Ministério da Saúde
Fundação Oswaldo Cruz
Escola Nacional de Saúde Pública Sérgio Arouca
Departamento de Epidemiologia e Métodos Quantitativos em Saúde

DOENÇA PERIODONTAL E PREMATURIDADE E/OU BAIXO PESO AO NASCER

Tese apresentada para a banca examinadora para a obtenção do Grau de Doutor em Saúde Pública

Mario Vianna Vettore

Orientadora: Maria do Carmo Leal
Orientadores externos: Anna Thereza Thomé Leão
Aubrey Sheiham

Rio de Janeiro, agosto de 2006

Dedicatória

Dedico este trabalho à Gabriela, minha esposa e ao meu filho Hugo, as duas grandes razões da minha vida. Agradeço a Deus todos os dias por ter vocês ao meu lado.

"... e me fala de coisas que eu acredito que não deixarão de existir

amizade, palavra, respeito, caráter, bondade, alegria e amor ..."

Milton Nascimento e Fernando Brant

Agradecimentos

À Deus, pelo dom da vida, por estar presente em todos os meus caminhos, e especialmente, por conceder-me mais esta conquista.

À Professora Maria do Carmo Leal, Duca, minha querida orientadora, professora e conselheira, quero manifestar meu reconhecimento e profunda gratidão pelo estímulo ao meu desenvolvimento profissional e pessoal, por partilhar seus conhecimentos e experiências, por sua competência, dedicação e valiosa orientação, que me conduziram aos caminhos da pesquisa na saúde pública e epidemiologia.

À Professora Anna Thereza Thomé Leão, pela orientação e colaboração neste trabalho. Sua ajuda na elaboração do projeto, realização do estudo piloto e implementação do trabalho de campo foram imprescindíveis, e pelo seu senso crítico na redação e discussão dos artigos, e por sua amizade e carinho.

Ao Professor Aubrey Sheiham, pela orientação em todas as etapas da tese, por ter me recebido no Departamento de Epidemiologia e Saúde Pública da University College London. Agradeço imensamente pelas nossas freqüentes discussões sobre conceitos, análise de dados e durante a redação dos artigos da tese na etapa sanduíche do doutorado, que ficarão para sempre em minha memória. Acima de tudo, agradeço ao Professor Sheiham por ter me ensinado a sua filosofia de Saúde Pública.

I wish to thank Professor Aubrey Sheiham for his guidance during all steps of this thesis and for giving me the opportunity to study in the Department of Epidemiology and Public Health, University College London. Our frequent discussions about concepts, the data analyses and during the writing the papers for the thesis are gratefully acknowledge and will be remembered for ever. Above all, I thank Professor Sheiham for teaching me about his philosophy of Public Health.

À Professora Magda Feres, pela sua orientação em toda etapa laboratorial deste estudo, por ter me recebido no Laboratório de Microbiologia Oral da Universidade Guarulhos (SP), e pelas valiosas correções nos artigos que envolveram os dados microbiológicos desta tese.

À Professora Angela Maria Monteiro da Silva, por auxiliar na aplicação e avaliação das escalas de depressão e ansiedade deste estudo.

À Professora Silvana Granado Nogueira da Gama, pela amizade e incentivo ao longo do meu percurso na ENSP.

À Izilvânia Barreto, técnica do Laboratório de Microbiologia Oral da Universidade Guarulhos (SP), que realizou os experimentos de análises microbiológicas.

Aos professores da Escola Nacional de Saúde Pública Sérgio Arouca – Fundação Oswaldo Cruz: Ana Glória Vasconcelos, Evandro Coutinho, Geraldo Marcelo, Luís Antônio Camacho, Paulo Barata e Rosalina Koifmann.

Aos cirurgiões-dentistas Filipe Brand Thomaz e Mônica Argento, pela supervisão do trabalho de campo.

Aos estagiários Anna Carolina Riqueza, Arthur Schilithz, Clara Elisa, Danielle de Carvalho, Edgard Valente, Érika Dias, Érika Santana, Fernanda Torres, Fernanda Peçanha, Ingrid Babiski, Marcelo Marini, Rafael de Lima, por participarem da coleta e montagem do banco de dados.

Aos colegas do Curso de Doutorado em Saúde Pública da Escola Nacional de Saúde Pública Sérgio Arouca / Fundação Oswaldo Cruz, especialmente Eliana Bender e Rita Checon, pelos momentos científicos e não-científicos compartilhados, pelas emoções, alegrias e conquistas divididas.

Ao Lúcio Gonçalves, meu grande amigo, pelo exemplo profissional, incentivo na vida acadêmica, pela amizade e pelo carinho.

Ao Rodrigo Carvalho, pela amizade, pela troca de experiência acadêmica e por compartilhar idéias, sempre construtivas.

À Professora Cristina Maria Rabelais Duarte, pelo exemplo profissional, incentivo na vida acadêmica e pela amizade.

Aos meus pais, pela dedicação, amor e cuidado, fundamentais durante todo o desenvolvimento de minha vida.

Aos meus irmãos, Marilene e Marcelo, pelo amor e pela amizade.

À Professora Mirella Giongo e à Dra Kátia Ratto, pela ajuda nos passos iniciais desse trabalho, abrindo as portas da Secretaria de Saúde do município do Rio de Janeiro para que este estudo pudesse ser realizado.

Às agências de fomento CNPq e CAPES pela concessão de bolsa de estudos para a realização do doutorado.

À FAPERJ e à OPAS/OMS pelo financiamento da pesquisa de campo do doutorado.

Às puérperas, seres humanos em plenitude, cuja participação possibilitou este estudo.

Resumo

Esta tese é apresentada sob a forma de artigos. Os quatro trabalhos apresentados neste estudo avaliaram a associação entre a doença periodontal (DP) e a prematuridade e/ou o baixo peso ao nascer (P/BPN). O primeiro artigo é uma revisão sistemática sobre DP e P/BPN. O segundo é o desenvolvimento e validação de um novo método epidemiológico para avaliação da DP, denominado *Periodontal Inflammatory Load* (PIL). O terceiro é um estudo caso-controle sobre a associação de medidas clínicas da DP com a P/BPN e o quarto, um estudo caso-controle aninhado que avaliou a relação da microbiota periodontal materna com a P/BPN.

A revisão sistemática mostrou a ausência de controle sobre variáveis de confusão em muitos estudos, e uma heterogeneidade metodológica em relação às formas de mensuração da DP e aos desfechos de P/BPN investigados. As limitações metodológicas na maioria dos estudos não permitem apropriadas conclusões sobre a real associação entre a DP e a P/BPN.

Um estudo caso-controle em puérperas com idade 30 anos (n=542) foi realizado sobre a associação entre medidas clínicas e microbiológicas da DP com a P/BPN. A medida PIL desenvolvida mostrou-se válida para avaliar a DP em estudos epidemiológicos, devido à sua significativa correlação com patógenos periodontais. Além disso, foram empregados todos os métodos utilizados nos estudos anteriores para avaliar a extensão da DP. Quatro grupos de casos foram analisados: prematuros, baixo peso ao nascer (BPN), prematuros e/ou com BPN, e prematuros e com BPN.

Os resultados clínicos mostraram que os níveis de DP foram maiores nos grupos controles do que nos grupos de casos de P/BPN. A DP não aumentou o risco para a P/BPN utilizando 15 diferentes medidas clínicas de DP. Os casos de P/BPN apresentaram uma tendência de menores níveis de DP em relação aos controles quando a medida PIL foi empregada. A comparação da microbiota periodontal materna entre casos e controles em uma sub-amostra (n=116) não detectou diferenças em relação à média de proporções dos complexos microbianos. A média de contagens do patógeno periodontal *Treponema socranskii* foi menor nas puérperas com neonatos prematuros, e prematuros e com BPN, comparados aos controles.

Concluiu-se que a doença periodontal não foi um fator de risco para a prematuridade, BPN, prematuridade e/ou BPN, e prematuridade e BPN em mulheres com 30 anos de idade ou mais.

Palavras-chave: doença periodontal, prematuridade, baixo peso ao nascer, estudo caso-controle, patógenos periodontais, epidemiologia periodontal.

Abstract

The four studies for this thesis assessed the relationship between periodontal disease (PD) and preterm low birth weight (PTLBW). The first is a systematic review on periodontal disease and PTLBW. The second is the development and validation of a new epidemiologic method of assessing PD, the Periodontal Inflammatory Load (PIL) method. The third is a case-control study on the association between clinical parameters of PD and PTLBW and fourthly, a nested case-control study testing the relationship between maternal periodontal microbiota and PTLBW.

The systematic review showed that many studies did not control for confounders and there was a clear heterogeneity among studies on how periodontal disease was measured and on what type of measure of PTLBW was used as the outcome. The limitations in methodologies used in most studies do not allow reliable conclusions to be drawn about real associations between periodontal disease and PTLBW.

A case-control study on puerperal women aged 30 years (n=542) was conducted on the association between clinical and microbiological measures of PD and PTLBW. The PIL measure was shown to be a valid measure of periodontal disease in epidemiologic studies, as PIL was significantly related to known periodontal bacterial pathogens. In addition, all measures of the extent of PD employed by other workers were used. Four groups of cases were analyzed: preterm, low birth weight (LBW), preterm and/or LBW, and preterm and LBW. The clinical results showed that PD levels were higher in controls than PTLBW cases. The extent of periodontal disease did not increase the risk for PTLBW using 15 different measures of periodontal disease. PTLBW cases tended to have less Periodontal Inflammatory Load (PIL) measures than controls.

The comparison of maternal periodontal microbiota between cases and controls in a sub-sample (n=116) did not show differences in means of proportions of microbial complexes. The mean counts of the periodontal pathogen *Treponema socranskii* were lower in puerperal women with preterm, and preterm and LBW compared with controls.

In conclusion, based upon the studies reported in the four papers that constitute this thesis, periodontal disease was not a risk factor for preterm, LBW, preterm and/or LBW, and preterm and LBW in women aged 30 years or over.

Key words: periodontal disease, preterm, low birth weight, case control study, periodontal pathogens, periodontal epidemiology.

Sumário

Lista de tabelas e figuras

Lista de anexos

Lista de siglas

Sigla	**Significado**
AL	*Attachment loss*
ATCC	*American Type Culture Collection*
BMI	*Body mass index*
BOP	*Bleeding on probing*
BPN	Baixo peso ao nascer
CAL	*Clinical attachment level*
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CCI	Coeficiente de Correlação Intra-Classe
CEP	Comitê de Ética em Pesquisa
CI	*Confidence interval*
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
CPI	*Community Periodontal Index*
CPITN	*Community Periodontal Index for Treatment Needs*
DNA	Ácido desoxirribonucléico, *Deoxyribonucleic acid*
DP	Doença periodontal
DUM	Data da última menstruação
E. coli	*Escherichia coli*
EDTA	*Ethylenediaminetetraacetic acid*
ENSP	Escola Nacional de Saúde Pública Sérgio Arouca, *National School of Public Health Sérgio Arouca*
EPAL	*Estimated Physical Activity Level*
ESI	*Extension and Severity Index*
FAPERJ	Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro
FIOCRUZ	Fundação Oswaldo Cruz, *Oswaldo Cruz Foundation*
GA	*Gestational age*
GCF	*Gingival crevicular fluid*
GPD	*Generalized periodontal disease*
GUI	*Genito-urinary infection*
HCl	Ácido Clorídrico
HIV	Vírus da imunodeficiência humana, *Human imunodeficiency virus*
ICC	*Intraclass Correlation Coeficient*
ID	*Incidence difference*

Sigla	Significado
Ig	*Immunoglobulin*
IL	Interleucina, *interleukin*
IL	Illinois
IN	Indiana
IP	Índice de placa
ISS	Índice de sangramento à sondagem
LA	*Loss of attachment*
LBW	*Low birth weight*
LILACS	Literatura Latino-Americana e do Caribe em Ciências da Saúde
LMP	*Last menstrual period*
LPS	Lipopolissacarídeos
MA	*Massachusetts*
MD	*Maryland*
$MgCl_2$	Cloreto de Magnésio
MP	*Mild periodontitis*
MS	*Marital status*
MSP	*Moderate to severe periodontitis*
NaCl	Cloreto de Sódio
NaOH	Hidróxido de Sódio
NCI	Nível clínico de inserção
ND	*No disease*
NSD	*Lack of difference among groups*
NP	*Data not presented*
OR	*Odds ratio*
P	*Periodontitis*
P. acnes	*Propionybacterium acnes*
PAHO	*Pan American Health Organization*
PB	*Preterm birth*
P/BPN	Prematuridade e/ou baixo peso ao nascer
PBS	Profundidade de bolsa à sondagem
PC	*Prenatal care*
PD	*Periodontal disease*
PDI	*Periodontal Disease Index*
PG	Prostaglandina, *prostaglandin*

Sigla	Significado
P. gingivalis	*Porphyromonas gingivalis*
PH	*Periodontal health*
PI	*Plaque Index*
PIL	*Periodontal Inflammatory Load*
PLBW	*Preterm and low birth weight*
PMH	*Pregnancy medical history*
PPD	*Periodontal pocket depth*
PROM	*Premature rupture of membranes*
PTLBW	*Preterm and/or low birth weight*
RNA	Ácido ribonucléico
Scielo	*Scientific electronic library online*
SD	*Significant difference among groups*
SES	*Socio-economic status*
SP	São Paulo
SPE/PNE	*Sequential physical examinations and post-natal examination*
SPSS	*Statistical Package for the Social Sciences*
SRP	*Scaling and root planing*
S. mitis	*Streptococcus mitis*
TAI	*Trait Anxiet Inventory*
T. cruzi	*Trypanosoma cruzi*
T. denticola	*Treponema denticola*
T. forsythia	*Tanerella forsythia*
Teste K	Teste Kappa
TNF	Fator de necrose tumoral, *Tumor necrosis factor*
TPL	*Threatened preterm labor*
T. socranskii	*Treponema socranskii*
UE	*Ultrasound examination*
UFRJ	Universidade Federal do Rio de Janeiro
UNG	Universidade Guarulhos
UPO	*Undesirable pregnancy outcomes*
USA	*United States of America*
V. parvulla	*Veilonella parvula*
VPI	*Visible Plaque Index*
WHO	*World Heatlh Organization*

1 – Introdução

Investigações sobre alterações periodontais em gestantes têm sido conduzidas há muitos anos. Entretanto, do ponto de vista epidemiológico, existe uma nítida inversão na direção de uma possível relação de causa e efeito entre essas duas condições, ao analisarmos o curso histórico dos estudos. Isto porque, a cronologia observada nas pesquisas publicadas sobre esse tema define dois momentos.

Entre a década de 30 até meados da década de 90 o foco das pesquisas era direcionado para as manifestações periodontais decorrentes da gestação [1, 2, 3, 4, 5, 6, 7, 8]. Entre 1994 e 1996 a publicação de experimentos em animais [9, 10] e, principalmente um estudo epidemiológico [11] redirecionou todas as atenções para os possíveis efeitos deletérios da doença periodontal (DP) sobre os desfechos gestacionais. Seguiu-se então uma série de estudos com o objetivo de verificar se a DP pode ou não ser considerada um fator de risco para a prematuridade e/ou baixo peso ao nascer (P/BPN). São considerados prematuros os neonatos oriundos de gestações com menos de 37 semanas completas. Recém-nascidos com menos de 2500 g são considerados de baixo peso [12].

1.1 – Condições periodontais em gestantes

Estudos publicados desde o início da década de 30 descrevem a maior prevalência e severidade da gengivite em gestantes em relação à não gestantes [8, 13, 14, 15].

As pesquisas subseqüentes confirmaram os achados iniciais [1, 2, 3, 4, 5, 6, 7, 16]. Geralmente, as alterações inflamatórias iniciam no segundo mês de gravidez e aumentam de severidade no decorrer da gestação até o oitavo mês, seguida de uma redução acentuada no último mês [6, 17, 18]. Observa-se clinicamente a presença de edema, vermelhidão e sangramento nos tecidos gengivais [19, 20].

Na década de 60 os pesquisadores buscaram conhecer as melhores formas de prevenção da gengivite em gestantes [3, 7], denominada "gengivite gravídica" [21]. Isto porque, em virtude do conceito de cronicidade da DP que prevaleceu até a década de 80, acreditava-se que todos os quadros de gengivite evoluíam para a periodontite e posterior perda do elemento dentário [22].

Entre o final da década de 60 e meados da década de 90 importantes estudos descreveram a etiopatogenia da gengivite em gestantes [2, 6, 15, 19, 23, 24, 25]. A inflamação gengival era descrita como transitória [2, 6, 16, 20, 23, 25], não estava relacionada a um aumento dos níveis de placa dental, e era considerada autolimitante, pois não evoluía para uma DP destrutiva [2, 16, 23, 25]. O quadro inflamatório estava diretamente relacionado

ao aumento de hormônios esteroidais no fluido gengival durante os primeiros meses da gravidez [20, 23].

Alterações na ecologia do biofilme subgengival e a diminuição da resposta imune local são atribuídas como os principais fatores desencadeadores da gengivite em gestantes [20, 23, 24]. Estudos têm demonstrado que a composição do biofilme dental sofre modificações qualitativas decorrentes de mudanças hormonais durante a gestação, como o aumento de hormônios esteroidais [20, 23]. No segundo trimestre de gestação encontrou-se uma correlação positiva entre os níveis plasmáticos de estrogênio e progesterona com níveis de *Prevotella melaninogenica* [23] e *Prevotella intermedia* [5, 20, 23] no biofilme dental de gestantes com sinais clínicos de gengivite. Além disso, observou-se um aumento da proporção entre bactérias anaeróbias e aeróbias em mulheres com gengivite gravídica [23]. As reduções das respostas linfocitárias de células T [26], células B e macrófagos [2, 24] observadas durante o curso da gestação resultaram em uma supressão temporária do sistema imunológico, potencializando os efeitos bacterianos sobre os tecidos periodontais.

1.2 – Infecções subclínicas maternas e o critério de associação com a doença periodontal

Acredita-se que o critério de associação epidemiológica tenha despertado o interesse sobre a possível associação entre a doença periodontal e a P/BPN, uma vez que é bem estabelecida a associação de infecções vaginais e geniturinárias com a P/BPN [27, 28, 29, 30, 31].

Infecções subclínicas como as do trato urinário podem exercem um efeito adverso em gestantes alterando o curso normal da gestação [27, 30]. A associação entre infecções e a P/BPN é sustentada por estudos que detectaram elevados níveis de bacteróides e microorganismos Gram negativos no líquido amniótico e nas membranas corioamnióticas em gestantes com P/BPN [31, 32, 33, 34]. Além disso, evidências do aumento de marcadores bioquímicos de infecção (citocinas) no líquido amniótico e no soro, a presença de infiltrado de neutrófilos nas membranas corioamnióticas (corioamnionite histológica) e a presença de infecções clínicas nos neonatos prematuros sustentam esta relação [30, 35].

As infecções podem levar a P/BPN por mecanismos diversos [36]:

- motivando a hipertermia (infecção aguda), que influencia diretamente o aumento da cinética uterina;

- acometendo diretamente o concepto ou a placenta de modo que o feto pode vir a falecer, interrompendo-se a gestação antes do termo;
- determinando corioamnionite (inflamação das membranas materno-fetais) e conseqüente rotura prematura das membranas ovulares, gerando a perda de líquido amniótico antes do início do trabalho de parto;
- reduzindo o suprimento vascular na região placentária pela ação de mediadores inflamatórios produzidos pela infecção.

Os partos prematuros e os neonatos com baixo peso ao nascer (BPN) que decorrem de infecções subclínicas são mediados pelo deslocamento de produtos bacterianos como endotoxinas (lipopolissacarídeos - LPS) e pela liberação exacerbada de mediadores inflamatórios como a interleucina-1 (IL-1), prostaglandina-E_2 (PG-E_2) e fator de necrose tumoral-α (TNF-α) [30, 37] (Anexo 1). O LPS é o principal componente da parede celular de bactérias Gram negativas. Os mecanismos de ação da IL-1, PG-E_2 e TNF-α sobre a placenta estão associados ao início precoce das contrações uterinas e à intensa redução de capilares sanguíneos, limitando assim a absorção de nutrientes pelo feto e retardando o seu desenvolvimento [38, 39, 40].

A DP é uma infecção anaeróbica Gram negativa que promove a destruição dos tecidos de suporte dos dentes. Os mecanismos que determinam os danos a essas estruturas, e a perda clínica de inserção periodontal, são determinados pela ação direta de produtos bacterianos do biofilme subgengival, e danos indiretos causados por mediadores inflamatórios a partir da resposta imunológica induzida pelas bactérias [41, 42].

Estudos sobre a patogênese periodontal demonstraram que componentes bacterianos (LPS e endotoxinas) dos microorganismos envolvidos na atividade da DP desencadeiam respostas imunoinflamatórias locais caracterizadas pela produção e liberação de mediadores inflamatórios como proteínas do sistema complemento, produtos do ácido aracdônio (PG-E_2) e citocinas (IL-1 e TNF-α) [42, 43].

O modelo biológico da associação entre infecções subclínicas e P/BPN sugere que a DP pode afetar o curso normal da gestação por ser uma fonte potencial de componentes bacterianos como endotoxinas (LPS), que desencadeiam a liberação de moduladores imunológicos como IL-1, PG-E_2 e TNF-α.

1.3 - Estudos em animais sobre a influência de patógenos periodontais sobre desfechos da gestação

Experimentos laboratoriais descrevem a influência da inoculação de endotoxinas bacterianas [44, 45] e de bactérias Gram negativas, principalmente a *Escherichia coli* [9, 44, 46,

[47, 48], sobre os embriões em animais prenhes. Os efeitos das inoculações bacterianas observados sobre *hamsters* [45], ratos [46, 47] e coelhas [48], incluem a redução no número de fetos, a presença de septicemia materna, malformações fetais, necrose neural, retardo no crescimento intra-uterino e abortos espontâneos [45, 46, 47, 48].

Além da *E. coli*, o *Bacteroides bivius*, *Fusobacterium necrophorum* [48], e o *Trypanosoma cruzi* [49] induziram o aumento de mediadores inflamatórios nas placentas dos animais infectados. A inoculação intracervical de *E. coli* e *T. cruzi* durante a gestação de coelhas gerou o aumento de IL-1□, IL-1□, TFN-□ e PG-E_2 nos fluidos amnióticos [49, 50], e a administração de endotoxinas bacterianas em camundongos aumentou a síntese de PG-F [44].

Evidências científicas em modelos animais da associação entre os patógenos periodontais e desfechos gestacionais indesejáveis foram descritas inicialmente em 1994 [9, 10]. Infecções subcutâneas por *Porphyromonas gingivalis*, um reconhecido patógeno periodontal [51], em *hamsters* grávidas causaram efeitos deletérios nos fetos, incluindo uma redução significativa de quase 25% no peso ao nascer [9, 10]. A restrição do crescimento embrionário esteve associada a um aumento nos níveis de PG-E_2 e TNF-□, que parecem determinar a magnitude da resposta no retardo do crescimento [10, 52]. Pesquisas posteriores confirmaram a restrição no crescimento intra-uterino em camundongos inoculados com *P. gingivalis* e a sua associação com o aumento de TNF-□ no soro [53, 54]. Em outro estudo em camundongos, a exposição ao *Campylobacter rectus* induziu a inflamação placentária e aumentou a letalidade neonatal [55].

1.4 – O início dos estudos epidemiológicos da doença periodontal como fator de risco para os desfechos indesejáveis da gestação e o ressurgimento da Teoria da Infecção Focal

Em 1996, ocorreu uma inversão no modelo de associação entre alterações periodontais e a gestação. Um estudo realizado na América do Norte detectou uma *odds ratio* (OR) maior que 7 para a ocorrência de P/BPN entre as gestantes com DP quando comparadas àquelas sem DP [11]. Desta forma, além dos estudos conduzidos pelo mesmo grupo de pesquisadores dois anos antes que encontraram uma redução significativa no peso ao nascer em *hamsters* grávidas infectadas por patógenos periodontais [9, 10], o resultado encontrado foi a primeira evidência de associação entre a DP e a P/BPN em seres humanos. Ressalta-se ainda que a elevada *odds ratio* encontrada entre a DP e a P/BPN neste estudo foi semelhante àquelas obtidas para os fatores de risco que apresentam as maiores estimativas de risco para a P/BPN.

