**, USA, v. 32, n. 1, p. 938-46, 2005.

MACHTEI, Eli E. et al. The effect of menstrual cycle on periodontal health. **Journal of Periodontology**, USA, v. 75, n. 1, p. 408-12, 2004.

MACONES, George A. et al. A polymorphism in the promoter region of TNF and bacterial vaginosis: preliminary evidence of gene-environment interaction in the etiology of spontaneous preterm birth. **American Journal of Obstetrics & Gynecology**, Netherlands, v. 190, n. 1, p. 1504-8, 2004.

MANAU, Carolina et al. Periodontal disease definition may determine the association between periodontitis and pregnancy outcomes. **Journal of Clinical Periodontology**, USA, v. 35, n. 1, p. 385-97, 2008.

MARKOU, Eleni et al. The influence of sex steroid hormones on gingiva of women. **The Open Dentistry Journal**, UAE, v. 3, n. 1, p. 114-9, 2009.

MARTIN, John A. Periodontal diagnosis affected by variation in terminology. **Journal of Periodontology**, USA, v. 84, n. 5, p. 606-13, 2013.

MARTÍNEZ-PABÓN, María Cecilia et al. Características fisicoquímicas y microbiológicas de la saliva durante y después del embarazo. **Revista de Salud Pública**, Colombia, v. 16, n.1, p. 128-38, 2014.

MATEVOSYAN, Naira Roland. Periodontal disease and perinatal outcomes. **Archives of Gynecology and Obstetrics**, USA, v. 283, n. 4, p. 675-86, 2011.

MATOBA, Nana et al. Differential Patterns of 27 Cord Blood Immune Biomarkers Across Gestational Age. **Pediatrics**, EUA, v. 123, n. 1, p. 1320-8, 2009.

MATSUOKA, Takashi et al. Increase of cord blood cytokine-producing T cells in intrauterine infection. **Pediatrics International**, USA, v. 43, n. 5, p. 453-7, 2001.

MESA, Francisco et al. Relationship between periodontal parameters and plasma cytokine profiles in pregnant woman with preterm birth or low birth weight. **Clinical Oral Investigations**, [s.l], 2015. doi: 10.1007/s00784-015-1553-x.

MINOZZI, F. et al. Odontostomatological approach to the pregnant patient. **European Review for Medical Pharmacological Sciences**, Italy, v. 12, n. 6, p. 397-409, 2008.

MIYAGI, Masaharu; MORISHITA Masayuki; IWAMOTO Yoshifumi. Effects of sex hormones on production of prostaglandin E2 by human peripheral monocytes. **Journal of Periodontology**, USA, v. 64, n. 11, p. 1075-8, 1993.

MOIMAZ, Suzely Adas Saliba et al. Periodontal status during pregnancy in a group of Brazilian women. **Ciência Odontológica Brasileira**, São José dos Campos (SP), v. 9, n. 4, p. 59-66, 2006.

MOIMAZ, Suzely Adas Saliba; SALIBA, Nemre Adas; GARBIN, Cléa Adas Saliba. **Odontologia para gestante: guia para o profissional da saúde**. Araçatuba, SP: Ed. Gráfica Moço, 2009, cap. 1, p. 15-24.

MORELI, Jusciele Brogin et al. Interleukin 10 and tumor necrosis factor-alpha in pregnancy: aspects of interest in clinical obstetrics. **ISRN Obstetrics and Gynecology**, USA, v. 2012, 2012.

MOORE, S. et al. An investigation into the association among preterm birth, cytokine gene polymorphisms and periodontal disease. **Bjog**: **an International Journal of Obstetrics and Gynaecology**, USA, 2004, v. 111, n. 2, p. 125-32, 2004.

MOSS, Kevin L.; BECK, James D.; OFFENBACHER, Steven. Clinical risk factors associated with incidence and progression of periodontal conditions in pregnant women. **Journal Clinical of Periodontology**, USA, v. 32, p. 492-8, 2005.