O impacto dos resultados deste primeiro estudo foi de grande magnitude na comunidade científica. Por um lado, a DP poderia explicar parte da ocorrência de P/BPN, uma vez que em muitos casos não se conhece a sua causa [56]. Por outro, intervenções odontológicas para controlar a doença periodontal poderiam reduzir novos casos de P/BPN, considerados importantes problemas para a saúde pública em todo o mundo. A Teoria da Infecção Focal descrita em 1910 [57], na qual microorganismos patogênicos e seus produtos teriam a capacidade de migrar e causar efeitos deletérios em outros órgãos e sistemas ressurgia. Diversos centros de pesquisa na América do Norte e Europa iniciaram estudos sobre o possível efeito da DP sobre a P/BPN.

Por um lado, existe adequada evidência da plausibilidade biológica demonstrada em estudos em animais [9, 10, 53, 54]. Entretanto, a atividade de DP, que está associada à produção de citocinas, ocorre em episódios intermitentes de "surtos periodontais", com longos períodos de quiescência [22]. Mesmo considerando a ocorrência de atividade da DP durante a gestação, tais citocinas produzidas na cavidade bucal precisam se deslocar sistemicamente e alcançar níveis suficientes na região placentária para acelerar o trabalho de parto e restringir os nutrientes para o feto [58].

1.5 – Modelo teórico da associação entre a doença periodontal e a prematuridade e/ou baixo peso ao nascer

O modelo teórico da associação entre a doença periodontal e a prematuridade e/ou o baixo peso ao nascer é apresentado no Anexo 1.

Dentre os fatores de risco para a prematuridade e baixo peso ao nascer apresentado neste modelo, a idade, o tabagismo e a diabetes *mellitus* são considerados potenciais variáveis de confusão[28]. Mulheres com idade menor que 18 anos e maior que 35 anos têm maiores riscos de terem recém-natos prematuros e de baixo peso, e a doença periodontal apresenta maior prevalência e severidade com o aumento da idade. O tabagismo e a diabetes *mellitus* são fatores de risco tanto para a doença periodontal quanto para desfechos da gestação[28]. Potenciais fatores de risco incluem raça negra, alcoolismo, fatores psicossociais e baixo nível socioeconômico.

Os mecanismos que explicam a plausibilidade biológica da associação entre a doença periodontal e a prematuridade e o baixo peso ao nascer, descritos no item 1.2, também são apresentados neste modelo. Além da ação de componentes da membrana celular de bactérias como LPS, os mediadores inflamatórios ($PG\text{-}E_2$, IL-1 e TNF-α) produzidos pela doença periodontal têm a capacidade de modular da cinética uterina e a restrição de nutrientes para o feto.

2 – Justificativa

A prematuridade e o baixo peso ao nascer constituem relevantes desafios para a saúde pública no Brasil. Apesar dos esforços para se reduzir os nascimentos prematuros e com baixo peso, as taxas ainda são consideradas elevadas para o país. Diferentes estratégias têm sido empregadas para seu controle. Além da reconhecida necessidade da melhoria da assistência pré-natal, destaca-se como promissora a caracterização de novos possíveis fatores de risco para a P/BPN. A identificação de novas exposições que aumentem o risco para P/BPN e o seu respectivo controle poderão promover uma alteração nas condutas durante o pré-natal, e, desta forma, reduzir efetivamente as suas taxas.

A revisão dos estudos sobre o possível efeito da DP sobre a P/BPN é apresentada no primeiro artigo desta tese. A inconsistência nos achados dos estudos analisados na revisão sistemática sobre a associação entre a doença periodontal e a prematuridade e/ou baixo peso ao nascer (P/BPN), associados às limitações metodológicas em muitas pesquisas não permitem considerar a doença periodontal como um fator de risco para a P/BPN. Justifica-se a realização de mais pesquisas pela escassez de estudos epidemiológicos com adequada metodologia, incluindo tamanho amostral apropriado, controle de potenciais variáveis de confusão, e mensurações válidas da doença periodontal, idade gestacional e do peso ao nascer. Não existe ainda evidência científica para o rastreamento e tratamento da doença periodontal em gestantes com o objetivo de reduzir a incidência de P/BPN. Poucos estudos testaram a associação da DP com a P/BPN em amostras da população brasileira, o que também justifica a realização desta pesquisa.

Essa tese é apresentada no formato de artigos científicos. O primeiro artigo é uma revisão sistemática sobre os estudos epidemiológicos que associaram a DP como fator de risco para a P/BPN. Os demais artigos são os resultados do projeto intitulado: "Infecção periodonal e prematuridade e/ou baixo peso ao nascer: um estudo caso-controle". Neste estudo, puérperas foram investigadas em maternidades públicas do município do Rio de Janeiro entre junho de 2003 e junho de 2005.

A elaboração do projeto, a investigação de campo e o trabalho laboratorial foram realizados através de um consórcio interinstitucional entre o Departamento de Epidemiologia e Métodos Quantitativos em Saúde da Escola Nacional de Saúde Pública Sérgio Arouca / Fundação Oswaldo Cruz, o Departamento de Epidemiologia e Saúde Pública da Universidade de Londres, o Departamento de Clínica Odontológica da

Universidade Federal do Rio de Janeiro (UFRJ), o Departamento de Periodontia da Universidade Guarulhos e a Secretaria Municipal de Saúde do Rio de Janeiro.

O projeto recebeu financiamento da Organização Pan-Americana de Saúde / Organização Mundial de Saúde (*Graduate Thesis Grants in Public Health*), da Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), processo n^0 E-26/ 170.421/2003 e da Hu-Friedy do Brasil. Durante o curso, o doutorando foi bolsista do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), e no estágio de doutorado no exterior recebeu uma bolsa sanduíche da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

A hipótese e os objetivos são apresentados nos próximos capítulos.

3 – Projeto: "Infecção periodonal e prematuridade e/ou baixo peso ao nascer: um estudo caso-controle"

3.1 – Teste de hipótese

A hipótese do estudo é que puérperas com prematuridade e/ou que tiveram recém-natos com baixo peso ao nascimento apresentem maior prevalência e severidade de doença periodontal em relação àquelas de parto a termo e/ou com bebês com peso normal. Em refutação a hipótese alternativa, a hipótese "nula" seria que não existem diferenças estatísticas significativas para prevalência e severidade da doença periodontal entre as puérperas com recém-natos prematuros e/ou com baixo peso ao nascimento e aquelas com bebês a termo e/ou com peso normal.

3.2 – Objetivos

3.2.1 – Desenvolver e validar um método epidemiológico de avaliação da doença periodontal.

3.2.2 – Investigar a relação de parâmetros clínicos da doença periodontal com a prematuridade e/ou o baixo peso ao nascer em puérperas com 30 anos de idade ou mais.

3.2.3 – Avaliar a associação entre os níveis e proporções de 39 espécies bacterianas do biofilme subgengival com a prematuridade e/ou o baixo peso ao nascer em puérperas com 30 anos de idade ou mais.

3.3 – Métodos

3.3.1 – Trabalho de campo e coleta de dados

O trabalho de campo do presente estudo envolveu puérperas com idade igual ou superior a 30 anos com ou sem DP, selecionadas em maternidades públicas de alto risco no município do Rio de Janeiro. O período de coleta de dados foi de junho de 2003 até junho de 2005.

Inicialmente foi feita a seleção das maternidades com as maiores incidências de prematuridade e BPN entre as unidades da rede Municipal de Saúde do Rio de Janeiro. Foram selecionadas quatro maternidades de alto risco: Hospital da Mulher Fernando Magalhães, Maternidade Alexander Fleming, Maternidade Carmela Dutra e Maternidade XV de Novembro. Em seguida foi realizado um estudo piloto para o treinamento e calibração dos examinadores para coleta de dados. Posteriormente o estudo principal foi conduzido.

3.3.1.1 - Desenho do estudo

Um estudo caso-controle utilizando dados de base primária e secundária foi delineado. Quatro definições de casos foram considerados: prematuros, BPN, prematuros e com BPN, e prematuros e/ou com BPN. Em estudos epidemiológicos analíticos a comparação entre os grupos é explícita, uma vez que o investigador aloca sistematicamente grupos de indivíduos com objetivos específicos e determina se o risco para um determinado desfecho é diferente ou não entre pessoas expostas e não expostas a um ou mais fatores de interesse.

Baseado no fato de que a prematuridade e o BPN têm prevalências em torno de 7% [59] e a DP em torno de 15% no Rio de Janeiro [60], o delineamento caso-controle foi escolhido para o estudo da associação entre a prevalência e a severidade da DP e a prematuridade e o BPN.

3.3.1.2 – Cálculo amostral

O tamanho da amostra de puérperas foi estabelecido em 551 puérperas com proporção de casos para controles de 1:3 para detectar diferenças de pelo menos 10% entre os grupos, considerando um nível de significância de 5% e um poder de 80%. O tamanho amostral foi estabelecido para comparar proporções entre amostras com tamanhos diferentes [61] considerando a prevalência de DP de 15% [60].

Uma sub-amostra de puérperas foi selecionada aleatoriamente para coleta de biofilme subgengival e posterior análise microbiológica de acordo com os recursos disponíveis.

3.3.1.3 - Critérios de inclusão e exclusão das puérperas

Foram selecionadas mães com idade maior ou igual a 30 anos com até 3 dias de pós-parto imediato em maternidades de alto risco da rede Municipal de Saúde do Rio de Janeiro. O critério da idade foi definido devido à baixa prevalência da DP em mulheres com menos de 30 anos. O período de 3 dias de pós-parto foi estabelecido porque normalmente as mães recebem alta após esse período, e aquelas que permanecem nos leitos geralmente apresentam complicações médicas no puerperio. Os outros critérios de inclusão foram: gestação atual com recém-nato vivo não gemelar, presença de pelo menos 15 dentes naturais, ausência de doenças sistêmicas ou utilização de medicamentos relacionados a alterações periodontais, ausência de uso de psicotrópicos e distúrbios psiquiátricos, não ter recebido limpeza dentária profissional ou tratamento periodontal nos últimos 6 meses e não ter usado antibióticos na última semana.

Foram excluídas as mulheres HIV positivas, aquelas com hipertensão crônica e diabetes *mellitus*. As mães que apresentavam histórico de febre reumática, endocardite bacteriana ou prolapso de válvula mitral também foram excluídas, pois incorreria em antibióticoterapia prévia ao exame periodontal.

3.3.1.4 - Considerações éticas

Este projeto foi submetido e aprovado pelo Comitê de Ética em Pesquisa da Escola Nacional de Saúde Pública Sérgio Arouca - ENSP/FIOCRUZ (Parecer nº 78/02) (Anexo 2). Ao ser convidada para participar da pesquisa, uma descrição verbal e escrita do estudo era apresentada para cada puérpera selecionada. O Termo de Consentimento Livre e Esclarecido (Anexo 3) foi empregado para descrever os objetivos e benefícios esperados da participação da mãe. Todas as puérperas foram informadas que poderiam desistir da participação no estudo quando quisessem durante a entrevista, exame periodontal e coleta de biofilme subgengival.

3.3.1.5 - Definição de casos e controle

Foram considerados prematuros os neonatos oriundos de gestações com menos de 37 semanas completas, ou seja, menos de 259 dias de período gestacional. Recém-nascidos com menos de 2500 g foram considerados de BPN [12].

A aferição da idade gestacional foi determinada pela data da última menstruação (DUM). A DUM é calculada subtraindo-se a data do primeiro dia do último ciclo menstrual relatado pela mãe da data do parto. A regra de Naegele é empregada somando sete dias e subtraindo três meses ou somando nove meses em relação ao primeiro dia do último ciclo menstrual para calcular a data provável do nascimento [62]. Somente semanas completas foram consideradas na definição da idade gestacional. Nas participantes com ausência da informação da DUM, o método do Capurro foi usado [63]. Isso ocorreu em 20% das puérperas.

O método do Capurro é determinado utilizando-se características somáticas ou características somáticas associadas às neurológicas do recém-nato (Anexo 4). O cálculo é feito em dias, através da soma de uma constante "K" aos valores encontrados de cinco sinais somáticos (K=204 dias). O outro modo seria somar a constante "K" aos sinais somáticos e dois sinais neurológicos encontrados (K=200 dias). As variáveis somáticas incluem a textura da pele, forma da orelha, glândula mamária, pregas plantares e formação do mamilo. Os sinais neurológicos são: o sinal do cachecol e posição da cabeça ao levantar (Anexo 4) [63]. Por se tratar de maternidades de referência para gestações de alto risco todos os pediatras desses estabelecimentos são treinados na utilização desse escore. A análise de confiabilidade entre a DUM e o escore do Capurro foi testada empregando-se o Coeficiente de Correlação Intra-Classe. A concordância entre os métodos foi de 0,92.

O peso de todos os recém-nascidos foi registrado com balanças calibradas imediatamente após o parto (em gramas). As informações das estimativas da idade gestacional pela DUM, pelo método do Capurro, e o peso ao nascer foram obtidos do prontuário médico.

3.3.1.6 - Exame clínico periodontal

As medidas clínicas periodontais utilizadas neste estudo foram: índice de placa visível, presença de cálculo visível, índice de sangramento à sondagem (ISS) [64], profundidade de bolsa à sondagem (PBS) e nível clínico de inserção (NCI). Foram registradas 4 medidas por dente (mesial, vestibular, distal e lingual) para índice de placa visível, presença de cálculo visível e ISS. Nas medidas de PBS e NCI foram realizadas 6 mensurações por dente, correspondentes às faces mesiovestibular, mediovestibular, distovestibular, mesiolingual, mediolingual e distolingual. Todas as medidas clínicas foram realizadas em todos os dentes, excluindo os terceiros molares.

O índice de placa visível, presença de cálculo visível, ISS são medidas dicotômicas de acordo com a presença ou ausência de placa dental visível, cálculo e sangramento após sondagem periodontal, respectivamente.

As medidas de PBS foram registradas em milímetros da margem gengival livre até o fundo do sulco gengival ou da bolsa periodontal. Nas mensurações de NCI a junção cemento-esmalte foi usada como ponto de referência. As medidas de PBS e NCI foram registradas com uma sonda periodontal modelo Carolina do Norte (Hu-Friedy®, Chicago, IL, USA), com marcações a cada milímetro em um total de 15 mm, cuja extremidade apresenta 0,35mm de diâmetro. Nos casos em que a margem gengival ou a junção cemento-esmalte estava entre duas marcações da sonda periodontal, era registrado o valor correspondente à marcação mais profunda. Para facilitar o exame clínico periodontal foram usados espelhos bucais planos número 5 (Hu-Friedy®, Chicago, IL, USA) e focos de luz fixados à região frontal do examinador (Head light, modelo 8720, Trilhas & Rumos®, Rio de Janeiro, RJ, Brasil).

3.3.1.7 – Coleta de amostras de biofilme

Amostras de biofilme bacteriano subgengivais foram obtidas de 2 sítios/pacientes de puérperas selecionadas aleatoriamente da amostra total. A coleta das amostras de biofilme ocorreu sempre anteriormente às medições clínicas periodontais. Nas pacientes com DP, amostras de biofilme foram coletadas dos 2 sítios com maior PBS, preferencialmente não adjacentes, em diferentes quadrantes. Nas pacientes que não apresentavam bolsas periodontais, as amostras foram coletadas de 2 sítios aleatórios localizados em diferentes quadrantes. Uma sondagem periodontal prévia à coleta das amostras de biofilme bacteriano foi conduzida apenas para a identificação dos sítios mais profundos.

Após a remoção do biofilme bacteriano supragengival com gaze estéril¸ cada amostra subgengival foi retirada com uma cureta periodontal Gracey estéril (Hu-Friedy®, Chicago, IL, USA) e colocada em um tubo de Eppendorf contendo 0,15 ml de solução tampão TE (10 mM Tris-HCL, 1 mM EDTA, pH 7,6). Em seguida a cada tubo de Eppendorf contendo a amostra de placa subgengival, foi adicionado 0,10 ml de 0,5 M de hidróxido de sódio. Os tubos de Eppendorf com as amostras foram congelados e transportados para o Laboratório de Microbiologia Oral da Universidade Guarulhos, Guarulhos (SP) aonde as amostras foram processadas.

3.3.1.8 - Co-variáveis

Os dados das co-variáveis foram coletados dos prontuários médicos ou através de entrevistas estruturadas. As co-variáveis incluídas neste estudo foram características antropométricas e sociodemográficas maternas, condições de moradia, hábitos maternos, atividades físicas e violência durante a gravidez, fatores psicossociais, satisfação com a gravidez, história obstétrica, cuidados pré-natais e doenças ocorridas durante a gestação.

3.3.1.8.1 - Características antropométricas e sociodemográficas maternas

As características antropométricas e sociodemográficas maternas registradas foram idade, altura, índice de massa corporal prévio à gestação, raça, escolaridade, estado matrimonial. Informações sobre trabalho e renda incluíram tipo de ocupação, renda e número de dias que parou de trabalhar antes do parto.

3.3.1.8.2 - Condições de moradia

O nível de condição de moradia das participantes foi avaliado a partir de 14 características relacionadas à moradia, incluindo condições sanitárias, abastecimento de água e características físicas da moradia. Cada característica foi analisada separadamente em relação ao risco para prematuridade e BPN. As características que apresentaram *odds ratio* (OR) maior que 1 foram selecionadas para compor a variável "condições de moradia". A moradia foi considerada "inadequada" quando uma ou mais das características a seguir estavam presentes: ausência de banheiro com descarga, paredes feitas de madeira, plástico ou metal, ausência de piso revestido, ausência de água encanada dentro de casa, ausência de esgoto ligada à rede geral e presença de valão a céu aberto na rua em que a mulher mora.

3.3.1.8.3 - Hábitos maternos

As puérperas responderam a perguntas sobre consumo de bebidas alcoólicas, hábito de fumar antes e durante a gestação e uso drogas ilícitas. Associados às perguntas sobre o consumo de bebidas alcoólicas foram empregados os instrumentos CAGE [65] e T-ACE [66], validados para se determinar o alcoolismo das puérperas, sendo o T-ACE sugerido com instrumento próprio para a gravidez. O CAGE e o T-ACE possuem 4 perguntas cada um, sendo que 3 são comuns aos dois: 1) "As pessoas a aborreciam criticando o seu modo de beber?" - T-**A**CE / CA**G**E. 2) "Você sentiu que deveria ter parado de beber durante a gravidez?" - T-A**C**E / **C**AGE. 3) "Alguma vez precisou de

uma dose de bebida para começar o dia?" - T-AC**E** **/** CAG**E**. O CAGE inclui uma pergunta sobre sentimento de culpa: "Você teve sentimentos de culpa sobre a bebida?" - CA**G**E, e o T-ACE uma sobre tolerância ao álcool: "Quantas doses são necessárias para deixar você "alta"?" – **T**-ACE.

3.3.1.8.4 - Atividades físicas e violência durante a gravidez

O nível de atividades físicas durante o período gestacional foi mensurado pelo questionário *Estimated Physical Activity Level* (EPAL) [67]. Foi perguntado às mães se elas sofreram agressões físicas durante a gravidez.

3.3.1.8.5 - Fatores psicossociais

Ansiedade e depressão foram avaliadas através de instrumentos psicométricos validados para a população brasileira. A ansiedade foi avaliada pelo Inventário de Ansiedade Traço [68]. A escala de traço de ansiedade consiste em 20 afirmações, as quais requerem que os sujeitos descrevam como geralmente se sentem. A escala A-traço fornece bons recursos para avaliar a propensão à ansiedade ou traço de personalidade relativo à ansiedade, que é relativamente estável.

O Inventário Multifásico Minesolta de Personalidade é um teste que mostra o perfil e os traços mais importantes da personalidade. São 566 proposições das quais 60 medem a depressão [69].

3.3.1.8.6 - Satisfação com a gravidez

As mulheres foram investigadas quanto ao uso de métodos de contraceptivos para evitar a gravidez, bem como sobre a satisfação da mãe e do pai em relação à gravidez atual.

3.3.1.8.7 - História obstétrica, cuidados pré-natais e doenças ocorridas durante a gestação

Informações sobre a gestação atual incluindo idade gestacional, peso do recém-nato ao nascer, tipo de parto, sexo do recém-nato, comprimento e proporção peso/idade gestacional foram coletadas do prontuário médico.

A história obstétrica relativa ao número de partos anteriores, história de prematuridade, BPN prévio, intervalo entre o último parto e o atual foi perguntada à mãe. A atenção pré-natal foi avaliada pela realização ou de pré-natal, último mês em

que fez pré-natal, número de consultas no pré-natal, e com o uso do Índice de Kotelchuck modificado [70].

A ocorrência de hipertensão gestacional, pré-eclâmpsia, anemia, diabetes gestacional, infecção urinária e outras infecções durante a gravidez foi obtida do prontuário médico.

3.3.1.9 - Estudo Piloto

O estudo piloto foi realizado para que informações fidedignas e válidas fossem obtidas de maneira padronizada da população de estudo. Seis alunos de graduação em odontologia da UFRJ foram selecionados para a coleta de dados deste estudo. O estudo piloto teve por objetivo adaptar e testar os questionários para a entrevista, treinar os examinadores para a coleta das amostras de biolfilme subgengivais e conduzir a calibração dos examinadores para a realização do exame clínico periodontal.

Os questionários foram adaptados de um estudo prévio conduzido na mesma população de referência sobre a associação de fatores associados à assistência ao parto e à morbi e mortalidade perinatal no município do Rio de Janeiro [71]. A apresentação gráfica, compreensão e vocabulário dos itens dos questionários foram testados através de entrevistas com quarenta puérperas na Maternidade Fernando Magalhães. As modificações relacionadas à compreensão foram feitas. Após as entrevistas as mulheres receberam orientações sobre cuidados com a sua saúde bucal e a de seu bebê.

Os examinadores foram treinados para a coleta de biofilme subgengival e calibrados para o exame clínico periodontal no Departamento de Clínica Odontológica da UFRJ. Trinta pacientes com pelo menos 4 sítios periodontais com PBS > 4 mm foram selecionados e examinados. O treinamento para coleta de amostras de biofilme subgengivais foi feito em 6 pacientes. A calibração clínica avaliou a variabilidade clínica intra e interexaminadores para os parâmetros clínicos periodontais. Na calibração intra-examinador, cada examinador examinou 3 pacientes, com intervalos de 30 minutos entre os exames no mesmo paciente. Doze pacientes que não participaram da calibração intra-examinador foram selecionados para a calibração interexaminador. Estes pacientes foram submetidos a três exames periodontais, sendo feitos por diferentes examinadores. O teste Kappa e o Coeficiente de Correlação Intra-classe foram usados para aferir a calibração clínica periodontal. Os resultados da calibração intra e interexaminadores estão apresentados na tabela apresentada no Anexo 5. Os resultados do teste Kappa e do Coeficiente de Correlação Intra-classe para medidas de

PBS foram □ 0,78 e □ 0,72 para calibração intra-examinador, e □ 0,77 e □ 0,72 para calibração interexaminador.

Todos os examinadores foram mascarados em relação aos objetivos do estudo principal.

3.3.1.10 – Estudo Principal

O estudo principal constituiu uma investigação epidemiológica observacional analítica do tipo caso-controle. Foram entrevistadas e examinadas 542 puérperas com até 3 dias de pós-parto imediato em maternidades de alto risco da rede Municipal de Saúde do Rio de Janeiro. Os dados foram obtidos através de entrevistas estruturadas, de informações disponíveis no prontuário médico e de exames clínicos periodontais.