MOSS, Kevin L. et al. Third molars and progression of periodontal pathology during pregnancy. **Journal of Oral and Maxillofacial Surgery**, Netherlands v. 65, n. 6, p. 1065-9, 2007.

MURPHY, Shaun P. et al. Uterine NK cells mediate inflammation-induced fetal demise in IL-10-null mice. **The Journal of Immunology**, USA, v. 175, n. 6, p. 4084-90, 2005

MURPHY, Shaun P. et al. Evidence for participation of uterine natural killer cells in the mechanisms responsible for spontaneous preterm labor and delivery. **American Journal of Obstetrics Gynecology,** v. 200, n. 3, p. 308.e1-e9, 2009.

NABET, Cathy. et al. Maternal periodontitis and the causes of preterm birth: the case–control Epipap study. **Journal Clinical of Periodontology**, USA, v. 37, n. 1, p.37-45, 2010.

OFFENBACHER, Steven et al. Progressive periodontal disease and risk of very preterm delivery. **Obstetrics & Gynecology**, USA, v. 107, n. 1, p. 29-36, 2006.

OTENIO, C. C. M. et al. Expression of IL-1β, IL-6, TNF-α and iNOS in pregnant women with periodontal disease. **Genetics and Molecular Research**, Ribeirão Preto, SP, v. 11, n. 4, p. 4468-78, 2012.

PARK, Yang Hee et al. Effect of early postnatal neutropenia in very low birth weight infants born to mothers with pregnancy-induced hypertension. **Korean Journal of Pediatrics**, Korea, v. 55, n. 12, p. 462-9, 2012.

PASSINI JR, Renato et al. Brazilian Multicentre Study on Preterm Birth study group. Brazilian multicentre study on preterm birth (EMIP): prevalence and factors associated with spontaneous preterm birth. **PLoS One**, California, v. 9, n. 10, p. e109069, 2014.

PATIL, Shankargouda et al. Microbial Flora in Oral Diseases. The **Journal of Contemporany Dental Practice**, New Delhi, v. 14, n. 6, p. 1202-8, 2013.

PEARCE, B. D. et al. Interrelationship of cytokines, hypothalamic-Pictuitary-Adrenal Axis Hormones, and Psychosocil Variables in the Prediction os Preterm Birth. **Gynecologic and Obstetric Investigation**, Switzerland, v. 70, n. p. 40-6, 2010.

PEREIRA, Thaís Basso de Brito et al. Regulatory cytokine expression and preterm birth: case-control study nested in a cohort. **PloS One**, California, v. 11, n. 8, p. e0158380,

PIMENTEL, G. J. Lopes de Oliveira et al. Effect of periodontal treatment on the incidence of preterm delivery: a systematic review. **Minerva Stomatologica**, Torino, v. 59, n. 10, p. 543-50, 2010.

PNUD. Programa das Nações Unidas para o Desenvolvimento. Disponível em: <http://www.atlasbrasil.org.br/2013/>. Acesso em: 20.01.2015.

POLYZOS, Nikolaus P. et al. Effect of periodontal disease treatment during pregnancy on preterm birth incidence: a metaanalysis of randomized trials. **American Journal of Obstetrics & Gynecology**, Netherlands, v. 200, n. 3, p. 225-32, 2009.

POLYZOS Nikolaus P. et al. Obstetric outcomes after treatment of periodontal disease during pregnancy: systematic review and meta-analysis. **The BMJ**, London, v. 341, n. 1, p. 1-10, 2010.

RAHMAN, Latifah A.; HAIRI, Noran N.; SALLEH, Nooriah. Association between pregnancy induced hypertension and low birth weight; a population based case-control study. **Asia-pacific Journal of Public Health**, UK, v. 20, n. 2, p. 152-8, 2008.

RAMCHANDANI, Manisha et al. Proteomic signature of periodontal disease in pregnancy: Predictive validity for adverse outcomes. **Bioinformation**, Stanford, v. 5, n. 7, p. 300-3, 2011.