Uma sub-amostra de 116 puérperas foi selecionada aleatoriamente para coleta de amostras de biofilmes subgengivais. Houve uma defasagem entre o início da pesquisa e o início da coleta de amostras de biofilmes subgengivais, devido à disponibilidade do material para armazenamento das amostras. Entretanto, a seleção das puérperas da sub-amostra foi equilibrada, uma vez que foram selecionadas 49 participantes para a coleta de biofilme na primeira metade da amostra (n = 271) e 67 na segunda metade. O procedimento utilizado para assegurar a aleatoriedade da sub-amostra foi a disponibilização periódica do material para armazenamento das amostras ao longo do período de trabalho de campo. A distribuição da sub-amostra de puérperas que tiveram coleta de biofilme subgengival em relação a todas as participantes está apresentada no Anexo 6.

Entrevistas individuais utilizando questionários pré-testados foram conduzidas para coletar os dados relativos às características sociodemográficas, condições de moradia, hábitos maternos, atividades físicas, violência durante a gravidez, fatores psicossociais, satisfação com a gravidez, história obstétrica e cuidados pré-natais. Informações sobre a gestação atual, características antropométricas e doenças ocorridas durante a gestação foram obtidas dos prontuários médicos.

Todos os examinadores seguiram uma seqüência padronizada e previamente estabelecida para a coleta de dados. Inicialmente os examinadores inspecionavam os prontuários médicos e selecionavam as mães que tiveram seus filhos dentro de 3 dias e que tinham pelo menos 30 anos de idade. Todas as mães pré-selecionadas foram convidadas e receberam informações sobre os objetivos do estudo, procedimentos para a coleta de dados e da sua participação voluntária no estudo, empregando-se o Termo de Consentimento Livre e Esclarecido (Anexo 3). Após a obtenção da assinatura de

consentimento, o questionário de seleção das mães era preenchido (Anexo 7). Se a mãe apresentasse uma ou mais características de exclusão a entrevista era encerrada ao final do questionário de seleção das mães. Se a mãe preenchesse todos os critérios de inclusão, a entrevista prosseguia, e um segundo questionário era utilizado para a coleta de dados das co-variáveis (Anexo 8). Após as entrevistas, parte das mães foi submetida às coletas subgengivais de amostra de biofilme bacteriano, sendo realizada uma sondagem periodontal prévia para identificação dos sítios periodontais mais profundos. Em seguida o exame clínico periodontal era realizado em todas as puérperas e uma ficha periodontal preenchida (Anexo 9). Posteriormente, as mães recebiam orientações sobre cuidados com a sua saúde bucal e a do seu bebê. Os casos de diagnóstico de DP eram encaminhados para tratamento em unidades odontológicas da Secretaria Municipal de Saúde do Rio de Janeiro. Finalmente, os examinadores coletavam as informações dos prontuários médicos (Anexo 8).

As entrevistas, coletas de amostras de biofilmes subgengivais e os procedimentos de exame clínico periodontal foram realizados nos leitos das enfermarias, estando as mulheres sentadas em suas camas.

3.3.2 - Determinação microbiológica do biofilme bacteriano subgengival

3.3.2.1 - Determinação dos microorganismos bucais utilizando a técnica de "*Checkerboard DNA-DNA hybridization*"

A identificação dos microorganismos subgengivais foi realizada por meio da técnica do "*checkerboard DNA-DNA hybridization*" [72, 73].

As suspensões bacterianas presentes nos tubo de Eppendorf contendo as amostras de placa subgengival foram fervidas em banho-maria por 10 minutos e em seguida neutralizadas pela adição de 0,8 ml de 5 M de acetato de amônia. Cada amostra contendo DNA livre foi depositada nas fendas do "Minislot 30" (Immunetics, Cambridge, MA, USA) e o DNA concentrado em uma membrana de nylon (15 x 15 cm) com carga positiva (Boehringer Mannheim, Indianápolis, IN, USA) (Anexo 10). O DNA depositado na membrana foi então fixado na mesma por aquecimento em forno a 120°C por 20 minutos. As duas últimas canaletas do "Minislot 30" foram reservadas para a colocação dos controles, contendo uma mistura das espécies de microorganismos investigadas pelas sondas de DNA, em duas concentrações, 10^5 e 10^6 células bacterianas.

3.3.2.2 - Hibridização das membranas com as sondas de DNA

Após fixação do DNA nas membranas, essas foram pré-hibridizadas a 42°C por 1 hora numa solução de 50% de formamida, 1% de caseína, 5 X SSC, 25 mM de fosfato de sódio (pH 6,5) e 0,5 mg/ml de RNA de levedura. Em seguida, cada membrana foi colocada sob a placa acrílica do "Miniblotter 45" (Immunetics), com as linhas contendo o DNA fixado perpendiculares às canaletas do "Miniblotter 45" (Anexo 11). O "Miniblotter" contém 45 canaletas que servem cada uma para a colocação de uma sonda de DNA.

3.3.2.3 - Sondas de DNA

As sondas de DNA foram confeccionadas usando o "*Random primer digoxigenin labeling kit*" (Boehringer Mannheim), como descrito por Feinberg & Vogelstein [75]. Sondas de DNA específicas para 39 espécies foram usadas no presente estudo (Anexo 12). Essas espécies foram selecionadas devido a sua associação com saúde e com diferentes tipos de doenças periodontais [75, 76].

Anteriormente ao seu uso, as sondas foram testadas com uma mistura controle contendo as espécies investigadas, numa concentração de 10^4 células bacterianas. Suas concentrações foram ajustadas de tal modo que as intensidades dos sinais de todas as sondas fossem semelhantes. Cada canaleta do "Miniblotter 45" foi preenchida com 130 µl de uma determinada sonda, contida numa solução de hibridização (45% de formamida, 5 X SSC, 20 mM de fosfato de sódio, pH 6,5), 0,2 mg/ml de RNA de levedura, 10% de sulfato de dextrano, 1% de caseína e 20 ng/ml de sonda de DNA). As sondas hibridizaram perpendicularmente às linhas contendo o DNA bacteriano fixado, propiciando um formato de xadrez, com as linhas contendo o DNA bacteriano no sentido horizontal e as sondas no sentido vertical (Anexo 13). A hibridização das membranas com as sondas ocorreu a 42°C, durante um período mínimo de 20 horas.

3.3.2.4 - Detecção das espécies

Após a hibridização com as sondas, as membranas foram removidas do "Miniblotter 45" e lavadas por 5 minutos em temperatura ambiente, seguido de duas lavagens de 20 minutos, a 68°C, numa solução de fosfato (0,1 X SSC, 0,1% SDS), a fim de remover sondas que não hibridizaram completamente. Em seguida, as membranas foram imersas por 1 hora em uma solução contendo 0,1 M de ácido maleico, 3 M de NaCl, 0,2 M de NaOH, 0,3% de Tween 20, 0,5% de caseína, pH 8,0; e por 30 minutos na mesma solução contendo o anticorpo anti-digoxigenina conjugado à fosfatase

alcalina (Boehringer Mannheim), numa diluição de 1/15.000. As membranas foram, então, lavadas com uma solução de 0,1 M de ácido maleico, 3 M de NaCl, 0,2 M de NaOH, 0,3% de Tween 20, pH 8,0, 2 vezes por 20 minutos; e uma vez por 5 minutos em 0,1 M de Tris HCl, 0,1 de NaCl, 50 mM de $MgCl_2$, pH 9,5. Em seguida, as membranas foram incubadas em uma solução detectora, CDP-*Star* (Boehringer Mannheim), por 45 minutos a 37°C. Finalmente, as membranas foram colocadas em um cassete sob um filme radiográfico (Kodak X-OMAT) por aproximadamente 40 minutos. O filme foi posteriormente revelado (Anexo 14) manualmente pelo método convencional temperatura-tempo, de acordo com orientações do fabricante. As soluções utilizadas foram da marca Kodak, sempre mantidas à temperatura de 20ºC.

A leitura dos filmes radiográficos foi realizada por um único examinador treinado, da seguinte forma: cada sinal produzido por uma determinada sonda na amostra de biofilme foi comparado em intensidade ao sinal produzido pela mesma sonda nos dois controles contendo 10^5 e 10^6 bactérias. Desta forma, o número 0 foi registrado quando não houve detecção do sinal; 1 equivaleu a um sinal menos intenso que o controle de 10^5 células; 2 equivaleu a aproximadamente 10^5 células; 3, entre 10^5 e 10^6 células; 4 aproximadamente 10^6 células e 5, mais do que 10^6 células (Anexo 15). Estes registros foram então utilizados para determinar os níveis das diferentes espécies investigadas nos sítios periodontais.

3.3.3 – Desenvolvimento do método *Periodontal Inflammatory Load* (PIL)

O método *Periodontal Inflammatory Load* (PIL) foi desenvolvido com dados clínicos e microbiológicos periodontais da sub-amostra de 116 puérperas, com o objetivo de mensurar adequadamente a doença periodontal. A medida PIL é um parâmetro clínico calculado somando-se todas as medidas de PBS acima de três milímetros de sítios que apresentam perda de inserção periodontal.

As mensurações de PBS e NCI são realizadas com uma sonda periodontal milimetrada, em seis sítios por dente (mesiovestibular, mediovestibular, distovestibular, distolingual, mediolingual e mesiolingual) de todos os dentes, excluindo os terceiros molares. As medidas de PBS e NCI são variáveis contínuas, que podem ser expressas como um vetor [x_1, x_2, x_3, x_4, … x_n], onde xi é uma medida (em milímetros), e n é o número total de sítios a serem medidos em cada indivíduo.

Um sítio periodontal é considerado uma área patológica quando a medida de PBS é ≥ 4 mm e está associada à perda clínica de inserção. Se a PBS de um sítio for ≥ 4 mm e a margem gengival livre estiver abaixo ou no mesmo nível da junção cemento-

esmalte, este sítio é incluído no cálculo da PIL. Sítios com PBS □ 3 mm e sítios com PBS ≥ 4 mm sem perda de inserção (“falsas” bolsas periodontais) não são incluídas na medida de PIL.

Para mensurar a PIL, os sítios com PBS ≥ 4 mm associados à NCI ≥ 4 mm são somados fornecendo uma medida contínua da DP. O vetor [*d*i] é gerado:

*d*i = *x*i se *x*i ≥ 4 (*x*i é uma medida de PBS em milímetros em sítios com NCI ≥ 4 mm)

$$\text{PIL} = \sum_{i=1}^{n} di$$

A PIL é expressa pela soma de todas as PBS ≥ 4 mm em sítios com NCI ≥ 4 mm. A PIL pode ser usada tanto como variável contínua como variável categórica. Para usar a PIL como variável categórica, a amostra deve ser sub-agrupada de acordo com os quartis de distribuição da PIL na amostra total.

3.3.3.1 – Validação da PIL como medida da doença periodontal

A medida *proxy* da doença periodontal deve representar a carga de patógenos periodontais. Para considerar a PIL como uma medida clínica da DP, a PIL deve estar relacionada somente com os patógenos periodontais em análises isoladas e agregadas. Além disso, a PIL não deve estar associada com espécies bacterianas benéficas.

Na validação da PIL foram usados os patógenos periodontais *Porphyromonas gingivalis*, *Treponema denticola* e *Tanerella forsythia*, e diferentes grupos de microorganismos bucais agregados em complexos, descritos por Socransky et al. [51]. Os diferentes grupos de microorganismos bucais são rotulados por cores específicas (Anexo 12). Algumas bactérias que não estão associadas a nenhum complexo e as sondas de DNA de novas espécies foram analisadas em um único grupo (grupo cinza).

As análises foram feitas em dois estágios. Primeiro, a medida PIL foi avaliada como medida contínua. Para validar a PIL, foram testadas as correlações entre a PIL, média de PBS, freqüência de PBS □ 4 mm, freqüência de PBS □ 5 mm, média de NCI, freqüência de NCI □ 3 mm e freqüência de PBS □ 4 mm em sítios com NCI □ 4, com as contagens dos complexos microbianos [51] e com as contagens dos patógenos periodontais, empregando-se o coeficiente de correlação de Spearman. Segundo, a amostra foi dividida em 4 grupos de acordo com os quartis de distribuição da medida PIL. Os quartis de distribuição da PIL foram: Nível 1: 0 a 7 mm, Nível 2: 8 a 45 mm, Nível 3: 45 a 100 mm e Nível 4: 101 mm ou mais.

Com o objetivo de comparar as contagens de cada complexo microbiano entre quartis de distribuição da PIL, os dados foram apresentados com contagens vezes 10^6, sendo feita a média para cada indivíduo e posteriormente a média para cada grupo. As diferenças entre os grupos para as médias de contagens dos complexos bacterianos foram analisadas pelo teste de Kruskal-Wallis.

As contagens das sondas de DNA foram computadas em cada sítio de cada indivíduo e a proporção da contagem de cada complexo bacteriano foi determinada. O teste de Kruskal-Wallis foi empregado para comparar as médias de proporções dos diferentes complexos microbianos [51] (Anexo 12) nas amostras de biofilme subgengivais entre os 4 grupos formados pelos quartis da distribuição da PIL.

O nível de significância estabelecido para todas as análises foi de 5% ($p \leq 0.05$).

3.3.4 – Análise estatística dos dados da associação entre doença periodontal e prematuridade e/ou baixo peso ao nascer

Todos os testes estatísticos foram realizados utilizando-se o programa estatístico SPSS ("Statistical Package for the Social Sciences", versão 11.0, SPSS Inc., Chicago, IL, USA). O nível de significância estatística estabelecido para todas as análises foi de 5% ($p \leq 0,05$).

A normalidade da distribuição das variáveis contínuas foi testada usando o teste de Kolmogorov-Smirnov. Variáveis contínuas com distribuição normal foram comparadas utilizando-se o teste *t*. No caso de não normalidade das variáveis, o teste de Mann-Whitney foi empregado. Variáveis categóricas foram analisadas empregando-se o teste Qui-quadrado e o teste exato de Fisher.

3.3.4.1 – Análise estatística dos parâmetros clínicos periodontais

Quatro estratégias foram empregadas para testar a associação entre parâmetros clínicos da DP e a P/BPN. No primeiro procedimento, procedeu-se a comparação de parâmetros clínicos periodontais. A média e a média de freqüência de cada parâmetro clínico foram feitas individualmente e, posteriormente, para cada grupo. Comparou-se entre os grupos a média e a média de freqüências de sítios com placa dental visível, cálculo visível, sangramento à sondagem, médias de PBS e NCI. Além disso, compararou-se as diferenças entre os parâmetros clínicos de PBS □ 4 mm, □ 5 mm e □ 6 mm, e NCI □ 3 mm, □ 4 mm, □ 5 mm e □ 6 mm, e usando a combinação de PBS □

4 mm e NCI □ 3 mm. As diferenças estatísticas entre os grupos foram determinadas utilizando-se o teste de Mann-Whitney.

O segundo procedimento para testar a hipótese do presente estudo foi feito utilizando-se 13 diferentes definições de DP baseados em estudos prévios sobre esse assunto. As ORs foram calculadas para cada definição de DP. As 13 definições de DP são apresentadas abaixo:

1. **Definição 1**: pelo menos 1 sítio com PBS □ 5 mm em cada quadrante, não considerando as PBS nas faces distais dos dentes mais posteriores de cada quadrante [77],
2. **Definição 2**: pelo menos 1 sítio com PBS □ 4 mm, e pelo menos 50% dos sítios com ISS igual a 1 [78, 79],
3. **Definição 3**: pelo menos 4 sítios com PBS □ 3,5 mm [79],
4. **Definição 4**: média de PBS, índice de placa e ISS acima da mediana [80],
5. **Definição 5**: mais de 3 sítios com NCI □ 3 mm [81],
6. **Definição 6**: pelo menos 5 sítios com NCI □ 3 mm [82],
7. **Definição 7**: pelo menos 60% dos sítios com NCI □ 3 mm [11],
8. **Definição 8**: pelo menos 4 sítios com NCI □ 3 mm e PBS □ 4 mm [83],
9. **Definição 9**: pelo menos 4 dentes com 1 sítio ou mais com NCI □ 3 mm e PBS □ 4 mm no mesmo sítio [84],
10. **Definição 10**: mais de 5% dos sítios com PBS □ 5 mm e mais de 5% dos sítios com NCI □ 3 mm [85],
11. **Definição 11**: pelo menos 1 sítio com PBS □ 5 mm e 2 ou mais sítios com NCI > 6 mm e sangramento à sondagem > 5% [86],
12. **Definição 12**: Saúde periodontal: ausência de PBS > 3 mm e ausência de sítios com NCI > 2 mm, Periodontite leve: pacientes com menos doença do que o grupo moderado a severo e mais doença que o grupo saudável, Periodontite moderada a severa: pelo menos 4 sítios com PBS □ 5 mm e NCI □ 2 mm [87],
13. **Definição 13**: Ausência de doença: menos de 3 sítios com NCI □ 3 mm, Periodontite: pelo menos 3 sítios com NCI □ 3 mm, DP generalizada: pelo menos 90% dos sítios com NCI □ 3 mm [88].

A terceira estratégia de análise empregada foi comparar os diferentes quartis da distribuição de acordo com o número de PBS ≥ 4 mm. Os quartis de distribuição de PBS ≥ 4 mm foram calculados e as puérperas agrupadas em diferentes níveis para PBS. Nível 1: 0 a 1 sítio com PBS ≥ 4 mm, Nível 2: 2 a 10 sítios com PBS ≥ 4 mm, Nível 3: 11 a 23 sítios com PBS ≥ 4 mm e Nível 4: 24 sítios ou mais com PBS ≥ 4 mm.

O quarto procedimento analisou a DP em termos da carga de infecção periodontal (PIL), descrita previamente. Os quartis da distribuição da PIL foram comparados. Na terceira e quarta estratégias, as ORs foram calculadas utilizando o Nível 1 como categoria de referência.

3.3.4.2 – Análise estatística dos dados microbiológicos

Foram analisadas 232 amostras de biofilmes subgengivais de 116 puérperas (2 amostras de biofilmes subgengivais por puérpera) para contagens de 39 espécies bacterianas. Os dados microbiológicos foram expressos em nível médio de cada espécie (contagem) em cada paciente. Estes níveis foram computados por indivíduo e depois dentro de cada grupo avaliado. Diferenças nas médias de contagens dos microorganismos e nas médias de proporções dos complexos microbianos [51] (Anexo 12) foram avaliadas por meio do teste de Mann-Whitney.

3.3.4.3 - Análise estatística das co-variáveis

As co-variáveis foram computadas para cada sujeito, e posteriormente para cada grupo. As comparações entre os grupos foram analisadas com o teste Qui-quadrado e o teste exato de Fisher para as variáveis expressas em proporções. Para as variáveis contínuas utilizou-se o teste de Mann-Whitney e o teste *t*.

As consistências internas das escalas de depressão e de ansiedade foram aferidas através do Coeficiente *alpha* de Cronbach.

3.3.4.4 – Análises multivariadas entre a doença periodontal e prematuridade e/ou baixo peso ao nascer

Análises multivariadas de regressão logística foram realizadas entre a doença periodontal e a prematuridade e/ou baixo peso ao nascer. Nestas análises foram consideradas as 13 definições de doença periodontal (apresentadas no item 3.3.1) e quatro desfechos gestacionais: prematuridade, baixo peso ao nascer, prematuridade e baixo peso ao nascer, e prematuridade e/ou baixo peso ao nascer.

Em todas as análises, a doença periodontal entrou no modelo de regressão logística em um primeiro nível, e as co-variáveis em um segundo nível. Este procedimento permitiu analisar a associação entre a doença periodontal e a prematuridade e/ou o baixo peso ao nascer ajustando para todas as co-variáveis, uma vez que a doença periodontal foi forçada a permanecer no modelo até a última etapa da

regressão logística. A regra de decisão utilizada na modelagem dos dados para as co-variáveis foi o valor de p □ 0,30.

Os resultados das análises multivariadas são apresentados no Anexo 16.

Artigos

Artigo I - Periodontal infection and undesirable pregnancy outcomes: a systematic review of epidemiologic studies.

Artigo II - Periodontal Inflammatory Load (PIL): a new periodontal epidemiologic method.

Artigo III - The relationship between periodontitis and preterm low birth weight.

Artigo IV - The relationship between maternal periodontal microbiota and preterm low birth weight.

4 – Artigo I

Periodontal infection and undesirable pregnancy outcomes: a systematic review of epidemiologic studies.

Mario Vianna Vettore[1,2], Gabriela de Almeida Lamarca[3], Anna Thereza Leão[4], Filipe Brand Thomaz[3], Aubrey Sheiham[2], Maria do Carmo Leal[1]

1 – Department of Epidemiology and Quantitative Methods in Health, National School of Public Health, Oswaldo Cruz Foundation (ENSP/FIOCRUZ) – RJ, Brazil

2 - Department of Epidemiology and Public Health, University College London, London, England

3 – Section of Periodontology, Brazilian Dental Association, Regional Petropolis-RJ, Brazil

4 - Department of Dental Clinic, Federal University of Rio de Janeiro (UFRJ) – RJ, Brazil

Aceito para publicação em Fevereiro de 2006 no Cadernos de Saúde Pública.

Resumo

O objetivo desta revisão sistemática foi avaliar os estudos analíticos que relacionaram a doença periodontal como possível fator de risco para desfechos indesejáveis da gestação. Uma busca bibliográfica foi conduzida nas bases de dados MEDLINE, SciELO, LILACS e banco de teses da CAPES em Dezembro de 2005. Uma revisão sistemática dos estudos epidemiológicos sobre doença periodontal e desfechos indesejáveis da gestação foi feita. Dentre os 964 estudos identificados, 36 preencheram os critérios de inclusão. Vinte e seis estudos encontraram associações entre a doença periodontal e desfechos indesejáveis da gestação. Observou-se uma heterogeneidade entre os estudos em relação ao método de mensuração na doença periodontal e dos desfechos indesejáveis da gestação, não sendo possível realizar uma meta-análise. A maioria dos estudos apresentou falta de controle de variáveis de confusão que tornam suas conclusões duvidosas. Assim como suas limitações metodológicas não permitem adequadas conclusões sobre o real efeito da doença periodontal sobre os desfechos da gestação. Uma possível relação causal permanece desconhecida. Estudos analíticos com maior rigor metodológico, empregando medidas confiáveis para avaliar a exposição e desfecho serão úteis nas pesquisas futuras.

Palavras-chave: revisão sistemática, doença periodontal, desfechos indesejáveis da gestação, prematuridade, baixo peso ao nascer.

Abstract

The objective of this systematic review was to evaluate analytic studies on periodontal disease as a possible risk factor for undesirable pregnancy outcomes (UPO). A literature search of the bibliographic databases MEDLINE, SciELO, LILACS and CAPES thesis database, was conducted up December 2005. A systematic review was done of epidemiological studies about periodontal disease and UPO. Of the 964 papers identified, 36 analytical studies met the inclusion criteria. Twenty-six epidemiological studies reported associations between periodontal disease and UPO. There was a clear heterogeneity among studies concerning how periodontal disease was measured as well as what type of undesirable pregnancy outcome was used as the outcome. So a meta-analysis was not performed. Most studies did not control for confounders, casting serious doubts on their conclusions. The limitations in the methodology used in most studies do not allow conclusions to be drawn about the effects of periodontal disease on undesirable pregnancy outcomes. Larger, methodologically rigorous, analytical studies using reliable outcomes and exposure measures are recommended.

Key words: systematic review, periodontal disease, undesirable pregnancy outcomes, preterm, low birth weight.

4.1 - Introduction

The possibility that pathogenic microorganisms and their products from infectious foci, including those from the mouth, can be disseminated to other parts of the body, triggering different pathologies, was first suggested by Hunter in 1910, in his "Focal Infection Theory" [1]. The lack of good scientific evidence for the theory was criticized and it was refuted. Currently, a similar theory has been put forward, namely, an association between periodontal disease and undesirable pregnancy outcomes.

The improvements in epidemiology, biostatistics and molecular biology during the last three decades and the concern among dental researchers to assess the effects of the mouth on general health provoked a rebirth of the "Focal Infection Theory". Considerable improvements in research methodology are the main triggers for this "rebirth". The methodological improvements include a more rational analysis of the biological plausibility, inferences about causality, valid evaluation of statistical analysis, and control for bias and confounding and interactional variables. Because of these developments scientific studies about the relationship of dental infections with chronic and multifactorial diseases can be better developed. Periodontal medicine is a new branch in periodontology involving the study of the periodontal illness as a possible risk factor for several diseases, including coronary heart diseases, diabetes, and undesirable pregnancy outcomes.