REIS, Denise Moreira et al. Health education as a strategy for the promotion of oral health in the pregnancy period. **Ciência e Saúde Coletiva**, Rio de Janeiro, v. 15, n. 1, p. 269-76, 2010.

RESSLER-MAERLENDER, Jessamyn; KRISHNA Ranjitha; ROBISON Valerie. Oral health during pregnancy: current research. **Journal of Women's Health**, Washington, DC, v. 14, n. 10, p. 880-2, 2010.

ROBERTSON, Sarah A.; CARE, Alison S.; SKINNER, Rebecca J. Interleukin 10 regulates inflammatory cytokine synthesis to protect against lipopolysaccharide-induced abortion and fetal growth restriction in mice. **Biology of Reproduction**, Washington, DC, v. 76, n. 5, p. 738-48, 2007.

RODRIGUES, A. S. et al. Preterm and low birth weight and the relationship with periodontal disease: clinical, microbiological and immunological aspects. **Revista de Odontolologia da UNICID**, São Paulo, v. 16, n. 1, p. 55-61, 2004.

ROMERO, R. et al. Meta-analysis of the relationship between asymptomatic bacteriuria and preterm delivery/low birth weight. **Obstetrics & Gynecology**, Netherlands, v. 73, n. 4, p. 576-82, 1989.

ROSA, Maria Inês da et al. Periodontal disease treatment and risk of preterm birth: a systematic review and meta-analysis. Cadernos de Saúde Pública, Rio de Janeiro, v. 28, n. 10, p. 1823-33, 2012.

RUIZ, RJ. et al. Seconder trimester maternal plasma levels of cytokines IL-1Ra, IL-6 and IL-10 and preterm birth. **Jornal of Perinatology**, USA, v. 32, n. 7, p. 483-90, 2012..

RUSSELL, S. L.; MAYBERRY, L. J. Pregnancy and Oral Health: A Review and Recommendations to Reduce Gaps in Practice and Research. MCN, The **American Journal of Maternal/Child Nursing**, USA, v. 33, n. 1, p. 32-7, 2008.

SANTA CRUZ, Isabel et al. Association between periodontal status and pre-term and/or low-birth weight in Spain: clinical and microbiological parameters. **Journal of Periodontal Research**, USA, v. 48, n. 4, p. 443-51, 2013.

SCHMIDT-WEBER, Carsten B.; BLASER, Kurt. Regulation and role of transforming growth factor-β in immune tolerance induction and inflammation. **Current Opinion in Immunology**, Netherlands, v. 16, n. 6, p. 709-16, 2004.

SHAARAWY, Mohamed; EL MELEIGY, Mohamed; RASHEED, Khaled. Maternal serum transforming growth factor beta-2 in preeclampsia and eclampsia, a potential biomarker for the assessment of disease severity and fetal outcome. **Journal of the Society for Gynecologic Investigation**, UK, v. 8, n. 1, p. 27-31, 2001.

SHANTHI, Vanka et al. Association of pregnant women periodontal status to preterm and low-birth weight babies: A systematic and evidence-based review. **Dental Research Journal,** Isfahan, v. 9, n, 4, p. 368-80, 2012.

SHUB, Alexis et al. Maternal periodontal disease and perinatal mortality. **Australian and New Zealand Journal of Obstetrics and Gynaecology**, Victoria, Australia, v. 49, n. 2, p. 130-6, 2009.

SILK, Hugh et al. Oral health during pregnancy. **American Family Physician**, Leawood, KS, v. 77, n. 8, p. 1139-44, 2008.

SILNESS, J.; LÖE, H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. **Acta Odontologica Scandinavica**, United States, v. 22, n.1, p. 121-35, 1964.

SILVA, Antonio Augusto Moura da et al. Can we explain why Brazilian babies are becoming lighter? **Internationa Journal of Epidemiology**, Oxford, v, 33, n. 4, p. 821-8, 2004.

SILVA, Antonio Augusto Moura da et al. Changes in perinatal health in two birth cohorts (1997/1998 and 2010) in São Luís, Maranhão State, Brazil. **Cadernos de Saúde Pública**, Rio de Janeiro, v. 31, n. 7, p. 1437-50, 2015.

SILVA, Antonio Augusto Moura da et al. Perinatal health and mother-child health care in the municipality of Sao Luis, Maranhao State, Brazil. **Cadernos de Saúde Pública**, Rio de Janeiro, v.17, n. 6, p.1413-23, 2001.

SILVA, Antonio Augusto Moura da et al. A protocol to identify non-classical risk factors for preterm births: the Brazilian Ribeirão Preto and São Luís prenatal cohort (BRISA). **Reproductive Health**, London, v. 11, n. 1, p. 79, 2014.

SILVER Karlee L et al. Circulating soluble endoglin levels in pregnant women in Cameroon and Malawi: associations with placental malaria and fetal growth restriction. **PloS One**, California, v, 6, n, 9, p. e24985, 2011.

SINGH, Nina; PERFECT, John R. Immune reconstitution syndrome and exacerbation of infections after pregnancy. **Clin Infectious Diseases**, Oxford, v. 45, n. 9, p. 1191-9, 2007.

SOCRANSKY, S. S. et al. "Checkerboard" DNA-DNA hybridization. **Biotechniques**, NY, v, 17, n. 4, p. 788-92, 1994.

STADELMANN, P. et al. The potential association between gingival crevicular fluid inflammatory mediators and adverse pregnancy outcomes: a systematic review. **Clinical Oral Investigation**, [s.l], v. 17, n. 6, p. 1453-63, 2013.

SYKES, Lynne et al. The Th1: Th2 dichotomy of pregnancy and preterm labour. **Mediators of Inflammation**, London, v. 2012, id. 967629, 2012.

SZARKA, András et al. Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array. **BMC Immunology**, London, v. 11, n. 1, p. 1, 2010.

TAANI, DQ et al. The periodontal status of pregnant women and its relationship with socio-demographic and clinical variables. **Journal Oral Rehabilitation**, USA, v. 30, n. 4, p. 440-5, 2003.

TATAKIS, Dimitris N.; TROMBELLI, Leonardo. Modulation of clinical expression of plaque-induced gingivitis. I. Background review and rationale. **Journal Clinical of Periodontology**, USA, v. 31, n. 4, p. 229-38, 2004.

THAXTON, Jessica E.; SHARMA, Surendra. Interleukin-10: A Multi-Faceted Agent of Pregnancy. **American Journal of Reproductive Immunology**, USA, v. 63, n. 6, p. 482-91, 2010.

THOMAZ, Erika Bárbara Abreu Fonseca et al. Perinatal outcomes and changes in the oral cavity: Brazilian cohorts of Ribeirão Preto and São Luís. **Revista Brasileira de Epidemiologia**, São Paulo, v. 18, n. 4, p. 966-70, 2015.

TILAKARATNE, A. et al. Periodontal disease status during pregnancy and 3 months posth-partum, in a rural population of Sri-Lanka women. **Journal Clinical of Periodontology**, USA, v. 27, n. 10, p. 787-92, 2000.

TONELLO, Aline Sampieri; ZUCHIERI, Maria Angélica Botega Ortuzal; PARDI, Vanessa. Assessment of oral health status of pregnant women participating in a family health program in the city of Lucas do Rio Verde – MT – Brazil. **Brazilian Journal of Oral Sciences**, São Paulo, v. 6, n. 20, p. 1265-8, 2007.

TSIARTAS, P. et al. Prediction of spontaneous preterm delivery in women with threatened preterm labour: a prospective cohort study of multiple proteins in maternal serum. **An International Journal of Obstetrics & Gynaecology**, USA, v. 119, n.7, p. 866-73, 2012.

TSUKIMORI, K. et al. Neutrophil function during pregnancy: is nitric acid production correlated with superoxide production? **American Journal Reproductive Immunology**, USA, v. 55, p. 99-105, 2006.