The studies linking periodontal disease with undesirable pregnancy outcomes began in 1996, when Offenbacher and coworkers claimed to find a strong relationship between these diseases [2]. Their findings aroused interest mainly because of the impressive odds ratio of 7.9 of women with periodontal disease having a preterm low birth weight baby. Since that study, several investigations and reviews have been conducted on the relationship between periodontal disease and undesirable pregnancy outcomes. However, different study designs were used to assess the relationship between periodontal disease and undesirable pregnancy outcomes. Some investigations have serious shortcomings. For example, confounding variables have not routinely been analyzed. And there is a lack of a balanced view of the possible relationship between periodontal disease and undesirable pregnancy outcomes. The objective of this review is to conduct a critical review of analytic studies regarding periodontal disease as a possible risk factor for undesirable pregnancy outcomes.

4.2 - Methods

The methods applied in this systematic review cover the search strategy and inclusion criteria.

Search strategy

We searched the PubMed, Scielo, LILACS and CAPES thesis databases. Standardized methodological filters were used to identify analytical studies and paper reviews included the following keywords: ((Low birth weight OR pre term OR preterm OR prematur* OR immatur*) OR ((labor OR pregnancy OR birth OR neonatal OR fetal OR intrauterin*) AND (complication* OR disease* OR adverse)) OR PLBW) AND (periodont*). We also searched reference lists of identified articles and abstracts. The search was limited to human studies written in English or Portuguese. Identified studies published before 21 December 2005 were included.

Inclusion criteria

Studies were considered for inclusion if they addressed different aspects and measures of destructive periodontal disease like clinical, microbiological or immunological, and clinical undesirable pregnancy outcomes. The analytical studies had to include an estimate of the effect of periodontal disease on pregnancy outcomes and/or statistical tests for comparison of groups. There was a clear heterogeneity among studies concerning how periodontal disease was measured as well as what type of UPO was the outcome so a meta-analysis was not performed.

Exclusion criteria

Cross-sectional studies reporting periodontal conditions in puerperal women, case reports, ecological studies, experimental animal studies and previous reviews on this subject were excluded.

4.3 - Results

Of the 964 papers identified, 36 analytical studies met inclusion criteria. One of the 36 studies was excluded from this analytical review [3] because it was a duplicate of the Dasanayake and co-worker's study [4]. One cohort data set was analysed twice in the present review because the study compared both the incidence of preterm birth between treated and untreated women for periodontal disease (clinical trial design) and secondly, compared periodontal disease between preterm birth and non-preterm birth (nested case control in a cohort study analysis) [5]. Therefore, that cohort was included as two studies. Overall, 36 studies were considered in the present systematic review. Twenty-six showed positive associations between periodontal disease and undesirable pregnancy

outcomes. Figure 1 displays the epidemiological studies included and those showing positive association, by type of epidemiological design.

The main characteristics of the methodology applied in analytical studies are described according the study design in Table 1 – Case control studies, Table 2 – Cohort studies and Table 3 – Clinical trials. There was a clear heterogeneity in the methodology and in the sample sizes in analytical studies, which may have affected the power and precision in some studies. A wide range of clinical parameters and indices to assess periodontal disease have been used.

The results are presented according to type of study. Overall relevant findings about outcome of interest, periodontal disease measurements, confounding and statistical issues are also presented

Case control studies

The findings from the case control studies were analyzed separately according the outcome investigated (Table 1). They are:

Low birth weight babies

Low birth weight was the outcome in 8 case control studies [4, 6, 7, 8, 9, 10, 11, 12, 13]. Contradictory findings were reported in 2 studies that used Community Periodontal Index for Treatment Needs (CPITN) scores to assess periodontal disease [8, 10]. Similar differences were observed in the results between 2 studies using seric levels of Immunoglobulin G (IgG) for periodontal pathogens species [4, 10], and between 2 studies using clinical attachment level to define periodontal disease [10, 13]. While the small sample size may be responsible for the lack of power to detect association in Louro's study [8], this may not be the case in the other studies not finding an association between periodontal disease and low birth weight [9, 10, 11, 12].

Preterm birth

Nine studies considered preterm birth as the outcome of interest [9, 10, 11, 12, 14, 15, 16, 17, 18]. A strong relationship between red and orange microbial complex organisms in periodontal pockets and preterm birth was reported [14, 15]. However, no evidence was found to support the systemic dissemination of periodontal pathogens and its products throughought the body, as evidenced by the lack of difference of IgG seric levels in umbilical cord IgG for periodontal pathogens in mothers [14]. Other studies differed in their findings. On the one hand, Jarjoura et al [10] found a significant association between mean periodontal attachment loss and higher prevalence of periodontitis with preterm deliveries , whilst, on the other hand, in a large sample of 3738 women, Moore and co-worker's [9] found similar levels of periodontal disease between cases and controls. The

absence of an association between periodontal disease and preterm birth were also reported by other studies [11, 12, 17, 18].

Preterm low birth weight babies

Preterm low birth weight is the term used to combine the two previous criteria. However, the criteria for preterm low birth weight were not the same in all the studies reviewed.

Preterm and low birth weight babies

Eight case control studies considered preterm low birth weight when the newborn was preterm in addition to being low birth weight [2, 19, 20, 21, 22, 23, 24, 25]. Davenport's study [20] did not find a difference for CPITN periodontal scores between cases and controls. Similarly, Offenbacher et al [19] , Noack et al [24] and Budunelli et al [25] found similar periodontal disease levels between cases and non cases. Noack et al [24] also reported no differences for periodontal pathogens between groups.

However, crevicular levels of interleukins and periodontopathogens were higher in women with preterm and low birth babies [19]. Four other case control studies reported an increased risk in the periodontal disease group despite using different definitions for periodontal disease [2, 21, 22, 23]. Carta et al [23] also found differences for prostaglandin E-2 (PGE-2) and interlukin-1 □ (IL-1 □) crevicular levels between groups with and without a preterm and low birth weight birth.

Preterm or low birth weight babies

In five studies, the definition for the outcome was preterm babies or low birth weight babies [5, 11, 26, 27, 28]. Mokeen et al [27] and Dörtbudak et al [28] reported different levels of periodontal disease between cases and controls, whereas other authors did not find differences in periodontal status between the groups [5, 11, 26]. In one other study, puerperal women with clinical periodontitis had an increased risk of low birth weight or preterm birth or premature rupture of membranes or threatened preterm labor [29].

Four studies reported differences in amounts of periodontal pathogens in mothers of preterm low birth and non-preterm low birth weight babies [5, 19, 25, 28]. Yet, in 3 of the studies there were no differences in parameters for periodontal disease. The great variability in methods of periodontal disease assessment, the possible presence of confounders and different methods to assess the outcome may explain the disagreements between the case control studies.

Cohort studies

Six cohort studies were identified [30, 31, 32, 33, 34, 35] (Table 2). Preterm birth was the outcome in 3 studies. Jeffcoat and co-worker's [31] found a strong dose-response

relationship between periodontal attachment loss and gestational age at birth. The authors used odds ratio as a measure of association instead of relative risk, which may have distorted the association since preterm birth cannot be considered an uncommon event. Holbrook et al [33] and Marin et al [34] did not find an association between periodontal disease and preterm births.

López et al [30] found a 3.5 odds ratio for having a low birth weight or preterm baby for women with moderate periodontal disease. As in Jeffcoat's cohort study [31], the use of odds ratios to estimate the association can bias the findings. Offenbacher et al [32] detected the same risk of low birth weight and preterm birth among women with moderate periodontal disease. Nonetheless, the risk of low birth weight and preterm was twice as high in women with severe periodontal disease compared to periodontally healthy women.

Of six cohort studies, only one presented the numbers of women lost to follow-up. Twelve percent of women were lost in the Chile cohort study [30]. The frequency of women excluded were 11.5% and 11.2% in the periodontally healthy women and periodontal disease groups respectively. As losses to follow-up represented 72% of excluded women, it is probable that the groups compared had similar losses [30]. Other cohort studies did not report losses to follow-up [31].

Clinical trial studies

Three clinical trials were conducted to evaluate the effect of periodontal therapy on reduction of undesirable pregnancy outcomes [5, 36, 37]. Of the 3 clinical trials, only one showed that periodontal intervention in pregnant women decreased the risk of having a "preterm birth", "low birth weight" or "preterm or low birth weight" birth [36] (Table 3). The risk for preterm birth was not decreased in women submitted to scaling and root planing and those having scaling and root planing plus metronidazole [5, 37].

Of the 3 clinical trials, only one conducted intention-to-treat analysis [36]. The intention-to-treat analysis odds ratios were higher than those obtained from the protocol analysis. Random assignment of who received periodontal therapy was described in two studies [36, 37].

Among three clinical trials analyzed, two reported numbers lost to follow-up. Mitchell-Lewis et al [5] did not present separately the loss for each group. The overall percentage of the recruited sample lost to follow-up was 8%. In the other clinical trial, a total of 12.7% of women were lost during the follow-up. While the treatment group lost 18.5%, the control group lost 6% [36].

Three cohorts and one clinical trial study did not present any information about losses to follow-up [31, 32, 33, 36]. They account for more than 50% of prospective studies conducted to date. If the proportion of women lost in those studies were large, the validity of the studies would be affected.

Outcome measures of interest

The majority of studies did not present information on how birth weight and gestational age were assessed. It is probable that all studies employed calibrated scales for birth weight assessment. However, the influence of the time of post delivery weight assessment on the newborn weight registered in the medical records is well recognized.

Fourteen of the 36 studies analyzed reported the method for gestational age assessment. Ultrasound measurement of the fetus was the most common method used. Last menstrual period was used together with ultrasound in 3 studies [30, 36, 37] and clinical methods were used in 3 studies [2, 21, 30]. Only 5 studies reported more than one method for gestational age assessment, an important procedure to avoid bias of classification [2, 30, 32, 36, 37].

Problems with last menstrual period recall, irregularity of menstruation, oral contraceptive use, and bleeding in early pregnancy usually affect the accuracy of the gestational age assessment method [38]. When compared with last menstrual period, clinical estimate of newborn has a higher mean overestimation of preterm babies.

There may be bias in the estimate of risks in analytical studies of the impact of putative etiologic factors on the risk of preterm when ultrasound is used. If the exposures of interest interfere with early fetal growth, as suggested as the influence of periodontal disease in preterm births, then the associated risk of preterm delivery will be overestimated when gestational age is assessed by ultrasound [39]. The validity and precision of ultrasound to estimate the gestational age is seriously affected when ultrasounds are conducted after 18th week gestational age and can be an important source of misclassification [39].

Periodontal disease measurements

Thirteen different definitions of periodontal disease were used in the 36 selected studies. Furthermore, the assessment of periodontal disease was carried out by two periodontal indexes. The CPITN and the Periodontal Disease Index. The Community Periodontal Index for Treatment Needs was employed to assess periodontal status in 5 case control studies [6, 7, 20, 23, 27] despite the fact that CPITN is considered an unsuitable index to measure periodontal severity and prevalence in clinical studies. Periodontitis and gingivitis are related but different, and CPITN mixes both and may be a source of

misclassification of exposure. The Periodontal Disease Index (PDI), used by Konopka and coworkers [22] has similar limitations. The misclassification produced by CPITN and PDI index is very important because those considered as non-exposed to periodontal disease can be incorrectly classified as exposed to periodontal disease due to overestimation of periodontal disease, biasing the analysis.

Because measurement of periodontal disease is so important, it is surprising that only 3 studies provided information on diagnostic reliability for periodontal disease assessment [11, 12, 24]. Few studies reported the exact percentage of agreement of clinical calibration for periodontal examination [6, 26, 31, 36]. However, that method is not an adequate statistical test for analysing measurement reproducibility.

Confounding

The most interesting feature observed in analyzing all the studies that tested the association between periodontal disease and undesirable pregnancy outcomes together, is the inconsistency in controlling for confounders. Psychological stress, physical activities, weight gain by pregnant, violence and social support are important risk factors for undesirable pregnancy outcomes. Nevertheless, in only one study [24] was stress assessed, and the other important risk factors were not taken into account in any study linking periodontal disease with undesirable outcomes in pregnancy. This is a major shortcoming and casts doubts on the conclusions of all such studies.

Statistical issues

The positive associations between periodontal disease and undesirable pregnancy outcomes found in 5 studies may be confounded by the effect of potential variables for undesirable pregnancy outcomes because, only bivariate analysis were performed [15, 19, 23, 28, 34]. This statistical technique is unsuitable for making statistical inferences due to lack of control for several confounders (Figure 2). In all other studies the statistical analysis were performed using multivariate analysis, which allows determination of the independent contribution of each risk factor to the development of undesirable pregnancy outcomes.

Few confounders were taken into account when logistic multivariate analysis were performed and no study reported procedures in modeling data. Neither did they test if the model fitted the data (residual analysis).

4.4 - Discussion and Conclusion

The development of strategies to reduce neonatal and infant mortality due to preterm birth and low birth weight babies have been supported by evidence-based

neonatal medicine. Researchers in periodontal medicine contributed to information on this subject, since studies linking periodontal disease and undesirable pregnancy outcomes may help to clarify another unknown risk factor of undesirable pregnancy outcomes. In fact, the ultimate goal of the dental studies is to find evidence to support dental screening of pregnant women and determine if treatment of their periodontitis can lower the risk of adverse pregnancy outcomes.

Although the majority of the studies analyzed found a positive association between periodontal disease and increased risk for undesirable pregnancy outcomes, the shortcomings in the methods used casts serious doubts on the validity of the outcomes and conclusions. There is considerable variation in methodological quality, with virtually every study having serious methodological limitations. These include small sample size, limited number of statistical analyses, inadequate control for potential confounders, inadequate assessment of gestational age and measurment of periodontal disease, and reliance on cross-sectional data.

A meta-analysis was not performed in this systematic review because the heterogeneity in the methods mentioned above. Such statistical procedure is considered a powerful tool to obtain a summary measure of association when systematic reviews are conducted. A recent systematic review found a strong association between periodontal disease and PTLBW [40]. However, their findings are probably biased as they included only 5 studies compared with the 36 studies analyzed in the present review, suggesting failures in their search strategy and inclusion criteria.

A complication in the combined analysis of epidemiological studies in the present review is caused by the diversity of periodontal disease measures used to describe and quantify periodontal diseases and the lack of consensus as to a uniform definition and classification of periodontal disease. Robust measures for periodontal disease must use periodontal pocket depth measures associated with clinical attachment level. A pilot study is essential to ensure that all examiners are properly calibrated.

Future studies should use more than one method for gestational age assessment. In antenatal care programs the overall validity of methods to estimate gestational age appears to be high because the vast majority of babies are born at or near term. However, this is misleading in epidemiological studies, because the high percentage of selected preterm births and the gestational age assessment in preterm births is frequently subject to errors.

Extensive literature reviews have highlighted at least 43 potential determinants for undesirable pregnancy outcomes (summarized in Figure 2) [41]. Of all the 43 cited risk

factors mentioned, 11 can be considered confounders in the studies of the possible role of periodontal disease in undesirable pregnancy outcomes. So, it is vital to control for as many confounding factor as possible using different strategies like restriction, matching, randomization and statistical analysis in order to avoid spurious associations.

The serious limitations in the methodology used in most studies casts serious doubts on their findings. They do not allow suitable conclusions to be drawn about the genuine effects of periodontal disease on undesirable pregnancy outcomes. In conclusion, although 26 of the 36 studies included in this review consider there is a positive relationship between periodontal pathology and undesirable pregnancy outcomes there is no sound scientific justification to recommend periodontal disease screening in pregnant women to reduce poor pregnancy outcomes.

Funding

This research was supported by PAHO/WHO (Graduate Thesis Grants in Public Health), CNPq, FAPERJ grants E-26/ 170.421/2003 and Hu-Friedy do Brasil.

4.5 - Bibliographic references

1. Billings F. Chronic focal infection and their etiologic relations to arthritis. Arch Intern Med 1912; 9: 484-98.

2. Offenbacher S, Katz V, Fertik G, Collins J, Boyd D, Maynor G, et al. Periodontal infections as a risk factor for preterm low birth weight. J Periodontol 1996; 67:1103-13.

3. Dasanayake AP, Russell S, Boyd D, Madianos PN, Forster T, Hill E. Preterm low birth weight and periodontal disease among African Americans. Dent Clin North Am. 2003; 47:115-25.

4. Dasanayake AP, Boyd D, Madianos PN, Offenbacher S, Hills E The association between Porphyromonas gingivalis-specific maternal serum IgG and low birth weight. J Periodontol 2001; 72:1491-7.

5. Mitchell-Lewis D, Engebretson SP, Chen J, Lamster IB, Papapanou PN. Periodontal infections and pre-term birth: early findings from a cohort of young minority women in New York. Eur J Oral Sci 2001; 109:34-9.

6. Dasanayake AP. Poor periodontal health of the pregnant woman as a risk factor for low birth weight. Ann Periodontol 1998; 3:206-12.

7. Sembene M, Moreau JC, Mbaye MM, Diallo A, Diallo PD, Ngom M, et al. Periodontal infection in pregnant women and low birth weight babies OdontostomatolTrop 2000; 89:19-22.

8. Louro PM, Fiori HH, Louro Filho P, Steibel J, Fiori RM Doença periodontal na gravidez e baixo peso ao nascer. J Pediatr 2001; 77:23-8.

9. Moore S, Ide M, Coward PY, Randhawa M, Borkowska E, Baylis R, et al. A prospective study to investigate the relationship between periodontal disease and adverse pregnancy outcome. Br Dent J 2004; 197:251-8.

10. Jarjoura K, Devine PC, Perez-Delboy A, Herrera-Abreu M, D'alton M, Papapanou PN. Markers of periodontal infection and preterm birth. Am J Obstet Gynecol 2005; 2:513-9.

11. Lunardelli AN, Peres MA. Is there an association between periodontal disease, prematurity and low birth weight? A population-based study. J Clin Periodontol 2005; 32:938-46.

12. Moreu G, Tellez L, Gonzalez-Jaranay M. Relationship between maternal periodontal disease and low-birth-weight pre-term infants. J Clin Periodontol 2005; 32:622-7.

13. Cruz SS, Costa MCN, Filho ISGF, Vianna MIP, Santo CT. Maternal periodontal disease as a factor associated with low birth weight. Rev Saude Publica 2005; 39:782-7.

14. Madianos PN, Lieff S, Murtha AP, Boggess KA, Auten RLJr, Beck JD, et al. Maternal periodontitis and prematurity. Part II: Maternal infection and fetal exposure. Ann Periodontol 2001; 6:175-82.

15. Hasegawa K, Furuichi Y, Shimotsu A, Nakamura M, Yoshinaga M, Kamitomo M, et al. Associations between systemic status, periodontal status, serum cytokine levels, and delivery outcomes in pregnant women with a diagnosis of threatened premature labor. J Periodontol. 2003; 74:1764-70.

16. Goepfert AR, Jeffcoat MK, Andrews WW, Faye-Petersen O, Cliver SP, Goldenberg RL, et al. Periodontal disease and upper genital tract inflammation in early spontaneous preterm birth. Obstet Gynecol. 2004; 104:777-83.

17. Moore S, Randhawa M, Ide M. A case-control study to investigate an association between adverse pregnancy outcome and periodontal disease. J Clin Periodontol 2005; 32:1-5.

18. Moore S, Ide M, Randhawa M, Walker JJ, Reid JG, Simpson NA. An investigation into the association among preterm birth, cytokine gene polymorphisms and periodontal disease. BJOG 2004; 111:125-32.

19. Offenbacher S, Jared HL, O´Reilly PG, Wells SR, Salvi GE, Lawrence HP, et al. Potential pathogenic mechanisms of periodontitis-associated pregnancy complications. Ann Periodontol 1998; 3:233-50.

20. Davenport ES, Williams CE, Sterne JA, Murad S, Sivapathasundram V, Curtis MA Maternal periodontal disease and preterm low birthweight: case-control study. J Dent Res 2002; 81:313-8.

21. Moliterno LF, Monteiro B, Figueredo CMS, Fischer RG. Association between periodontitis and low birth weight: a case-control study. J Clin Periodontol 2005; 32:886-90.

22. Konopka T, Rutkowska M, Hirnle L, Kopec W, Karolewska E. The secretion of prostaglandin E2 and interleukin 1-beta in women with periodontal diseases and preterm low-birth-weight. Bull Group Int Rech Sci Stomatol Odontol 2003; 45:18-28.

23. Carta G, Persia G, Falciglia K, Iovenitti P. Periodontal disease and poor obstetrical outcome. Clin Exp Obstet Gynecol. 2004; 31:47-9.

24. Noack B, Klingenberg J, Weigelt J, Hoffmann T. Periodontal status and preterm low birth weight: a case control study. J Periodontal Res. 2005; 40:339-45.

25. Buduneli N, Baylas H, Buduneli E, Turkoglu O, Kose T, Dahlen G. Periodontal infections and pre-term low birth weight: a case-control study. J Clin Periodontol 2005; 32:174-81.

26. Cardoso EOC. O estudo das doenças periodontais em gestantes e seu impacto no nascimento de crianças prematuras e/ou de baixo peso. Tese de Mestrado. Universidade Federal do Rio de Janeiro 1999; 76.

27. Mokeem SA, Molla GN, Al-Jewair TS. The prevalence and relationship between periodontal disease and pre-term low birth weight infants at King Khalid University Hospital in Riyadh, Saudi Arabia. J Contemp Dent Pract. 2004 15; 5:40-56.

28. Dörtbudak O, Eberhardt R, Ulm M, Persson GR. Periodontitis, a marker of risk in pregnancy for preterm birth. J Clin Periodontol 2005; 32:45-52.

29. Radnai M, Gorzó I, Nagy E, Urbán E, Novák T, Pál A. A possible association between preterm birth and early periodontitis. J Clin Periodontol 2004; 31:736-41.

30. López NJ, Smith PC, Gutierrez J. Higher risk of preterm birth and low birth weight in women with periodontal disease. J Dent Res 2002; 81:58-63.

31. Jeffcoat MK, Geurs NC, Reddy MS, Cliver SP, Goldenberg RL, Hauth JC. Periodontal infection and preterm birth: results of a prospective study. J Am Dent Assoc 2001; 132:875-80.

32. Offenbacher S, Lieff S, Boggess KA, Murtha AP, Madianos PN, Champagne CM, et al. Maternal periodontitis and prematurity. Part I: Obstetric outcome of prematurity and growth restriction. Ann Periodontol 2001; 6:164-74.

33. Holbrook WP, Oskarsdottir A, Fridjonsson T, Einarsson H, Hauksson A, Geirsson RT. No link between low-grade periodontal disease and preterm birth: a pilot study in a healthy Caucasian population. Acta Odontol Scand 2004; 62:177-9.

34. Marin C, Segura-Egea JJ, Martinez-Sahuquillo A, Bullon P. Correlation between infant birth weight and mother's periodontal status. J Clin Periodontol 2005; 32:299-304.

35. Rajapakse PS, Nagarathne M, Chandrasekra KB, Dasanayake AP. Periodontal disease and prematurity among non-smoking Sri Lankan women. J Dent Res 2005; 84:274-7.

36. López NJ, Smith PC, Gutierrez J. Periodontal therapy may reduce the risk of preterm low birth weight in women with periodontal disease: a randomized controlled trial. J Periodontol 2002; 73:911-24.

37. Jeffcoat MK, Hauth JC, Geurs NC, Reddy MS, Cliver SP, Hodgkins PM, et al. Periodontal disease and preterm birth: results of pilot intervention study. J Periodontol 2003; 74:1214-18.

38. Berg AT, Bracken MB. Measuring gestational age: an uncertain proposition. Br J Obstet Gynaecol 1992; 99:280-2.

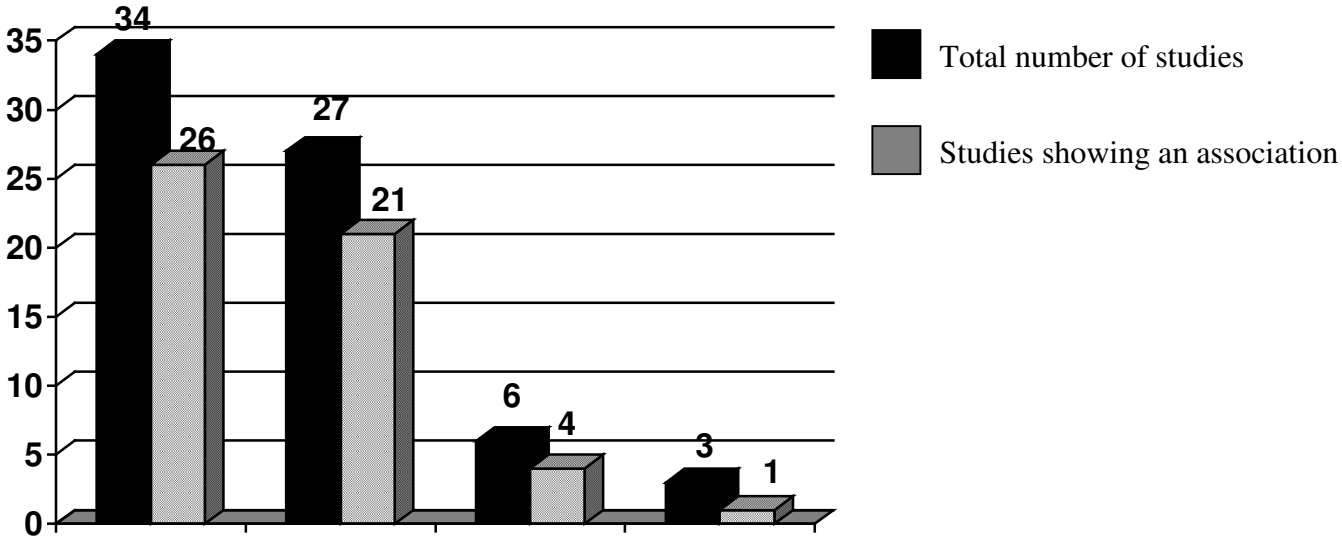
39. Henriksen TN, Wilcox AJ, Heedegaard M, Secher NJ. Bias instudies of preterm and postterm delivery due to ultrasound assessment of gestational age. Epidemiology 1995; 6:533-7.

40. Khader YS, Ta'ani Q. Periodontal diseases and the risk of preterm birth and low birth weight: a meta-analysis. J Periodontol 2005; 76:161-5.

41. Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. Bull WHO 1987; 65:663-737.

4.6 – Figuras e Tabelas

Figure 1. Numbers of studies conducted and showing positive associations between periodontal disease and undesirable pregnancy outcomes, by type of epidemiologic study design*.



* One study was included twice because the paper presented results from a clinical trial and a nested case control analysis in the same cohort study.

Figure 2. Risk factors for undesirable pregnancy outcomes

Risk factors for undesirable pregnancy outcomes	
Medical conditions	Social characteristics
Woman's obstetrical past	Mother's age – under 17 years of age and over 35 years
Multi-fetal pregnancy	Ethnic origin - African and Caucasian
Placental pathologies	Low socio-economic status
Smoking	Alcohol abuse
Diabetes mellitus	Drug use during pregnancy
Vaginal infections	Inadequate prenatal care
Immunological diseases	Psychological factors
Presence of anticardiolipin and lymphocytotoxic	Some types of mother's work during pregnancy

Table 1. Characteristics of case-control studies about the relationship between periodontal disease and undesirable pregnancy outcomes

Outcome	Parameter for periodontal disease	Author / year	PB and BW assessment	Sample size	Mean age	Variables controlled	OR*, SD(†), NSD(‡)
LBW	CPITN	Dasanayake 1998 [6]	BW: NP	110	27.2 ± 5.8	Age, diabetes, asthma, cardiac diseases, smoking, alcohol, caffeine, SES, GA, ethnicity	4.1 (1.3-12.8)*†
	CPITN	Sembene et al. 2000 [7]	BW: NP	113	68% <30 (15-39)	Age, GUI, previous abortion	NSD‡
	ESI	Louro et al. 2001 [8]	BW: NP	26	21.0 (14-36)	Age, diabetes, hypertension, GUI, race, smoking, alcohol, PMH, PC, SES, ethnicity	26.9% CPITN 3 associated with BW NSD for PD Extension Index ‡ 7.2 (0.4-125.4)* for PD Severity Index ‡
	Seric levels of specific IgG for periodontal pathogens	Dasanayake et al. 2001 [4]	BW: NP	448	21.7 ± 5.4	Age, race, drugs, smoking e alcohol, ethnicity	1.02 (1.01-1.04)* for *P. gingivalis* IgG † 1.15 (0.96-1.38)* for *T. forsythia* IgG ‡ 0.99 (0.95-1.02)* for *T. denticola* IgG ‡
	□ 4 tooth with AL □ 4 mm	Cruz et al. 2005 [13]	BW: NP	306	44% < 20	Age, parity, previous periodontal therapy, alcohol, smoking, hypertension, diabetes, MS, SES, GUI	2.2 (1.3-3.5)*† 4.0 (1.6-11.1)*† for schooling level ≤ 4 years
PB	Periodontal pathogens quantification, mothers IgG seric levels and umbilical cord IgM for periodontal pathogens	Madianos et al. 2001 [14]	PB: NP	812	26.7 ± 6.4	Age, GUI, race, smoking, PMH, PC, dietary habits, MS	4.3 (2.11-8.90)* for orange complex† 2.2 (1.48-3.79)* for red complex†
	CAL, PPD, BOP, periodontal pathogens semi-quantification	Hasegawa et al. 2003 [15]	PB: NP	88	29.7 ± 4.6	Age, PMH, GUI, smoking, parity, antibiotics, BMI	SD for PPD mean, % CAL □ 3 mm SD for *T. forsythia* total numbers†
	PI, BOP, PPD, CAL > 5% with PPD □ 5mm or > 5% sites with AL □ 3mm TNF- α and IL1 - β polymorphism	Moore et al. 2004 [18]	PB: NP	130	29.4 ± 6.4	Age, ethnicity, parity, hypertension, diabetes, SES	NSD for any periodontal parameters‡
	CAL, PPD, BOP, PI	Moore et al. 2005 [17]	PB: NP	154	29.4 ± 6.3	Age, parity, diabetes, antibiotics, hypertension, SES, GUI, ethnicity	% of sites PPD ≥ 5mm was lower in cases†
PB§	AL > 5 mm in any one sextant ESI	Goepfert et al. 2004 [16]	PB: NP	139	23.9 ± 5.4	Age, ethnicity, smoking, PMH, PC	2.6* (1.1-6.2)† SD for Extent 5†
LBW PB	□ 5 sites with CAL □ 3 mm, periodontal pathogens quantification, serum IgG levels against periodontal species	Jarjoura et al. 2004 [10]	PB: UE (before 20th week) BW: NP	203	28.6 ± 6.7	Age, GUI, race, smoking, BMI, PMH, SES	PB: 2.75 (1.01-7.54)* for AL > 3 mm† LBW: 1.99 (0.73-5.45)* for AL > 3 mm‡ NSD for periodontal pathogens quantification, serum IgG levels against periodontal species‡
	Mean of PPD and CAL % PPD □ 4 and □ 5 mm % LA □ 2 and 3 mm	Moore et al. 2004 [9]	PB: UE (at 12th week) BW: NP	3738	29.9 ± 5.5	Age, GUI, race, smoking, alcohol, PMH, antibiotics, SES	NSD for LBW‡ NSD for PB‡
	% BOP, % PI, PPD mean and % PPD > 3mm	Moreu et al. 2005 [12]	PB: NP BW: NP	96	29.32 (18-40)	Age, smoking, alcohol, drugs, parity, gestational weeks	PB: 0.99* (% PPD > 3mm‡); 0.87* (PPD mean‡) LBW: 1.99* (% PPD > 3mm†); 1.04* (PPD mean‡)
LBW PB LBW or PB	□ 1 sites with PPD □ 3.5 mm, □ 4 sites with PPD □ 3.5 mm	Lunardelli & Peres, 2005 [11]	PB: NP BW: NP	449	91.3% > 19 8.7% □ 19	Age, diabetes, cardiac disease, parity, race, SES, PMH, GUI, PC, drugs, smoking, BMI, ethnicity	2.7 (0.7-9.7)* for PB‡ 2.0 (0.8-4.8)* for LBW‡ 1.5 (0.5-4.4)* for PTLBW‡
LBW or PB	ESI	Cardoso 1999 [26]	PB: NP BW: NP	287	27.3 ± 4.1	Age, diabetes, hypertension, GUI, race, smoking, alcohol, PMH, PC, SES	NSD for ESI‡
	Mean of PPD and PI, % of sites with BOP, with calculus and periodontal pathogens semi-quantification	Mitchell-Lewis et al. 2001 [5]	PB: NP BW: NP	164	16.7 ± 1.4	Age, Diabetes, GUI, race, drugs, smoking, alcohol, PMH, PC, SES	NSD for clinical parameters‡ SD for *P. nigrescens; T. forsythensis, Camplobacter rectus, E. corrodens and E. nodatum*†
	Mean of PPD, BOP, calculus, CPITN	Mokeen et al. 2004 [27]	PB: NP BW: NP	90	29.3 ± 6.6	Age, diabetes, hypertension, GUI, smoking, PMH, antibiotics, parity, PC, Previous periodontal therapy, SES	4.21 (1.99-8.93)* for mean CPITN SD for Mean PPD, mean BOP, mean calculus, mean CPITN†
	□ 1 site with PPD □ 4 mm and □ 50% BOP	Radnai et al. 2004 [29]	PB: NP BW: NP PROM: NP TPL	85	27.9	Age, diabetes, hypertension, parity, MS, PC, SES, smoking, alcohol, drugs	5.46 (1.72-17.32)* †
	□ 1 site with PPD □ 5 mm in each quadrant and red and orange clusters	Dörtbudak et al. 2005 [28]	PB: NP BW: NP	36	31.1 ± 3.4	Age, PC, smoking, diabetes, alcohol, BMI	SD for % periodontitis between groups† SD for % of orange and red clusters†
LBW and PB	ESI with a 4 mm AL threshold value, PGE-2 and IL-1β GCF mean and periodontal pathogens quantification	Offenbacher et al. 1998 [19]	PB: NP BW: NP	44	NP	Age, PMH, smoking, alcohol, GUI	SD for PGE-2 and IL-1β crevicular levels SD for red complex† NSD for PD Extension Index DP‡
	CPITN	Davenport 2002 [20]	PB: NP BW: NP	743	26.8 (16->35)	Age, hypertension, GUI, race, smoking, alcohol, PMH, PC, dietary habits, SES	0.78 (0.64-0.99)*‡

□ 4 sites with CAL and PPD □ 3mm	Moliterno et al. 2005 [21]	PB: Capurro score BW: digital scale	151	25.0 ± 6.4	Age, diabetes, hypertension, GUI, race, drugs, smoking, alcohol, PMH, PC, SES, ethnicity	3.48 (1.17-10.36)*†
ESI	Offenbacher et al. 1996 [2]	PB: UE (at 24th week) and Dubowitz examination BW: NP	124	23.5 (14-40)	Age, diabetes, hypertension, GUI, race, drugs, smoking, alcohol, PMH, PC, parity	Primiparous: 7.9 (1.50-41.1)*† Non primiparous: 7.5 (1.95-28.8)*†
PDI, PGE-2 and IL-1β levels in serum and GCF	Konopka et al. 2003 [24]	PB: NP BW: NP	84	27.5 (16-41)	Age, parity, antibiotics, PMH, GUI, smoking	**Primiparous:** 3.90 (0.93-19.14) for PDI*† and SD for PGE-2 and IL1β GCF levels and for PGE-2 level in blood serum † **Non primiparous:** 1.26 (0.53-3.06) for PDI *‡ and DS for PGE-2 and IL-1β GCF levels †
CPITN, PGE-2 and IL-1β crevicular levels	Carta et al. 2004 [23]	PB: NP BW: NP	92	NP	Age, diabetes, hypertension, PMH, smoking, alcohol, GUI, PC	CPITN = 4: controls = 4.3%, cases=40% SD for PGE-2 and IL-1β †
PPD, BOP and PI mean, % of sites with BOP and plaque and periodontal pathogens semi-quantification	Buduneli et al. 2005 [25]	PB: LMP BW: NP	181	24.9 ± 4.9 (18-35)	Age, diabetes, hypertension, parity, smoking, GUI, Previous periodontal therapy, SES	NSD for periodontal clinical parameters‡ 0.35* for *P. nigrescens*† 0.18* for *A. actinomycetemcomitans*† 3.82* for *P. micros*† 7.15* for *C. rectus*†
PPD, LA, % sites AL ≥ 3 mm, BOP, IP mean, IL-1β crevicular and periodontal pathogens semi-quantification	Noack et al. 2005 [26]	PB: NP BW: NP	59	27.8-30.3	Age, BMI, GUI, diabetes, parity, smoking, drugs, alcohol, PMH, stress, SES, PC, antibiotic	NSD for any periodontal parameters‡ 0.73 (0.13-4.19)*‡

L**BW**: low birth weight; **PB:** preterm birth (< 37 weeks of gestation); **PB§**: preterm birth (< 32 weeks of gestation); **PROM:** premature rupture of membranes; **TPL:** threatened preterm labor; **CPITN**: Community Periodontal Index of Treatment Needs; **ESI**: Extension and Severity Index; **Ig**: Imunoglobulin; **PPD**: periodontal pocket depth; **CAL**: clinical attachment level; **LA:** loss of attachment; **BOP:** bleeding on probing; **PI:** plaque index; **PGE-$_2$**: prostaglandin E-$_2$; **IL-1β**:interleukin-1β; **GCF:** gingival crevicular fluid; **PDI**: Periodontal Disease Index; **UE**: ultrasound examination; **N.P.**: data not presented; **SES**: socio-economic status; **GA**: gestational age; **GUI**: genito-urinary infection; **PMH**: pregnancy medical history; **PC**: prenatal care; **MS**: marital status; **BMI:** body mass index; **OR**: Odds Ratio; **SD**(†): significant difference among groups ($P \leq 0.05$); **NSD**(‡): lack of difference among groups.

Table 2. Characteristics of cohort studies about the relationship between periodontal disease and undesirable pregnancy outcomes

Outcome	Parameter for periodontal disease	Author / year	PB and BW assessment	Sample size	Mean age	Variables controlled	OR*, RR**, SD(†), NSD(‡)
PB	Localized: □ 3 sites with AL □ 3 mm Generalized: 90% of sites or more with AL of 3 mm or more	Jeffcoat et al. 2001 [31]	PB: NP	1313	91% <30	Age, race, PMH, antibiotics, hypertension, PC	4.45 (2.16-9.18)***GA** < 37weeks† 5.28 (2.05-13.6)* **GA**<35weeks† 7.07 (1.70-27.4)* **GA**<32weeks†
	PPD □ 4mm in Ramfjord teeth	Holbrook et al. 2004 [33]	PB: UE (at18th -19th week)	96	NP	Age, parity, race, GUI, smoking, antibiotics, PMH	NSD for □ 4 PPD □ 4 mm‡
LBW PB	Gingivitis: BOP > 5% without □ 2 sites with CAL > 6mm and < 2 sites with PPD □ 5mm Periodontitis: □ 1 site with PPD □ 5mm and □ 2 sites with CAL > 6mm and BOP > 5%	Marin et al. 2005 [34]	PB: NP	152	23.3 ± 5.7	Age, smoking, diabetes, alcohol, SES, race, Previous periodontal therapy	SD for infant birth weight between healthy and periodontitis for women > 25 years old† NSD for % of preterm among groups‡
LBW or PB	□ 4 tooth with 1 or more sites with PPD=4 mm and AL=3 mm in the same site	López et al. 2002 [30]	PB: UE (NP), LMP, SPE/PNE, Ballard neonatal assessment	639	25.0 ± 4.5	Age, GUI, smoking, PMH, PC, MS, SES	3.5 (1.7-7.3)**†
LBW and PB	Moderate to severe: □ 4 sites with at least PPD=5 mm and AL=2mm Moderate: 1 to 4 sites with PPD > 3 mm and AL >2 mm Progression/Incidency: □ 4 sites with increasing PPD of □ 2 mm.	Offenbacher et al. 2001 [32]	PB: UE (before 20th weeks) pelvic exam BW: recorded in delivery or in the neonatal intensive care	812	26.7 ± 6.4	Age, GUI, race, smoking, PMH, PC, dietary habits, MS	PD moderate to severe: 2.23**† PD moderate: 1**‡ PD progression DP: 1.4**†
	Mean PPD, PI and BOP greater than the median	Rajapakse et al. 2005 [35]	PB: UE (NP)	227	24.2 ± 4.2	Age, smoking, diabetes, alcohol, SES, race, hypertension, Previous periodontal therapy, PC	1.9 (0.7-5.4)* ‡

L**BW**: low birth weight; **PB:** preterm birth; **AL**: attachment loss; **PPD**: periodontal pocket depth; **NP**: data not presented; **UE**: ultrasound examination; **SPE/PNE**: sequential physical examinations and post-natal examination; **LMP**: last menstrual period; **PMH**: pregnancy medical history; **PC**: prenatal care; **GUI**: genito-urinary infection; **MS**: marital status; **SES**: socio-economic status; **OR:** Odds Ratio; **SD(†)**: significantt difference among groups ($P \leq 0.05$); **NSD(‡)**: lack of difference among groups ($P > 0.05$); **SRP**: scaling and root planing.

Table 3. Characteristics of clinical trial studies about the relationship between periodontal disease and undesirable pregnancy outcomes

Outcome	Parameter for PD	Author / year	PB and BW assessment	Sample size	Mean age	Variables controlled	Intervention	OR*, SD(†), NSD(‡)
PB LBW LBW or PB	□ 4 tooth with 1 or more sites with PPD=4 mm and AL=3 mm in the same sites	López et al. 2002 [36]	PB: UE (9th to 24th weeks), LMP, SPE/PNE,	400	27.5 ± 4.4	Age, GUI, smoking, PMH, PC, MS, antibiotics, SES	SRP + plaque control instruction	PB: 5.48 (1.17-27.71) *† LBW: 6.26 (0.73-53.78) *† LBW or PB: 5.49 (1.65-18.22) *†
LBW or PB	PPD and Plaque index mean, % of sites with BOP, with calculus and periodontal pathogens semi-quantification	Mitchell-Lewis et al. 2001 [5]	PB: NP BW: NP	164	16.7 ± 1.4	Age, diabetes, GUI, race, drugs, smoking, alcohol, PMH, PC, SES	SRP + plaque control instruction	ID = 28.6% ‡
PB	> 3 sites with AL □ 3 mm	Jeffcoat et al. 2003 [37]	PB: UE (NP) and last menstrual period	366	22.5 ± 4.6	Age, GUI, race, MS, smoking, PMH, Previous periodontal therapy, antibiotics, mouthrinses use, body mass index,	SRP+placebo SRP+ metronidazole	**age<37weeks gestational** SRP+placebo: 0.5 (0.2-1.3)* ‡ SRP+ metronidazole: 1.4 (0.7-2.9)* ‡ **age<35weeks gestational** SRP+placebo: 0.2 (0.02-1.4)* ‡ SRP+ metronidazole: 0.7 (0.2-2.4)* ‡

L**BW**: low birth weight; **PB:** preterm birth; **PPD**: periodontal pocket depth; **AL**: attachment loss; **BOP:** bleeding on probing; **UE**: ultrasound examination; **SPE/PNE**: sequential physical examinations and post-natal examination; **NP**: data not presented; **GUI**: genito-urinary infection; **PMH**: pregnancy medical history; **PC**: prenatal care; **MS**: marital status; **SES**: socio-economic status; **SRP**: scaling and root planing; **OR:** Odds Ratio; **ID**: Incidence difference; **SD(†)**: significant difference among groups ($P \leq 0.05$); **NSD(‡)**: lack of difference among groups ($P > 0.05$).

5 – Artigo II

Periodontal Inflammatory Load (PIL): a new periodontal epidemiologic method.

Mario Vianna Vettore[1,2], Anna Thereza Leão[3], Magda Feres[4], Maria do Carmo Leal[1], Luciene Cristina de Figueiredo[4], Aubrey Sheiham[2]

1 – Department of Epidemiology and Quantitative Methods in Health, National School of Public Health, Oswaldo Cruz Foundation (ENSP/FIOCRUZ) – RJ, Brazil

2 - Department of Epidemiology and Public Health, University College London, London, England

3 – Department of Dental Clinic, Federal University of Rio de Janeiro (UFRJ) – RJ, Brazil

4 – Department of Periodontology, Guarulhos University - SP, Brazil

Submetido em Maio de 2006 ao Journal of Clinical Periodontology

Running title: Periodontal Inflammatory Load - PIL

Key words: periodontal diseases, periodontitis, epidemiologic method

Clinical Relevance:

Scientific rationale for study: In epidemiological studies periodontal disease is conventionally assessed using periodontal indices or clinical parameters. Those measures have shortcomings for assessing periodontal status. This study tested a new approach, the Periodontal Inflammatory Load (PIL), which is a continuous variable measuring the sum of periodontal pocket depths of 4 mm at sites with a clinical attachment level 4mm. **Principal findings**: PIL was shown to be a valid measure of periodontal condition due to its strong association with periodontal pathogens. **Practical implications**: PIL avoids bias on the assessment of periodontal status in studies of periodontal disease.

Abstract

Aim: To describe and validate an epidemiologic method for periodontal disease assessment named the Periodontal Inflammatory Load.

Material and Methods: Clinical parameters were recorded in 116 women at 6 sites per tooth for all teeth excluding third molars. The Periodontal Inflammatory Load (PIL) was the sum of all pocket depths measurements 4 mm in sites with Clinical Attachment Level 4 mm. Two subgingival plaque samples per subject were collected and analysed for 40 bacterial species using the Checkerboard DNA-DNA hybridization technique. The data was analysed using Spearman linear correlation coeficient and the Kruskal-Wallis test. **Results:** Periodontal Inflammatory Load scores were strongly correlated with periodontal pathogens from the red complex, *Porphyromona gingivalis*, *Tannerella forsythia* and *Treponema denticola*, individually or grouped ($p<0.05$); and PIL was not associated with beneficial species from the yellow, green, purple and blue complexes ($p>0.05$). The red complex was the only complex that showed a significant increase in proportions according to the quartile groups of distribution of the Periodontal Inflammatory Load (from 1 to 4). There was also a significant positive relationship between these quartiles and mean counts of red and orange complexes.

Conclusions: Periodontal Inflammatory Load appears to be a valid measure of periodontal status.

5.1 - Introduction

Most of the periodontal indices commonly used in epidemiologic studies, such as the Periodontal Index (Russell, 1956), the Periodontal Disease Index (Ramfjord 1959), the Community Periodontal Index of Treatment Need (CPITN) and the Community Periodontal Index (CPI) (Ainamo et al. 1982, WHO 1997) have limitations in the assessment of periodontal status. The Extent and Severity Index (Carlos et al. 1986) and partial mouth recording protocols (Albandar & Kingman 1999) also do not provide a detailed estimation of the periodontal condition.

Therefore, recent studies in periodontal medicine have used full-mouth measurements of pocket depth and attachment level to describe the periodontal condition of a given population (Buduneli et al. 2005, Hasegawa et al. 2003, Moore et al. 2004). This kind of assessment has lead to the creation of a wide range of cut-off points for the frequency of deep pockets and/or sites with attachment loss to assess associations between periodontal status and systemic conditions (Offenbacher et al. 1996, Radnai et al. 2004, Dörtbudak et al. 2005, Moliterno et al. 2005, López et al. 2002). For instance, studies evaluating the relationship between undesirable pregnancy outcomes and periodontitis have used thirteen different measures and cut-off points to characterize periodontal disease, indicating a lack of standardization on the definition of a person with periodontal disease (Vettore et al. 2006).