UPPAL, Anna et al. The effectiveness of periodontal disease treatment during pregnancy in reducing the risk of experiencing preterm birth and low birth weight: a meta-analysis. **Journal American Dental Association**, Canadá, v. 141, n. 12, p. 1423-34, 2010.

VASILIAUSKIENE, Ingrida. Oral Health Status of Pregnant Women. **Stomatologija, Baltic Dental and Maxillofacial Journal**, Tartu, Estônia, v. 5, n. 1, p. 57-61, 2003.

VENKATESHA, Shivalingappa et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. **Nature Medicine**, London, v. 12, n. 1, p. 642-9, 2006.

VERGNES JN, SIXOU M. Preterm low birth weight and maternal periodontal status: a meta-analysis. **American Journal of Obstetrics and Gynecology**, New York, v. 196, n. 2, p. 135, 2007.

VETTORE, Mario Vianna et al. Periodontal infection and adverse pregnancy outcomes: a systematic review of epidemiological studies. **Cadernos de Saúde Pública**, Rio de Janeiro, v. 22, n. 10, p. 2041-53, 2006.

VETTORE, Mario Vianna et al. The relationship between periodontal disease and preterm low birthweight: clinical and microbiological results. **Journal of Periodontal Research**, USA, v. 43, n. 6, p. 615-26, 2008.

WANG, PH et al. Severe bleeding from a pregnancy tumor. A case report. **The Journal of Reproductive Medicine**, USA, v. 42, n. 6, p. 359-62, 1997.

WANG, Xiaobin et al. Maternal cigarette smoking, metabolic gene polymorphism, and infant birth weight. **JAMA**, Chicago, v. 287, n. 2, p. 195-202, 2002.

WANG, Yen-L; LIOU, Jui-Der; PAN, Whei-Lin. Association between maternal periodontal disease and preterm delivery and low birth weight. **Taiwanese Journal of Obstetric & Gynecology**, New York, v. 52, n. 1, p. 71-6, 2013.

WATANABE, Maria Angelica Ehara etl al. Pregnancy: an immune challenge. **Semina: Ciências Biológicas e da Saúde**, Londrina (PR), v. 35, n. 2, p. 147-62, jul./dez. 2014.

WEISSENBACHER, Tobias et al. Diagnostic biomarkers of pro-inflammatory immune-mediated preterm birth. **Archives of Gynecology And Obstetrics**, USA, v. 287, n. 4, p. 673-685, 2013.

XAVIER, H. S; XAVIER, V. B. C. Introdução. In:_____. **Cuidados Odontológicos com a gestante**. 1. ed. São Paulo: Editora Santos, 2010, cap. 1, p. 3-7.

XIONG, X. et al. Periodontal disease and adverse pregnancy outcomes: a systematic review. **Bjog**: **an International Journal of Obstetrics and Gynaecology**, USA, v. 113, n. 2, p. 135-43, 2006.

XIONG, X et al. Periodontal disease and pregnancy outcomes: state-of-the-science. **Obstetrics Gynecol Survey**, USA, v. 62, n. 9, p. 605-15, 2007.

YOKOYAMA, M. et al. Relationship between Campylobacter rectus and periodontal status during pregnancy. **Oral Microbiology Immunology**, Denmark, v. 23, n. 1, p. 55-9, 2008.

YU, Ching Yun. **Evaluating cutoff criteria of model fit indices for latent variable models with binary and continuous outcomes**. 183f. Tese (Doutorado em Educação) - University of California, California. 2002.

YUAN, Kuo; LIN, Ming T. The roles of vascular endothelial growth factor and angiopoietin-2 in the regression of pregnancy pyogenic granuloma. **Oral Diseases**, USA, v. 10, n. 3, 179-85, 2004.

YUAN, Kuo; WING, Lih-Yu C.; LIN, Ming T. Pathogenic roles of angiogenic factors in pyogenic granulomas in pregnancy are modulated by female sex hormones. **Journal of Periodontology Online**, Chicago, v. 73, n. 7, 701-8, 2002.