The establishment of periodontal status based exclusively on clinical attachment loss could represent a problem, since this parameter does not take into account the extent of current inflammation of the periodontal tissues and might reflect only the history of disease. Socransky et al. (1984) have suggested that the use of periodontal attachment loss alone to characterize disease can result in classification bias since in many cases there is no periodontal disease activity at sites with loss of attachment but without deepening of the pocket. The problem with this methodology is the inclusion of subjects with a healthy periodontium as a “case”. This would occur if certain sites considered to have disease were actually isolated areas of gingival recession due to other reasons, such as traumatism of the gum from tooth brushing. This kind of methodological problem has generated conflicting results, especially in studies that deal with younger populations, such as those on the relationship between periodontal disease and preterm low birth weight (Lopez et al. 2002, Moore et al. 2004, Offenbacher et al. 1996).

Furthermore, even those studies that took into consideration different thresholds of periodontal pocket depth and/or clinical attachment level measures as the diagnostic

criteria for periodontal disease usually find no correlation between these parameters and the extent of periodontal inflammation. Thus, the periodontal disease "cases" might not represent subjects with high levels of inflammation.

Valid clinical indices to determine periodontal disease status should be associated with specific periodontal pathogens such as the microorganisms of the red complex, *Porphyromonas gingivalis*, *Treponema denticola*, and *Tanerella forsythia* (Socransky et al. 1998). It has been shown by several authors that these species are associated with periodontal tissue breakdown and active lesions of destructive periodontal disease (Klein & Gonçalves 2003, Mayanagi et al. 2004, Kuboniwa et al. 2004, Haffajee et al. 2005).

Conversely, a valid periodontal measure should not be related to bacterial species considered to be beneficial or host-compatible, such as those from the yellow (*Streptococcus spp*), purple (*V. parvula and Actinomyces odontolyticus)* and blue complexes (*Actinomyces spp).* These species are usually found in inactive and healthy periodontal sites (Haffajee et al. 1991, Dzink et al. 1988, Socransky et al. 1998, Socransky & Haffajee 2002).

The present study aimed to test a new epidemiological approach to determine the extent of the periodontal inflammation, the Periodontal Inflammatory Load (PIL),

5.2 - Methods

Experimental design

The study was approved by the Committee of Ethics and Research of the National School of Public Health - Oswaldo Cruz Foundation (FIOCRUZ, RJ, Brazil - protocol no. 78/02). Subjects were informed that they were free to withdraw from the study.

Periodontal measurements were recorded in 116 women with at least 30 years of age presenting at least 15 natural teeth and selected from public maternity hospitals at the city of Rio de Janeiro (RJ, Brazil). Subjects received clinical and microbiological assessment in one appointment of approximately one hour.

Clinical Assessment

Clinical attachment level (CAL) and periodontal pocket depth (PPD) measures were obtained at 6 sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual) for all teeth excluding third molars (Haffajee et al. 1997). Periodontal Pocket Depth (PPD) measures were recorded in millimeters from the free gingival margin to the depth of the gingival sulcus or periodontal pocket (Armitage

1995). Clinical attachment level (CAL) measures were recorded in millimeters from the cemento enamel junction to the depth of the gingival sulcus or periodontal pocket (Armitage 1995). The measures were recorded to the nearest higher millimetre using the North Carolina periodontal probe (Hu-Friedy®, Chicago, IL, USA), 15 mm in length and 0.35 mm in diameter. This evaluation was done by 6 calibrated examiners. Kappa test and Intraclass Correlation Coefficient of agreement for periodontal pocket depth were, respectively, □ 0.78 and □ 0.72 for intra-examiner, and □ 0.77 and □ 0.72 for inter-examiner.

Description of the Periodontal Inflammatory Load measure

PPD and CAL are continuous variables, which can be expressed as a measurement vector [x_1, x_2, x_3, x_4, … x_n] where xi is the value, in millimeters, and n is the number of sites to be measured for each individual examined.

Measurements of PPD ≥ 4 mm in sites with CAL ≥ 4 mm were summed and the vector [di] was generated as follows:

di = xi if xi ≥ 4 (xi is a measurement of PPD in millimetres in sites with CAL ≥ 4 mm).

$$\text{PIL} = \sum_{i=1}^{n} di$$

PIL (Periodontal Inflammatory Load) is expressed as the sum of all PPD ≥ 4 mm in sites with CAL ≥ 4 mm.

The PIL can be used as a continuous or a categorical measure. To use PIL as a categorical measure, the sample population should be evaluated into subgroups according to the quartiles of distribution of PIL in the total population (*See Statistical Analysis*).

Microbiological Assessment

Microbiological assessment was conducted following the clinical examination. After removing the supragingival plaque, 2 biofilm samples were collected with individual sterile Gracey curettes (Hu-Friedy®, Chicago, IL, USA) from each woman from the deepest periodontal sites, located in different teeth. When the subject had no periodontal pockets the biofilm samples were collected from two random sites in different quadrants of the mouth. The samples were stored in eppendorf tubes containing 150 µl of TE (10mM Tris-HCl, 1 mM EDTA, pH 7.6). 0.15 ml of 0.5 M NaOH was immediately added to each tube and the samples were dispersed using a

vortex mixer. These tubes were stored –at 2^0C and transported to the laboratory of Oral Microbiology of Guarulhos University, São Paulo, Brazil. Samples were individually analyzed for their content of 39 bacterial species using a modification (Haffajee et al., 1997) of the checkerboard DNA-DNA hybridization technique (Socransky et al. 1994). In brief, samples were boiled for 10 minutes and neutralized using 0.8 ml of 5 M ammonium acetate. The released DNA was then placed into the extended slots of a Minislot-30 apparatus (Immunetics, Cambridge, MA, USA), concentrated onto a 15x15 cm positively charged nylon membrane (Boehringer Mannheim, Indianapolis, IN, USA) and fixed to the membrane by baking at 120º C for 20 min. The membrane was placed in a Miniblotter 45 (Immunetics, Cambridge, MA, USA) with the lanes of DNA at 90º to the lanes of the device. Digoxigenin-labeled whole genomic DNA probes to 39 subgingival species were hybridized in individual lanes of the Miniblotter 45. After hybridization, the membranes were washed at high stringency and the DNA probes detected using antibody to digoxigenin conjugated with alkaline phosphatase and chemiluminescence detection. Two lanes in each run contained standards at concentrations of 10^5 and 10^6 cells of each species. The sensitivity of the assay was adjusted to permit detection of 10^4 cells of a given species by adjusting the concentration of each DNA probe.

Table 1 presents the 39 species evaluated in this study. The species are presented according to the microbial complexes described by Socransky et al. in 1998. Some bacterial species which were not associated with any complex and the DNA probes for new species were grouped together and were represented in the Figures by the grey colour.

Assessing the Validity of PIL

The *proxy* measure for inflammation of periodontal disease should represent the periodontal pathogen load. Therefore, to consider PIL as a clinical measure of inflammation of periodontal disease, it should be related only with the counts and proportions of periodontal pathogens in separated and aggregated analysis. In addition, PIL should not be associated with bacterial species considered to be beneficial or host compatible.

Statistical Analysis

The analysis was done in two stages. First, the PIL measure was examined as a continuous variable. The associations of PIL measures, mean PPD, frequency of PPD □

4 mm, frequency of PPD 5 mm, mean CAL, frequency of CAL 3 mm and frequency of sites with PPD 4 mm and CAL 4 mm, with counts of microbial complexes and counts of periodontal pathogens were examined by nonparametric Spearman linear correlation coefficients. Second, the PIL measures were divided into 4 groups according to PIL's quartiles of distribution as following: Level 1: 0 to 7 mm; Level 2: 8 to 45 mm; Level 3: 45 to 100 mm and level 4: 101 mm or more.

In order to compare the counts of each bacterial complex and individual periodontal pathogens, the data were expressed as counts times 10^6, averaged within a subject and then across subjects. Significance of differences between groups in mean counts of bacterial complexes and periodontal pathogens were analyzed using Kruskal-Wallis test. The total DNA probe count was also computed at each sampled site in each subject and the proportion of each bacterial complex was determined. The Kruskal-Wallis test was also used to compare the means of proportions of different microbial complexes (Socransky et al. 1998, Table 1) in subgingival biofilm samples among the 4 quartile groups of the distribution of PIL. The significance level established for all analysis was 5% (p 0.05).

5.3 - Results

The clinical features of the subjects are presented in Table 2. The mean age of the tested population was 34.1 ± 3.6 years. The correlation (Spearman coefficient) between microbial complexes and different categories of PPD, CAL and Periodontal Inflammatory Load (PIL) is shown in Table 3. A significant association was observed between the red complex and three of the measurements evaluated, PIL, frequency of PPD 4mm and frequency of PPD 4mm and CAL 4mm ($p \leq 0.01$).

Full-mouth mean PPD was associated with purple and yellow microbial complexes ($p < 0.05$). Negative correlations between frequency of PPD 5mm and blue, yellow and green complexes, and the group formed by other species and new DNA probes (grey group) were also observed ($p < 0.05$). Full-mouth mean CAL and frequency of CAL 3 mm were positively correlated with purple, yellow and orange microbial complexes.

The correlations between the individual members of the red complex, *P. gingivalis*, *T. denticola*, *T. forsythia* and periodontal disease measures are presented in Table 4. PIL and frequency of PPD 4 mm were significantly associated with all species of the red complex. The frequency of CAL 3 mm was positively correlated with *T. forsythia*.

The comparisons of mean counts and mean proportions of each microbial complex in subgingival plaque samples among the 4 quartile groups of distribution of PIL are shown in Figures 1 and 2, respectively. The differences in mean counts of orange and red complexes were significant among the different groups of distribution of PIL. As the PIL measure of periodontal disease increased (from level 1 to Level 4), the mean counts of red complex species also increased. The mean proportions of red complex in the four Periodontal Inflammatory Load groups were significantly different ($p < 0.01$). The proportion of this microbial group of pathogens progressively increased from Levels 1 to 4 of the PIL. The PIL Levels 3 and 4 showed higher proportions of orange complex and lower proportions of blue complex, compared with PIL groups Levels 1 and 2. However, these differences were not statistically significant.

5.4 - Discussion

There is a lack of standardization in the assessment of the periodontal disease status in epidemiological studies. This fact creates a problem on the evaluation of data from different research protocols, especially those studies on the relationship between periodontal and systemic diseases. Therefore, the present study tested the reliability of a new measure, the Periodontal Inflammatory Load (PIL), to determine the periodontal disease condition in epidemiological studies. The PIL measure is the sum of all periodontal pocket values equal or greater to 4 mm in sites with CAL □ 4 mm. The logic of this approach is that by including all potentially inflamed sites in the assessment, the PIL value should represent the extent of the periodontal inflammatory 'wound' the mouth.

The validity of the method was demonstrated by the strong association between PIL values and the periodontal pathogens from the red complex, *P. gingivalis*, *T. denticola* and *T. forsythia,* by isolated and aggregated analysis. The levels of these pathogens, individually or grouped, were significantly associated with PIL measurements (Tables 3 and 4). Moreover, the red complex was the only group of bacteria that showed statistical significant differences in proportions among the 4 quartile groups of distribution of the PIL. It was interesting to observe that mean proportions of this complex consistently grew with the increase in the degree of periodontal disease determined by the PIL levels from 1 to 4 (Figure 2). In terms of total counts of the microbial complexes there was a positive relationship between PIL levels, red and orange complexes. This is an important observation since red complex harbors the 3 most important periodontal pathogens and several species considered to be

possible pathogens belong to the orange complex (Figure 1). The lack of an association between host-compatible species from the yellow, green, purple and blue complexes and PIL values was also a relevant finding, and suggests that the measure is a valid measure to assess periodontal inflammation.

Misclassification of the periodontal status is commonly observed in studies of periodontal medicine. For example, up to thirteen different methods have been used in studies of periodontal disease and preterm low birth weight (Vettore et al. 2006). Some studies used cut-offs for periodontitis diagnosis which varied in terms of the number of teeth/sites involved, the clinical measurements used and the values used as thresholds. The risk of misclassification increases when the assessment of periodontal disease uses only loss of attachment measurements, which is considered an appropriate measure of periodontal status in terms of cumulative periodontal destruction over time. However, this parameter alone might reflect only gingival recession without periodontal infection. Some of the studies that used CAL to define periodontal infection did not show an association between red complex or other periodontal pathogens and the criteria employed, such as: 5 sites with CAL 3 mm (Jarjoura et al. 2005); 60% sites with CAL 3 mm (Offenbacher et al. 1996); or No disease: < 3 sites with CAL 3 mm, Periodontitis: 3 sites with CAL 3 mm, Generalized periodontal disease: 90 of sites with CAL 3 mm (Jeffcoat et al. 2001).

It is important to note that in the present study the parameter "% PPD ≥ 4 mm" also had a positive correlation with total counts of red complex pathogens, individually (Table 4) or as a group (Table 3), a similar pattern observed for the PIL.

However, the shortcoming in using the frequency of PPD 4 mm for periodontal status assessment is the fact that patients with the same frequency of PPD 4 mm can present different levels of periodontal disease, depending on the depth of these sites. With the PIL measure it is possible to distinguish the severity of periodontal disease in patients with the same frequency of PPD 4 mm, since the PIL measure is the sum of PPD ≥ 4 mm in sites with CAL 4 mm. So the frequency of PPD ≥ 4 mm refers to the extension of the disease and does not include information about severity. On the other hand, PIL refers to the extension and severity of periodontal disease.

In conclusion, the PIL appears to be a valid epidemiologic measure of periodontal disease status. Further studies testing different populations and evaluating greater numbers of plaque samples and subjects will be conducted to confirm the validity of the PIL measure.

5.5 - References

Ainamo, J., Barmes, D., Beagrie, G., Cutress, T., Martin, J. & Sardo-Infirri, J. (1982) Development of the World Health Organization (WHO) community periodontal index of treatment needs (CPITN). *International Dental Journal* **32**, 281-91.

Albandar, J. M. & Kingman, A. (1999) Gingival recession, gingival bleeding, and dental calculus in adults 30 years of age and older in the United States, 1988-1994. *Journal of Periodontology* **70**, 30-43.

Armitage, G. C. (1995) Clinical evaluation of periodontal diseases. *Periodontology 2000* **7**, 39-49.

Buduneli, N., Baylas, H., Buduneli, E., Turkoglu, O., Kose, T. & Dahlen, G. (2005) Periodontal infections and pre-term low birth weight: a case-control study. *Journal of Clinical Periodontology* **32**, 174-81.

Carlos, J. P., Wolfe, M. D. & Kingman A. (1986) The extent and severity index: a simple method for use in epidemiologic studies of periodontal disease. *Journal of Clinical Periodontology* **13**, 500-505.

Dörtbudak, O., Eberhardt, R., Ulm, M. & Persson, G. R. (2005). Periodontitis, a marker of risk in pregnancy for preterm birth. *Journal of Clinical Periodontology* **32**, 45-52.

Dzink, J. L., Socransky, S. S. & Haffajee, A. D. (1988) The predominant cultivable microbiota of active and inactive lesions of destructive periodontal diseases. *Journal of Clinical Periodontology* **15**, 316-23.

Haffajee, A. D., Japlit, M., Bogren, A., Kent, R. L, Jr., Goodson, J. M. & Socransky, S. S. (2005) Differences in the subgingival microbiota of Swedish and USA subjects who were periodontally healthy or exhibited minimal periodontal disease. *Journal of Clinical Periodontology* **32**, 33-9.

Haffajee, A. D., Cugini, M. A., Dibart, S., Smith, C., Kent, R. L. Jr. & Socransky, S. S. (1997) The effect of SRP on the clinical and microbiological parameters of periodontal diseases. *Journal of Clinical Periodontology* **24**, 324-34.

Haffajee, A. D., Socransky, S. S., Smith, C. & Dibart, S. (1991) Relation of baseline microbial parameters to future periodontal attachment loss. *Journal of Clinical Periodontology* **18**, 744-50.

Hasegawa, K., Furuichi, Y., Shimotsu, A., Nakamura, M., Yoshinaga, M., Kamitomo, M., Hatae, M., Maruyama, I. & Izumi, Y. (2003) Associations between systemic status, periodontal status, serum cytokine levels, and delivery outcomes in

pregnant women with a diagnosis of threatened premature labor. *Journal of Periodontology* **74**,1764-70.

Jarjoura, K., Devine, P. C., Perez-Delboy, A., Herrera-Abreu, M., D'alton, M. & Papapanou, P. N. (2005) Markers of periodontal infection and preterm birth. *American Journal Obstetrics and Gynecology* **2**, 513-9.

Jeffcoat, M. K., Geurs, N. C., Reddy, M. S., Cliver, S. P., Goldenberg, R. L. & Hauth, J. C. (2001) Periodontal infection and preterm birth: results of a prospective study. *Journal of American Dental Association* **132**, 875-80.

Klein, M. I. & Goncalves, R. B. (2003) Detection of *Tannerella forsythensis* (*Bacteroides forsythus*) and *Porphyromonas gingivalis* by polymerase chain reaction in subjects with different periodontal status. *Journal of Periodontology* **74**, 798-802.

Kuboniwa, M., Amano, A., Kimura, K. R., Sekine, S., Kato, S., Yamamoto, Y., Okahashi, N., Iida, T. & Shizukuishi, S. (2004) Quantitative detection of periodontal pathogens using real-time polymerase chain reaction with TaqMan probes. *Oral Microbiology and Immunology* **19**, 168-76.

López, N. J., Smith, P. C. & Gutierrez, J. (2002) Higher risk of preterm birth and low birth weight in women with periodontal disease. *Journal of Dental Research* **81**, 58-63.

Mayanagi, G., Sato, T., Shimauchi, H. & Takahashi, N. (2004) Detection frequency of periodontitis-associated bacteria by polymerase chain reaction in subgingival and supragingival plaque of periodontitis and healthy subjects. *Oral Microbiology and Immunology* **19**, 379-85.

Moliterno, L. F., Monteiro, B., Figueredo, C. M. & Fischer, R. G. (2005). Association between periodontitis and low birth weight: a case-control study. *Journal of Clinical Periodontology* **32**, 886-90.

Moore, S., Ide, M., Coward, P. Y., Randhawa, M., Borkowska, E., Baylis, R, & Wilson, R. F. (2004) A prospective study to investigate the relationship between periodontal disease and adverse pregnancy outcome. *British Dental Journal* **197**, 251-8.

Offenbacher, S., Katz, V., Fertik, G., Collins, J., Boyd, D., Maynor, G., McKaig, R. & Beck, J. (1996) Periodontal infections as a risk factor for preterm low birth weight. *Journal of Periodontology* **67**, 1103-1113.

Radnai, M., Gorzó, I., Nagy, E., Urbán, E., Novák, T. & Pál, A. (2004). A possible association between preterm birth and early periodontitis. *Journal of Clinical Periodontology* **31**, 736-41.

Ramfjord, S. P. (1959) Indices for prevalence and incidence of periodontal disease. *Journal of Periodontology* **30**, 51-59.

Russell, A. L. (1956) A system of classification and scoring for prevalence surveys of periodontal disease**.** *Journal of Dental Research* **35**, 350-359.

Socransky, S. S. & Haffajee, A. D. (2002) Dental biofilms: difficult therapeutic targets. *Periodontology 2000* **28,**12-55.

Socransky, S. S., Haffajee, A. D., Goodson, J. M. & Lindhe, J. (1984) New concepts of destructive periodontal disease. *Journal of Clinical Periodontology* **11**, 21-32.

Socransky, S. S., Haffajee, A. D., Cugini, M. A., Smith C. & Kent Jr., R. L. (1998) Microbial complexes in subgingival plaque. *Journal of Periodontology* **25**, 134-144.

Socransky, S. S., Smith, C. M., Martin, L., Paster, B. J., Dewhirst, F. E. & Levin, A. E. (1994) "Checkerboard" DNA-DNA hybridization. *Biotechniques* **17**, 788-792.

Vettore, M. V., Lamarca, G. A., Leão, A. T. T., Thomaz, F. B., Sheiham, A. & Leal, Mdo C. (2006) Periodontal infection and undesirable pregnancy outcomes: a systematic review of epidemiologic studies. *Cadernos de Saude Publica* **In Press**.

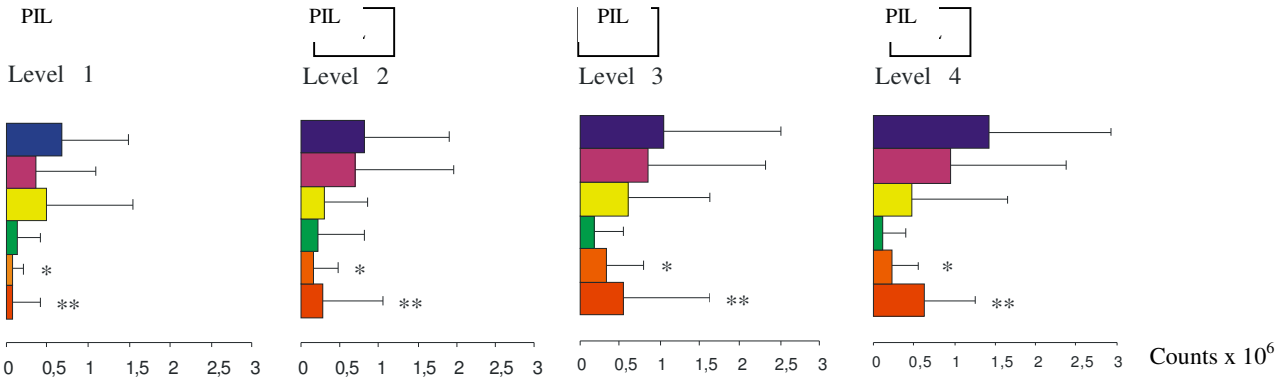
World Health Organization. Oral Health Surveys. Basic Methods. Geneva 1997: World Health Organization.

Acknowledgments

The authors thank Izilvania Barreto (Guarulhos University) for the laboratory analysis. This investigation was supported in part by CNPq, CAPES, FAPERJ Grant E-26/170.421/2003, Hu-friedy do Brasil and Pan American Health Organization (PAHO/WHO).

5.6 – Figuras e Tabelas

Figure 1. Mean counts (X 10^6) of different bacterial complexes in subgingival plaque samples of the 4 quartile groups of distribution of the Periodontal Inflammatory Load (PIL).



The bars represent the mean counts of each microbial complex described by Socransky et al. (1998). The different colors correspond to the colors of the complexes (See Table 1). Significance of differences in mean proportions between groups mean values for each complex was tested using the Kruskal-Wallis test. * $p < 0.05$ ** $p < 0.01$

PIL (Periodontal Inflammatory Load. Sum of all pockets $\geq$ 4 mm in sites with Clinical Attachment Level $\geq$ 4 mm):

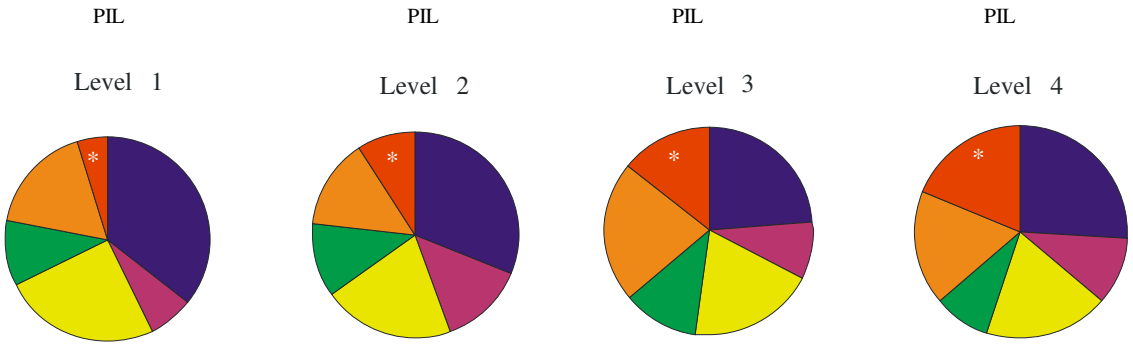
Level 1: 0 to 11 mm

Level 2: 12 to 55 mm

Level 3: 56 to 113 mm

Level 4: 114 mm and over

Figure 2. Pie charts of the mean proportions of each microbial complex in subgingival plaque samples from subjects in the 4 quartile groups of distribution of the Periodontal Inflammatory Load (PIL).



The colors in the pie diagrams correspond to colors of microbial complexes described by Socransky et al. (1998). Significance of differences in mean proportions between groups mean values for each complex was tested using the Kruskal-Wallis * $p < 0.01$ The different Periodontal Inflamatory Load Levels are described in Figure 1.

Table 1. Bacterial strains employed for the development of DNA probes.

		Campylobacter showae	51146
Blue complex			
Actinomyces gerencseriae	23860	*Eubacterium nodatum*	33099
Actinomyces israelii	12102	*Fusobacterium nucleatum sp. nucleatum*	25586
Actinomyces naeslundii genospecies 2	43146	*Fusobacterium nucleatum sp. polymorphum*	10953
		Fusobacterium nucleatum sp. vicentii	49256
Purple complex		*Fusobacterium periodonticum*	33693
Actinomyces odontolyticus	17929	*Micromonas micros*	33270
Veillonella parvula	10790	*Prevotella intermedia*	25611
		Prevotella nigrescens	33563
Yellow complex		*Streptococcus constellatus*	27823
Streptococcus gordonii	10558		
Streptococcus intermedius	27335	**Red complex**	
Streptococcus mitis	49456	*Tannerella forsythia*	43037
Streptococcus oralis	35037	*Porphyromonas gingivalis*	33277
Streptococcus sanguinis	10556	*Treponema denticola*	B1
Green complex		**Other species and new DNA probes**	
Actinobacillus actinomycetencomitans	43718		
serotypes a and *b*	29523	*Eubacterium saburreum*	33271
Capnocytophaga gingivalis	33624	*Gemella morbillorum*	27824
Capnocytophaga ochracea	33598	*Leptotrichia buccalis*	14201
Capnocytophaga sputigena	33612	*Neisseria mucosa*	19696
Eikenella corrodens	23834	*Prevotella melaninogenica*	25845
		Propionybacterium acnes I and II	11827 and 11828
Orange complex		*Selenomonas noxia*	43541
Campylobacter gracilis	33236	*Streptococcus anginosus*	33397
Campylobacter rectus	33238	*Treponema socranskii*	S1

All strains were obtained from the American Type Culture Collection (ATCC), (Rockville, MD), except *Treponema denticola* (B1) and *Treponema socranskii* (S1) which were obtained from the Forsyth Institute (Boston, MA). Microbial complexes were described by Socransky et al. (1998) and Socransky & Haffajee (2002). Probe from one species of the blue complex, *Actinomyces naeslundii genospecies 1*, was not included in this study.

Table 2. Clinical characteristics of subject group (n = 116).

	Mean (± SD)	Range
Number of teeth	23.3 ± 3.8	15 - 28
mean PPD (mm)	2.4 ± 1.0	1.0 – 4.8
mean CAL (mm)	2.5 ± 0.7	1.0 – 5.7
% sites with:		
VPI	52 ± 43	0 – 100
BOP	20 ± 28	0 - 100
Calculus	10 ± 22	0 - 100
PPD < 4 mm	88.1 ± 13.5	8 - 100
PPD 4-6 mm	11.8 ± 13.3	0 – 89
PPD > 6 mm	0.10 ± 0.6	0 - 11
CAL < 4 mm	85.0 ± 16.9	0 – 100
CAL 4-6 mm	14.6 ± 16.3	0 – 100
CAL > 6 mm	0.3 ± 1.5	0 - 24

VPI: Visible Plaque Index, BOP: Bleeding on Probing, PPD: Periodontal Pocket Depth, CAL: Clinical Attachment Level

Table 3. Correlation matrix of Periodontal Inflammatory Load (PIL) and clinical parameters of periodontal disease with mean counts of different microbial complexes.

Clinical parameters	**Microbial complexes (Total Counts)**						
	Blue complex	**Purple complex**	**Yellow complex**	**Green complex**	**Orange complex**	**Red complex**	**Other species and new DNA probes**
PIL	-0.094 (*p*=0.314)	0.035 (*p*=0.706)	-0.009 (*p*=0.924)	-0.038 (*p*=0.682)	-0.038 (*p*=0.682)	**0.289 (*p*=0.002)***	-0.019 (*p*=0.839)
Mean of PPD	0.048 (*p*=0.613)	**0.233 (*p*=0.012)***	**0.240 (*p*=0.010)***	0.074 (*p*=0.428)	0.178 (*p*=0.056)	0.161 (*p*=0.083)	0.072 (*p*=0.444)
% PPD □ 4 mm	-0.064 (*p*=0.494)	0.072 (*p*=0.441)	0.027 (*p*=0.770)	-0.016 (*p*=0.865)	0.175 (*p*=0.060)	**0.308 (*p*=0.001)***	0.010 (*p*=0.917)
% PPD □ 5 mm	**-0.214 (*p*=0.021)***	-0.178 (*p*=0.055)	**-0.333 (*p*<0.01)***	**-0.240 (*p*=0.009)***	-0.047 (*p*=0.615)	0.147 (*p*=0.115)	**-0.256 (*p*=0.006)***
Mean of CAL	0.070 (*p*=0.453)	**0.218 (*p*=0.019)***	**0.190 (*p*=0.041)***	0.105 (*p*=0.262)	**0.190 (*p*=0.041)***	0.166 (*p*=0.074)	0.076 (*p*=0.415)
% CAL □ 3 mm	0.145 (*p*=0.121)	**0.291 (*p*=0.002)***	**0.284 (*p*=0.002)***	0.164 (*p*=0.079)	**0.239 (*p*=0.010)***	0.181 (*p*=0.052)	0.147 (*p*=0.115)
% PPD □ 4 mm and CAL □ 4 mm	- 0.113 (*p*=0.256)	0.006 (*p*=0.949)	-0.008 (*p*=0.938)	0.013 (*p*=0.894)	0.165 (*p*=-0.094)	**0.252 (*p*=0.010)***	-0.002 (*p*=0.981)

PPD, periodontal pocket depth; CAL, clinical attachment level

* Spearman coefficient, $p < 0.05$

Table 4. Correlation matrix of Periodontal Inflammatory Load (PIL) and clinical parameters of periodontal disease with mean counts of selected periodontal pathogens.

Clinical parameters	**Periodontal pathogens (Total counts)**		
	P. gingivalis	***T. denticola***	***T. forsythia***
PIL	**0.242 (*p*=0.009)***	**0.237 (*p*=0.010)***	**0.294 (*p*=0.001)***
Mean of PPD	0.100 (*p*=0.287)	0.059 (*p*=0.528)	0.178 (*p*=0.056)
% PPD □ 4 mm	**0.255 (*p*=0.006)***	**0.250 (*p*=0.007)***	**0.312 (*p*=0.001)***
% PPD □ 5 mm	0.085 (*p*=0.363)	0.106 (*p*=0.259)	0.159 (*p*=0.088)
Mean of CAL	0.113 (*p*=0.228)	0.075 (*p*=0.423)	0.169 (*p*=0.069)
% CAL □ 3 mm	0.092 (*p*=0.325)	0.107 (*p*=0.252)	**0.183 (*p*=0.049)***
% PPD □ 4 mm and CAL □ 4 mm	**0.238 (*p* =0.015)***	0.161 (*p* =0.103)	**0.259 (*p*=0.008)***

PPD, periodontal pocket depth; CAL, clinical attachment level

* Spearman coefficient, $p < 0.05$

6 – Artigo III

The relationship between periodontitis and preterm low birth weight.

Mario Vianna Vettore[1,2], Maria do Carmo Leal[1], Anna Thereza Leão[3], Angela Maria Monteiro da Silva[4], Gabriela de Almeida Lamarca[5], Aubrey Sheiham[2]

1 – Department of Epidemiology and Quantitative Methods in Health, National School of Public Health, Oswaldo Cruz Foundation (ENSP/FIOCRUZ) – RJ, Brazil

2 - Department of Epidemiology and Public Health, University College London, London, England

3 – Department of Dental Clinic, Federal University of Rio de Janeiro (UFRJ) – RJ, Brazil

4 – Institute of Human and Social Sciences, Rural Federal University Rio de Janeiro (UFRRJ) – RJ - Brasil

5 - Section of Periodontology, Brazilian Dental Association, Regional Petropolis-RJ, Brazil

Submetido em Dezembro de 2005 ao Journal of Dental Research.

Abstract

There is no consensus about the possible influence of periodontal disease (PD) on preterm low birthweight (PTLBW). The objective was to investigate the relationship between PD and PTLBW. A case-control study with 542 post-partum women aged over 30 years of age was conducted. Four groups of cases were compared with normal controls (cases/controls): Preterm (110/422), Low birthweight (LBW) (96/446), Preterm and/or LBW (143/393), and Preterm and LBW (63/475). Periodontal clinical parameters and covariates were recorded. Periodontal disease levels were higher in controls than cases. The extent of PD did not increase risk for PTLBW using 13 different measures of PD. Frequency of periodontal sites with PPD □ 4 mm in women having LBW, Preterm, and Preterm and/or LBW was lower than controls. PTLBW cases tended to have less periodontal inflammatory load. In conclusion, periodontal disease was not more severe in women with PTLBW babies.

6.1 - Introduction

Preterm low birth weight (PTLBW) is one of the most important causes of morbidity and mortality in newborn children in Latin America. Despite intensive research on the etiology of PTLBW, in over 50% of clinical cases the cause remains unknown (Breborowicz *et al*., 1999).

It has been claimed that periodontal infection increased risk for PTLBW. Early evidence on this subject was from a study showing that women delivering PTLBW were almost 8 times more likely to have periodontal disease (Offenbacher *et al*., 1996). The "Focal Infection Theory" proposed by Hunter in 1910 was being resurrected. According to Hunter's theory, bacteria and their products from local infections could be disseminated throughout the body and so cause diseases in other organs (Billings, 1912).

The Offenbacher and co-workers study was followed by others. Most claimed a positive relationship (Dasanayake *et al*., 2001, Jeffcoat *et al*., 2001, Offenbacher *et al*., 2001). On the other hand the study by Davenport and coworkers (2002) reported no association and a systematic review on the subject reported limited evidence that periodontitis is associated with increased risk for PTLBW (Madianos *et al*., 2002). A systematic review we carried out, highlighted that most of the studies have methodological limitations, such as small sample size, lack of control for confounding variables, presence of bias, which limited their internal validity and could have promoted spurious conclusions (Vettore *et al*., unpublished observations).

The uncertainties regarding the relationship between periodontal infection and risks for PTLBW prompted us to carry out a methodologically robust case-control study to test the hypothesis.

The objective of this study was to investigate the relationship between clinical parameters of periodontal disease and preterm low birth in women over 30 years old.

6.2 - Subjects & Methods

A case-control study was conducted based on primary and secondary data in four public maternity hospitals at Rio de Janeiro city. The maternity units involved are referral centres for high risk pregnancies. Four definitions of cases were considered: Preterm birth (PB), low birth weight (LBW), PB and/or LBW, and PB and LBW.

Sample size was calculated to be 551 puerperal women based on the proportion case-control equal to 1:3 to detect 10% of differences between groups at least with type II error at 0.20 at 5% significance level. The sample size group for comparison was

established to compare proportions in samples with different size on the basis of a periodontal disease prevalence of 15% (Flores-de-Jacoby *et al*., 1992).

A pilot study was conducted to adapt and test the questionnaires. In addition, examiner calibration for periodontal clinical parameters was performed. Kappa test and Intraclass Correlation Coefficient of agreement findings for periodontal pocket depth were respectively □ 0.78 and □ 0.72 for intra-examiner, and □ 0.77 and □ 0.72 for inter-examiner. All examiners were masked concerning the purpose of the main study.

542 eligible women were recruited into the case-control study. The data were obtained from structured interviews, medical records and periodontal clinical examinations conducted by six examiners.

The inclusion criteria were women of at least 30 years of age who had given birth to a live child in the past 3 days. Other inclusion criteria were single birth mothers; presence of 15 or more natural teeth; absence of systemic conditions linked with periodontal disease or taking medicines related to periodontal changes or psychotropic drugs; did not have professional tooth cleaning or periodontal treatment during the last six months and had not taken antibiotics during the last week. Excluded women were those with HIV infection, chronic hypertension and chronic diabetes *mellitus*. Women that required prophylactic antibiotics for a periodontal examination were also excluded.

Babies delivered before 37 complete weeks of gestation were considered as preterm (PB). Estimation of gestational age was assessed from the last menstrual period (LMP) (Berg, 1991). When LMP data were missing, Capurro score was used to estimate gestational age (Capurro *et al*., 1978). Intraclass Correlation Coeficient between LMP and Capurro score was 0.92. Low birth weight newborns were infants weighing less than 2,500 g. at birth. All newborn were weight immediately after delivery by calibrated scales. Gestational age and infant weights were obtained from medical records.

Periodontal clinical measurements included Visible Plaque Index (VPI) (Ainamo and Bay, 1975), visible calculus, Bleeding on Probing Index (BOP) (Ainamo and Bay, 1975), Periodontal Pocket Depth (PPD) and Clinical Attachment Level (CAL) measured at 6 sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual) for all teeth excluding third molars.

Periodontal Pocket Depths (PPD) were registered in millimeters from the free gingival margin to base of gingival sulcus or periodontal pocket. CAL measurements were measured using the cemento-enamel junction as a reference point. PPD and CAL measures were recorded to nearest higher millimetre using the North Carolina periodontal probe (Hu-Friedy®, Chicago, IL, USA), 15 mm in length and 0.35 mm in

diameter. Oral plain mirrors (Hu-Friedy®, Chicago, IL, USA) and a head light (Head light, model 8720, Trilhas & Rumos®, Brazil) were used to facilitate periodontal examinations.

Covariate data were collected from medical records or through structured interviews. Anthropometric and socio-demographic characteristics included height, corporal mass index, ethnicity, education level, marital status, information on work and income. The overall level of housing conditions was assessed using 14 characteristics of housing relating to sanitation, water supply and physical environment of home. The women were questioned concerning cigarette consumption before and during pregnancy and alcohol intake. CAGE (Ewing, 1984) and T-ACE (Sokol *et al*., 1989) questionnaires were used to detect alcoholism and risky drinking, respectively. They answered questions about illicit drugs use, physical violence, contraceptive methods to avoid pregnancy, parental desire and satisfaction with the pregnancy. Physical activities during gestational period were assessed by EPAL questionnaire (Vasconcelos and Anjos, 2002). Anxiety and depression were assessed through the Spielberger Trait Anxiety Inventory (TAI) adapted to the Brazilian population (Biaggio *et al*., 1977), and The Minnesota Multiphasic Personality Inventory (Hathaway and McKinley, 1971).

Pregnancy information including gestational age, baby weight at birth, type of birth, sex of neonate, baby length and proportion weight/gestational age were transcribed from medical records. Occurrence of hypertension, pre-eclampsia, hepatitis B, anaemia, gestational diabetes, urinary infection and infections during pregnancy were also recorded. Prenatal care attendance was assessed by the modified Kotelchuck index (Leal *et al*., 2004).

The study was approved by the Committee of Ethics and Research of the National School of Public Health - Oswaldo Cruz Foundation (FIOCRUZ) (protocol no. 78/02). Subjects' rights were protected by an appropriate institutional review board and informed consent was obtained.

Statistical analysis

All statistical analyses were carried out using SPSS 10.0 (Statistical Package for Social Sciences for Windows®, SPSS Inc., Chicago, Illinois, USA). The significance level established for all analysis was 5% ($p \leq 0.05$).

Periodontal clinical parameters

Clinical parameters including number and percentage of sites with visible plaque and calculus, bleeding on probing as well as the average of PPD and CAL were computed for each subject and then averaged across subjects in the groups. Differences

among clinical parameters were examined in the subset of sites according to their PPD (□ 4 mm, □ 5 mm and □ 6 mm), CAL (□ 3 mm, □ 4 mm, □ 5 mm and □ 6 mm) and using a combination of both (PPD □ 4 mm and CAL □ 3 mm). The statistical significance of differences between the groups was checked by Mann-Whitney tests.

Thirteen different definitions for periodontal disease based on previous published studies on this subject were used to test the hypothesis (Table 3). In addition, the sample was divided into different quartiles of frequency distribution according to number of PPD ≥ 4 mm and sum of PPD ≥ 4 mm of sites with loss of attachment. The quartiles of distribution of PPD ≥ 4 mm and sum of all PPD ≥ 4 mm of sites with loss of attachment were calculated and subjects were grouped in 4 different levels of PPD. In both strategies the Odds Ratios were calculated using Level 1 as category of reference.

Covariates

Covariate variables were computed for each subject and then for each group. Comparisons between groups were tested by Chi-Square test and Exact's Fisher test for variables expressed in proportions and Mann-Whitney test and *t*-test for continuous variables. Internal consistencies for depression and anxiety scales were evaluated by α Cronbach coefficient.

6.3 - Results

Of the 2,561 puerperal women invited into the study, 172 (6.7%) refused. Of the 2,389 subjects that agreed, 1,847 were excluded for different reasons, resulting in 542 selected participants. Figure 1 shows the flow chart for selecting study subjects. Newborn weight at birth and gestational age were available in 100% and 98% of the medical records.

The main covariates are described in Table 1. Gestational age at birth and birth weight were significantly lower in all case groups. There were no differences in the type of birth, neonate's sex, mothers' age, ethnicity, alcohol consumption, depression and urinary infection between groups. Corporal mass index was significantly lower in LBW, preterm and/or LBW, and preterm and LBW (PLBW) groups compared to respective control groups. Married women tended to be higher in control groups; the difference was of borderline non-significance. All cases were more likely to have previous preterm and LBW babies. Inadequate prenatal care was more common in preterm, LBW, preterm and/or LBW and in preterm and LBW (PLBW) mothers (P<0.005). The LBW group had a significantly higher proportion of women who smoked during pregnancy. Number of cigarettes smoked per day was also greater in LBW mothers. Trait anxiety

scores were significantly associated with LBW. The frequency of gestational hypertension was higher in preterm, preterm and/or LBW, and preterm and LBW (PLBW) than controls. Pre-eclampsia was significantly associated with mothers who had preterm and LBW (PLBW) babies. Preterm subjects had a higher proportion who had gestational diabetes. A more detailed description of all covariates is in appendix.

Table 2 shows comparisons of periodontal clinical parameters between all pairs of cases and control groups. Number of teeth, bleeding on probing and calculus were similar in all comparisons between groups. Visible dental plaque scores were significantly higher in non-preterm compared to preterm cases (P=0.039).

The mean and frequency of CAL □ 3 mm were lower in women who had preterm births. Mean PPD and CAL, mean and frequency of PPD □ 4 mm, CAL □ 3 mm, and PPD □ 4 mm with CAL □ 3 mm were significantly lower LBW compared to non-LBW (P≤0.05). Mean of PPD and CAL, the mean of PPD □ 4 mm and PPD □ 4 mm with CAL □ 3 mm, and the mean and frequency of CAL □ 3 mm were lower in Preterm and/or non-LBW mothers. The PPD mean and the mean and frequency of CAL □ 3 mm was significantly lower in women who had preterm births and LBW babies (PLBW). Mean CAL tended to be lower in preterm and LBW mothers (P=0.056).

Unadjusted risk estimates [odds ratio (OR)] were calculated between periodontal disease and preterm LBW outcomes (Table 3). Periodontitis was more prevalent in non-preterm, non-LBW, and non-preterm and non-LBW controls compared with respective cases according to previous methods of defining periodontal disease. Frequency of periodontal sites with PPD □ 4 mm in women who had preterm, LBW, and preterm and/or LBW was significantly lower than controls. Periodontal inflammatory load was assessed through the percentiles of sums of all PPD ≥ 4 mm. Table 3 shows that the case subjects tended to have less periodontal inflammatory load than controls.

6.4 - Discussion

The hypothesis that periodontal disease is a risk factor for undesirable pregnancy outcomes was rejected. Unlike some other studies on the subject, clinical measures relating to periodontal disease, were not associated with preterm, LBW, preterm and/or LBW, and preterm and LBW. Furthermore, the controls had significantly more severe clinical measures related to periodontal disease.

There is a lack of consensus on a uniform definition of periodontal disease in studies on periodontal disease and PTLBW (Madianos *et al*., 2002). To overcome that,

we used all thirteen measures of extent of periodontal disease employed by workers in this research field.

For all thirteen measures used, woman who had preterm LBW had less periodontal disease than controls. Case definitions 14 and 15 facilitate assessment of a possible dose-response relationship between severity of destructive periodontal disease and pregnancy outcomes. There was a steady decrease in estimates of association between periodontal disease and preterm LBW among groups of subjects with more destructive periodontal disease. This finding can be considered strong evidence of no association between periodontal disease and pregnancy outcomes since the dose-response relationship is an important test in epidemiological studies identifying new causal factors.

The finding that periodontal clinical parameters in cases were not higher than in controls accords with some previous studies on the association between periodontal disease and PTLBW (Mitchell-Lewis *et al*., 2001; Moore *et al*., 2004). In addition, evidence of no association between periodontal disease and preterm LBW observed in this study were reported by other investigators (Davenport *et al*., 2002; Lunardelli and Peres 2005; Rajapakse *et al*., 2005).

The heterogeneity in diagnostic criteria for periodontal disease in previous studies is an important source of discrepancies (Vettore *et al*., unpublished observations). The strength of our study is that we used a wide range of measures of periodontal disease to avoid objections about measuring periodontal disease. Approaches included all those used by previous investigators of this subject. In contrast with our findings, several studies found positive associations between periodontal disease and PTLBW (Offenbacher *et al*., 1996, 2001; Lopez *et al*., 2002; Dörtbudak *et al*., 2005; Jarjoura *et al*., 2005; Marin *et al*., 2005; Moliterno *et al*., 2005; Radnai *et al*., 2005). One possible explanation for their positive findings is poor control for potential confounders in some studies. In our study the exclusion selection criteria for participants were relevant confounders such as chronic diseases like diabetes *mellitus* and previous periodontal treatment. Exclusion of women with chronic hypertension avoided bias of confounding as anti-hypertensive medications, are strongly related to periodontal status. Women using antibiotics in the last week were excluded because of their healing effects on inflamed periodontal tissues. Use of antibiotics in pregnancy is usually for genitor-urinary tract infections. This might explain lack of association between the association between genito-urinary infection and preterm LBW in the current study.

Classification bias is the most common systematic error compromising the validity of case-control studies. In our study gestational age was estimated through last menstrual period (LMP), considered as the gold standard method for gestational age assessment in epidemiological studies (Berg, 1991). LMP information was missing in 18% of medical records and Capurro score was used to estimate gestational age in such subjects, with high agreement of diagnostic reliability. The reliable sources of information for gestational age and weight at birth obtained from medical records avoided potential classification bias.

In summary, we conclude that periodontal disease is not a risk factor for preterm, low birth weight, preterm and/or low birth weight, and preterm and low birth weight.

Acknowledgments

Authors thanks to Mirella Galvão. Investigation supported in part by CNPq, CAPES, FAPERJ Grant E-26/170.421/2003, Hu-friedy do Brasil and PAHO/WHO.

6.5 - References

Ainamo J, Bay I (1975). Problems and proposals for recording gingivitis and plaque. *Int Dent J* 25: 229-235.

Berg A (1991). Menstrual cycle length and the calculation of gestational age. *Am J Epidemiol* 133:585-589.

Biaggio AMB, Natalício L, Spielberger CD (1977). Desenvolvimento da forma experimental em português do Inventário de Ansiedade Traço-Estado (IDATE). *Arq Bras Psic Apl* 29: 31-44.

Billings F (1912). Chronic focal infection and their etiologic relations to arthritis. *Arch Intern Med* 9: 484-98.

Breborowicz GH, Szymanjiewicz M, Anholcer A, Gadzinowsku J (1999). Wezesniactwo problem oilozniczy i neonatologiczny. *Medipress Ginekol Poloz* 5:3-12.

Capurro H, Konichezky S, Fonseca D, Caldeyro-Barcia R (1978). A simplified method for diagnosis of gestational age in newborn infant. *J Pediat* 93:120-122.