ZACHARIASEN, R. Ovarian hormones and oral health: pregnancy gingivitis. **Compendium of continuing education in dentistry**, Jamesburg, v. 10, n. 1, p. 508-12, 1989.

ZHONG, Q. Interleukin-10 gene polymorphisms and chronic/aggressive periodontitis susceptibility: A meta-analysis based on 14 case-control studies. **Cytokine**, Países Baixos, v. 60, n. 1, p. 47-54, 2012.

ANEXO A – PARECER DO COMITÊ DE ÉTICA EM PESQUISA

UNIVERSIDADE FEDERAL DO MARANHÃO
HOSPITAL UNIVERSITÁRIO
COMITÊ ÉTICA EM PESQUISA

HU-UFMA

PARECER CONSUBSTANCIADO

Parecer Nº223/2009
Pesquisador (a) Responsável: **Antônio Augusto Moura da Silva**
Equipe executora: **Antônio Augusto Moura da Silva, marco Antonio Barbieri, Heloisa Bettiol, Fernando Lamy Filho, Liberata Campos Coimbra, Maria Teresa Seabra S.B. e Alves, Raimundo Antonio da Silva, Valdinar Sousa Ribeiro, Vania Maria de Farias Aragão, Wellington da Silva Mendes, Zeni Carvalho Lamy, Mari Ada Conceição Saraiva, Alcione Miranda dos Santos, Arlene de Jesus Mendes Caldas, Cecilia Claudia Costa Ribeiro, Silma Regina P. Martins, Flávia Raquel F. Nascimentos, Marilia da Gloria Martins, Virginia P.L. Ferriani, Marisa Márcia M. Pinhata, Jacqueline P. Monteiro José S. Camelo Junior, Carlos Eduardo, Martinelli Júnior, Sonir Roberto R. Antonini e Aparecida Yulie Yamarmoto**
Tipo de Pesquisa: **Projeto Temático**
Registro do CEP: **350/08** Processo **4771/2008-30**
Instituição onde será desenvolvido: Hospital Universitário, Maternidade Marly Sarney, Clínica São Marcos, Maternidade Benedito Leite, Maternidade Maria do Amparo, Santa Casa de Misericórdia do Maranhão, Maternidade Nazira Assub, Clínica São José e Clínica Luiza Coelho.
Grupo: **III**
Situação: **APROVADO**

O Comitê de Ética em Pesquisa do Hospital Universitário da Universidade Federal do Maranhão analisou na sessão do dia **20.03.08** o processo Nº. **4771/2008-30**, referente ao projeto de pesquisa: "**Fatores etiológicos do nascimento pré-termo e conseqüências dos fatores perinatais na saúde de criança: coortes de nascimento em duas cidades brasileiras**", tendo como pesquisadora responsável **Antônio Augusto Moura da Silva**, cujo objetivo geral é "**Investigar novos fatores na etiologia da prematuridade, utilizando-se abordagem integrada e colaborativa em duas cidades brasileiras numa coorte de conveniência, iniciada no pré-natal**".

Tendo apresentado pendências na época de sua primeira avaliação, veio em tempo hábil supri-las adequada e satisfatoriamente de acordo com as exigências das Resoluções que regem esse Comitê. Assim, mediante a importância social e científica que o projeto apresenta a sua aplicabilidade e conformidade com os requisitos éticos, somos de parecer favorável à

UNIVERSIDADE FEDERAL DO MARANHÃO
HOSPITAL UNIVERSITÁRIO
COMITÊ ÉTICA EM PESQUISA

realização do projeto classificando-o como **APROVADO**, pois o mesmo atende aos requisitos fundamentais da Resolução 196/96 e suas complementares do Conselho Nacional de Saúde.

Solicita-se à pesquisadora o envio a este CEP, relatório parciais sempre quando houver alguma alteração no projeto, bem como o relatório final gravado em CD ROM.

São Luís,08 de abril de 2009.

Prof. Dr.João Inácio Lima de Souza
Coordenador do Comitê de Ética em Pesquisa
Hospital Universitário da UFMA
Ethica homini habitat est