Dasanayake AP, Boyd D, Madianos PN, Offenbacher S, Hills E (2001). The association between Porphyromonas gingivalis-specific maternal serum IgG and low birth weight. *J Periodontol* 72:1491-7.

Davenport ES, Williams CE, Sterne JA, Murad S, Sivapathasundram V, Curtis MA (2002). Maternal periodontal disease and preterm low birthweight: case-control study. *J Dent Res* 81:313-8.

Dörtbudak O, Eberhardt R, Ulm M, Persson GR (2005). Periodontitis, a marker of risk in pregnancy for preterm birth. *J Clin Periodontol* 32:45-52.

Ewing JA (1984). Detecting alcoholism. The CAGE questionnaire. *JAMA* 12:1905-7

Flores-de-Jacoby L, Bruchmann S, Mengel R, Zafiropoulos GGK (1991).. Periodontal conditions in Rio de Janeiro city (Brazil) using CPITN. *Community Dent Oral Epidemiol* 19:127-8.

Hathaway SR, McKinley, JC (1971). Inventário Multifásico Minnesota de Personalidade - Manual (Benko A, Simões RJP, Trads.) Rio de Janeiro: *Centro de Psicologia Aplicada*.

Jarjoura K, Devine PC, Perez-Delboy A, Herrera-Abreu M, D'alton M., Papapanou PN (2005). Markers of periodontal infection and preterm birth. *Am J Obstet Gynecol* 2:513-9.

Jeffcoat MK, Geurs NC, Reddy MS, Cliver SP, Goldenberg RL, Hauth JC (2001). Periodontal infection and preterm birth: results of a prospective study. *J Am Dent Assoc*132:875-80.

Jeffcoat MK, Hauth JC, Geurs NC, Reddy MS, Cliver SP, Hodgkins PM, *et al*. (2003). Periodontal disease and preterm birth: results of pilot intervention study. *J Periodontol* 74:1214-18.

Leal Mdo C, da Gama SG, Ratto KMN, Cunha CB (2004). Use of the modified Kotelchuck index in the evaluation of prenatal care and its relationship to maternal characteristics and birth weight in Rio de Janeiro, Brazil. *Cad Saude Publica* 20 (Sup1):S63-S72

Lunardelli AN, Peres MA (2005). Is there an association between periodontal disease, prematurity and low birth weight? A population-based study. *J Clin Periodontol* 32:938-946.

López NJ, Smith PC, Gutierrez J (2002). Higher risk of preterm birth and low birth weight in women with periodontal disease. *J Dent Res* 81:58-63.

Madianos PN, Bobetsis GA, Kinane DF (2002). Is periodontitis associated with an increased risk of coronary heart disease and preterm and/or low birth weight births? *J Clin Periodontol* 29(Supp.3):22-36.

Marin C, Segura-Egea JJ, Martinez-Sahuquillo A, Bullon P (2005). Correlation between infant birth weight and mother's periodontal status. *J Clin Periodontol* 32:299-304.

Mitchell-Lewis D, Engebretson SP, Chen J, Lamster IB, Papapanou PN (2001). Periodontal infections and pre-term birth: early findings from a cohort of young minority women in New York. *Eur J Oral Sci* 109:34-39.

Moliterno LF, Monteiro B, Figueredo CM, Fischer RG (2005). Association between periodontitis and low birth weight: a case-control study. *J Clin Periodontol* 32:886-90.

Moore S, Ide M, Coward PY, Randhawa M, Borkowska E, Baylis R, *et al*. (2004). A prospective study to investigate the relationship between periodontal disease and adverse pregnancy outcome. *Br Dent J* 197:251-8.

Offenbacher S, Katz V, Fertik G, Collins J, Boyd D, Maynor G, *et al*. (1996). Periodontal infections as a risk factor for preterm low birth weight. *J Periodontol* 67(10 Suppl):1103-1113.

Offenbacher S, Lieff S, Boggess KA, Murtha AP, Madianos PN, Champagne CM, et al. (2001). Maternal periodontitis and prematurity. Part I: Obstetric outcome of prematurity and growth restriction. *Ann Periodontol* 6:164-74.

Radnai M, Gorzó I, Nagy E, Urbán E, Novák T, Pál A (2004). A possible association between preterm birth and early periodontitis. *J Clin Periodontol* 31:736-741.

Rajapakse PS, Nagarathne M, Chandrasekra KB, Dasanayake AP (2005). Periodontal disease and prematurity among non-smoking Sri Lankan women. *J Dent Res* 84:274-7.

Sokol RJ, Martier SS, Ager JW (1989). The T-ACE questions: Practical prenatal detection of risk-drinking. *Am J Obstet Gynecol* 160:863-871.

Vasconcelos MTL, Anjos LA (2003). A simplified method for assessing physical activity level values for a country or study population. *Eur J Clin Nutr* 57:1025-33.

Vettore MV, Lamarca GA, Leão AT, Thomaz FB, Sheiham A, Leal MC Periodontal infection and undesirable pregnancy outcomes: a systematic review of the epidemiologic studies. *Cad Saude Publica*. unpublished observations.

6.6 – Apêndices do artigo 6

OBS: O periódico Journal of Dental Research estabelece um limite máximo de 2500 palavras. Assim, foram escritos dois apêndices, um sobre métodos e outro sobre resultados.

Apêndice 1. Methods

Population

The present investigation was carried out in four public maternity hospitals at Rio de Janeiro city. The maternity units involved are referral centres for high risk pregnancies and are administered by the Community Health Care System ("*Sistema Unico de Saude - SUS*"). All women aged 30 years old or over who had delivered live babies within the past 3 days were invited to take part in the study.

Type of study

A case-control study was conducted based on primary and secondary data. In analytic studies the comparison is explicit, since the investigator assembles groups of individuals for the specific purpose of systematically determining whether or not the risk of outcome is different for individuals exposed or not exposed to a serious of factors of interest. Based on the fact that preterm and low birth weight has a prevalence of around 7% in Rio de Janeiro, a case-control design was chosen to study the association between prevalence and severity of periodontal disease and preterm and low birth weight (PLBW) pregnancy outcomes.

Sample size calculation

The sample size was calculated to be 551 puerperal women based on the proportion case-control equal to 1:3 to detect 10% of differences between groups at least with type II error at 0.20 at 5% significance level. The sample size group for comparison was established to compare proportions in samples with different size (Fleiss, 1981) on the basis of a periodontal disease prevalence of 15% (Flores-de-Jacoby *et al.,* 1992).

Inclusion and exclusion criteria

The inclusion criteria to take part in the study were women of at least 30 years of age who had given birth to a live child in the past 3 days. The 30 years of age criteria was used because the prevalence of periodontal disease in women under 30 years of age is low. The 3 days post-partum period was used because women were frequently discharged after 3 days and those who stayed longer than 3 days were women who had complications in the pregnancy. Other inclusion criteria were single birth mothers;

presence of 15 or more natural teeth; absence of systemic conditions linked with periodontal disease or taking medicines related to periodontal changes or psychotropic drugs; did not have professional tooth cleaning or periodontal treatment during the last six months and had not taken antibiotics during the last week. Excluded women were those with HIV infection, chronic hypertension and chronic diabetes *mellitus*. Women that required prophylactic antibiotics for a periodontal examination were also excluded.

Ethical Issues

The study was approved by the Committee of Ethics and Research of the National School of Public Health - Oswaldo Cruz Foundation (FIOCRUZ) (protocol no. 78/02). The precise description of the study was presented orally and in writing to all subjects. Oral explanation and a written informed consent were employed to describe the objectives, purposes, expected benefits of clinical examination. Subjects were informed that they were free to withdraw from the study when they want.

Definitions of Preterm and Low Birth Weight

Babies delivered before 37 complete weeks of gestation were considered as preterm (PB) (WHO, 1977). The estimation of gestational age was assessed from the last menstrual period (LMP) (Berg, 1991). LMP is calculated by subtracting the last menstrual period from the date of delivery reported by the mother. The Naegele rule was applied by subtracting tree months and adding seven days to the first day of the last menstrual period to calculate the probable date of the delivery. Only full weeks are considered (Alexander *et al.,* 1995). When LMP data were missing, the Capurro score was used to estimate the gestational age (Capurro *et al.,* 1978). This score is registered by all paediatricians immediately after the delivery and is based on somatic and neurological characteristics of the newborn. The paediatricians are trained to score the Capurro score. The reliability analysis between LMP and Capurro score was tested by Intraclass Correlation Coeficient (ICC). The ICC of agreement findings was 0.92. Low birth weight newborns were infants weighing less than 2,500 g. at birth (WHO, 1977). All newborn were weighted after the delivery by calibrated scales. The gestational age estimative from LMP and Capurro score, and infant weights were obtained from medical records.

Case and control definitions

Four pair of groups of cases and controls were considered:

Preterm birth: gestational age at birth < 37 weeks *vs.* non-PB: gestational age at birth □ 37 weeks.

Low birth weight: infants weight < 2,500 g. *vs.* non-LBW: infants weight □ 2,500 g.

PB and LBW: gestational age at birth < 37 weeks with infants weighting < 2,500 g *vs.* non-PB and/or non-LBW: deliveries with gestational age □ 37 weeks and/or infants weight □ 2,500 g.

PB and/or LBW: gestational age at birth < 37 weeks and/or infants weighting < 2,500 g *vs.* non-PN and non-LBW: deliveries with gestational age □ 37 weeks with infants weighting □ 2,500 g.

All those case and control definitions were used in previous studies (Vettore *et al*., 2006).

Measurement of Periodontal Status

Periodontal clinical measurements included Visible Plaque Index (VPI) (Ainamo and Bay, 1975), Bleeding on Probing Index (BOP) (Ainamo and Bay, 1975), visible calculus, Periodontal Pocket Depth (PPD) and Clinical Attachment Level (CAL) measured at 6 sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual) for all teeth excluding third molars.

The Visible Plaque Index, Bleeding on Probing Index, visible calculus are dichotomized measures where the presence or absence of visible plaque, gingival bleeding after periodontal probing and visible calculus were registered respectively.

Periodontal Pocket Depths (PPD) were registered in millimeters from the free gingival margin to deep of gingival sulcus or periodontal pocket (Armitage, 1995). CAL measurements were measured using the cemento-enamel junction as a reference point (Ramfjord and Ash, 1979). PPD and CAL measures were recorded to the nearest higher millimetre using the North Carolina periodontal probe (Hu-Friedy®, Chicago, IL, USA), 15 mm in length and 0.35 mm in diameter. Oral plain mirrors (No. 5, Duflex-SSWhite®) and a head light (Trilhas & Rumos® model 8720) were used to facilitate the periodontal examinations.

Covariates

Covariate data were collected from medical records or through structured interviews. They included anthropometric and socio-demographic characteristics, housing conditions, harmful Maternal habits, physical activities and violence during pregnancy, psychosocial Factors, satisfaction with pregnancy, obstetric history, prenatal care and diseases during pregnancy.

Anthropometric and socio-demographic characteristics

Anthropometric and socio-demographic characteristics included height, corporal mass index, ethnicity, education level, marital status, information on work and income.

Housing conditions

Housing conditions are a reliable measure of material status. The overall level of housing conditions was assessed by 14 characteristics of housing relating to; sanitation, water supply and physical environment of home. Each characteristic was separately analysed in relation to preterm low birth weight risk. Those showing odds ratio over 1.0 were selected to compose the 'housing condition' variable. A house was considered 'inadequate' housing when one or more of the following housing characteristics were present: no flush toilet; walls of the house made with clay, straw, wood, plastic or metal; house without covered carpeted floor; water plumbing supply outside the house; lack of sewerage or pit sewerage; 'open rubbish pit' in the street of where the woman lives.

Harmful Maternal habits

Cigarette consumption before and during pregnancy were recorded. Alcohol intake during pregnancy was assessed as well as alcoholism. CAGE (Ewing, 1984) and T-ACE (Sokol *et al.,* 1989) questionnaires were used to detect alcoholism and risky drinking, respectively. They are based on four and five questions concerning self-perception on drinking habits. The women were requested to answer questions about illicit drugs use before and during the current pregnancy.

Physical activities and violence during pregnancy

Physical activities during gestational period were assessed by the EPAL questionnaire (Vasconcelos and Anjos 2002). Subjects were asked about physical violence during pregnancy.

Psychosocial Factors

Anxiety and depression were assessed through psychometric instruments. Depression was assessed using The Minnesota Multiphasic Personality Inventory (MMPI). The MMPI aims to detect the most important personality profile and traits. There are 566 items in the whole instrument and 60 are used to measure depression. The interviewer distinguishes the answers at three levels: "right", "wrong" or "I do not know" (Hathaway and McKinley, 1971).

Anxiety was assessed with the Spielberger Trait Anxiety Inventory (TAI) adapted to the Brazilian population by Biaggio *et al.* (1977). The Trait Anxiety Inventory is a reliable measure of anxiety propensity or personality trait relative to anxiety, which is

relatively stable. The TAI questionnaire consists, in one scale 20 items, followed by a four-point scale. The subjects have to check one among four options for each item: almost never, sometimes, frequently or almost ever. These scale measure trait dimensions of anxiety. The trait anxiety scale requires that subjects describe the way they generally feel. The range of possible score varies from a minimum score of 20 to a maximum score of 80 on the scale.

Satisfaction with pregnancy

The women were questioned concerning the use of contraceptive methods to avoid pregnancy. Parental desire and satisfaction with the pregnancy were also investigated.

Obstetric history, prenatal care and diseases during pregnancy

Pregnancy information including gestational age, baby weight at birth, type of birth, sex of neonate, baby length and proportion weight/gestational age were collected from medical records. The occurrence of hypertension, pre-eclampsia, hepatitis B, anaemia, gestational diabetes, urinary infection and infections during pregnancy were also collected. Prenatal care attendance was assessed with the modified Kotelchuck index for Brazilian population (Leal *et al.,* 2004)..The individual score provided by Kotelchuck Index is based on the month of starting the prenatal care and on the proportion of the number of prenatal care appointments over the number of expected appointments, according with the gestational age at birth (Kotelchuck, 1994).

Pilot Study

A pilot study was conducted to obtain reliable information concerning the study population in a standardized way by six examiners. The purpose of the pilot study was to adapt and test the questionnaires for interview. In addition, examiner calibration for periodontal clinical parameters was performed The questionnaires were adapted from a previous study about associated factors with perinatal morbidity in post-partum women in Rio de Janeiro (Leal *et al.,* 2005). Forty pregnant women were interviewed at the Fernando Magalhães Hospital to test the understanding and layout. Modifications were made related to comprehension and vocabulary. After the interview all women received instructions for proper self-performed tooth cleaning and information concerning how to take care of their child's oral health.

The examiners were calibrated for periodontal clinical examination at the Department of Dental Clinic at Federal University of Rio de Janeiro. 30 subjects with at least 4 sites with periodontal pocket depths > 4.0 mm were selected and examined. Clinical calibration tested the clinical variability among examiners for periodontal clinical parameters. At the intra-examiner calibration each examiner examined three

subjects. The examiners waited for 30 minutes between examinations of the same patient. Twelve patients that did not take part in the intra-examiner calibration were examined for inter-examiner calibration. For each subject three examinations were performed by three different examiners. Kappa test and Intraclass Correlation Coefficient of agreement findings for periodontal pocket depth were respectively □ 0.78 and □ 0.72 for intra-examiner, and □ 0.77 and □ 0.72 for inter-examiner. All examiners were masked concerning the purpose of the main study.

Main Study

Five hundred and forty two eligible women were recruited into the case-control study. The data were obtained from structured interviews, medical records and periodontal clinical examinations. Individual interviews using the pre-tested questionnaires were conducted to collect data concerning social-demographic characteristics, housing conditions, unhealthy maternal habits, physical activities, violence during pregnancy, psychosocial factors and satisfaction with pregnancy. Anthropometrical characteristics, obstetric history, prenatal care and diseases during pregnancy were gathered from hospital medical records.

A standardized sequence to collect data was established and followed by all interviewers.

1. First, the interviewers inspected the medical notes and chose admitted mothers who had delivered their babies within the past 3 days and were at least 30 years of age. All suitable patients received written information concerning the study aims, procedure and the voluntary character of their participation.
2. Once a written informed consent was signed, a questionnaire to include or exclude mothers was used.
3. If the woman had one or more characteristic that excluded her, the interview stopped.
4. If not, a second questionnaire was used to collect data about covariates.
5. After questionnaires were completed, patients underwent a full-mouth periodontal examination.
6. Finally, hospital medical records information was collected. All data were collected in the same day.
7. During the interview and clinical examination procedure the women were in prone position in their bed in the ward.
8. After that, instructions for proper self-performed tooth cleaning and information concerning how to take care of their child oral health were given to all women, and

those with clinical diagnosis of periodontitis were directed to public Dental Centres for treatment.

Statistical analysis

All statistical analyses were carried out using SPSS 10.0 (Statistical Package for Social Sciences for Windows, SPSS Inc., Chicago, Illinois). The significance level established for all analysis was 5% (p □ 0.05). The continuous variables were tested for normal distribution using the Kolmogorov-Smirnov test. Normally-distributed continuous variables were compared using t-test. In cases of a non-normal distribution the Mann-Whitney test was performed. Categorical data were analyzed by $\square^2$ and Fisher's exact test.

Periodontal clinical parameters

Four statistical approaches were conducted to test the association between periodontal disease and undesirable pregnancy outcomes. In the first one clinical parameters including number and percentage of sites with visible plaque and calculus, bleeding on probing as well as the average of PPD and CAL were computed for each subject and then averaged across subjects in the groups. Differences among clinical parameters were examined in the subset of sites according to their PPD (□ 4 mm, □ 5 mm and □ 6 mm), CAL (□ 3 mm, □ 4 mm, □ 5 mm and □ 6 mm) and using a combination of both (PPD □ 4 mm and CAL □ 3 mm). The values for each clinical parameter were averaged separately within each PPD and CAL category for each subject and then averaged across subjects in the groups. The statistical significance of differences between the groups was checked by Mann-Whitney tests.

The second procedure to test the hypothesis of the present study was conducted using 13 different definitions for periodontal disease based on previous published studies on this subject. The Odds Ratios were calculated for each case definition. The 13 definitions for periodontal disease were as follows:

14. Definition 1: □ 1 site with a PPD □ 5 mm in each quadrant not accounting for the PPD at the distal aspects of the most posterior tooth in the quadrant (Dörtbudak *et al.,* 2005),
15. Definition 2: □ 1 site with PPD □ 4 mm and □ 50% BOP (Radnai *et al.,* 2004; Lunardelli and Perez 2005),
16. Definition 3: □ 4 sites with PPD □ 3.5 mm (Lunardelli and Perez 2005),
17. Definition 4: Mean PPD, PI and BOP > than the median (Rajapakse *et al.,* 2005),
18. Definition 5: > 3 sites with CAL □ 3 mm (Jeffcoat *et al.,* 2003),
19. Definition 6: □ 5 sites with CAL □ 3 mm (Jarjoura *et al.,* 2005),

20. Definition 7: □ 60% sites with CAL □ 3 mm (Offenbacher *et al.,* 1996),
21. Definition 8: □ 4 sites with CAL □ 3 mm and PPD □ 4 mm (Moliterno *et al.,* 2005),
22. Definition 9: □ 4 teeth with □1 sites with CAL □ 3 mm and PPD □ 4 mm in the same site (Lopez *et al.,* 2002),
23. Definition 10: > 5% with PPD □ 5mm and > 5% sites with CAL □ 3mm (Moore *et al.,* 2004),
24. Definition 11: □ 1 site with PPD □ 5 mm and □ 2 sites with CAL > 6 mm and BOP > 5% (Marin *et al.,* 2005),
25. Definition 12: Periodontal health: absence of any PPD > 3 mm and no sites with CAL > 2 mm, Mild periodontitis: had less disease than the moderate to severe group and had more disease than the healthy group, Moderate to severe periodontitis: □ 4 sites with PPD □ 5 mm and CAL □ 2 mm (Offenbacher *et al.,* 2001),
26. Definition 13: No disease: < 3 sites with CAL □3 mm, Periodontitis: □ 3 sites with CAL □ 3 mm, Generalized periodontal disease: □ 90 of sites with CAL □ 3 mm (Jeffcoat *et al.,* 2001).

The third strategy applied in the analysis was to compare different quartiles of frequency distribution according to the number of PPD ≥ 4 mm. The quartiles of distribution of PPD ≥ 4 mm were calculated and the subjects were grouped in different levels for PPD. Level 1: 0 to 1 sites with PPD ≥ 4 mm, Level 2: 2 to10 sites with PPD ≥ 4 mm, Level 3: 11 to 23 sites with PPD ≥ 4 mm and level 4: 24 or more with PPD ≥ 4 mm.

The fourth procedure considered periodontal disease in terms of load of periodontal infection according percentiles of sum of all PPD ≥ 4 mm. For each subject all sites with PPD < 3 mm of were not included. Periodontal pocket depths ≥ 4 mm were summed giving a continuous measure of periodontal disease load. The quartiles of distribution of sum of all PPD ≥ 4 mm were Level 1: 0 to 7 mm, Level 2: 8 to 45 mm, Level 3: 45 to 100 mm and level 4: 101 mm or more. In the third and fourth strategies the Odds Ratios were calculated using Level 1 as the category of reference.

Covariates

Covariate variables were computed for each subject and then for each group. Comparisons between groups were tested by Chi-Square test and Exact's Fisher test for variables expressed in proportions and Mann-Whitney test and *t*-test for continuous

variables. Internal consistencies for the depression and anxiety scales were evaluated by the α Cronbach coefficient.

References

Ainamo J, Bay I (1975). Problems and proposals for recording gingivitis and plaque. *Int Dent J* 25: 229-235.

Alexander GR, Tompkins ME, Petersen DJ, Hulsey TC, Mor J. (1995) Discordance between LMP-based and clinically estimated gestational age: implications for research, programs, and policy. *Public Health Rep* 110:395-402.

Armitage GC (1995) Clinical evaluation of periodontal diseases. *Periodontol 2000* 7:39-49.

Berg A (1991). Menstrual cycle length and the calculation of gestational age. *Am J Epidemiol* 133:585-589.

Biaggio AMB, Natalício L, Spielberger CD (1977). Desenvolvimento da forma experimental em português do Inventário de Ansiedade Traço-Estado (IDATE). *Arq Bras Psic Apl* 29: 31-44.

Capurro H, Konichezky S, Fonseca D, Caldeyro-Barcia R (1978). A simplified method for diagnosis of gestational age in newborn infant. *J Pediat* 93:120-122.

Dörtbudak O, Eberhardt R, Ulm M, Persson GR (2005). Periodontitis, a marker of risk in pregnancy for preterm birth. *J Clin Periodontol* 32:45-52.

Ewing JA (1984). Detecting alcoholism. The CAGE questionnaire. *JAMA* 12:1905-7.

Fleiss JL (1981) *Statistical Methods for rates and proportions*. 2ªed New York, John Wiley & Sons.

Flores-de-Jacoby L, Bruchmann S, Mengel R, Zafiropoulos GGK (1991).. Periodontal conditions in Rio de Janeiro city (Brazil) using CPITN. *Community Dent Oral Epidemiol* 19:127-8.

Hathaway SR, McKinley, JC (1971). Inventário Multifásico Minnesota de Personalidade - Manual (Benko A, Simões RJP, Trads.) Rio de Janeiro: *Centro de Psicologia Aplicada*.

Jarjoura K, Devine PC, Perez-Delboy A, Herrera-Abreu M, D'alton M., Papapanou PN (2005). Markers of periodontal infection and preterm birth. *Am J Obstet Gynecol* 2:513-9.

Jeffcoat MK, Geurs NC, Reddy MS, Cliver SP, Goldenberg RL, Hauth JC (2001). Periodontal infection and preterm birth: results of a prospective study. *J Am Dent Assoc*132:875-80.

Jeffcoat MK, Hauth JC, Geurs NC, Reddy MS, Cliver SP, Hodgkins PM, *et al*. (2003). Periodontal disease and preterm birth: results of pilot intervention study. *J Periodontol* 74:1214-18.

Kotelchuck M (1994). An evaluation of Kessner adequacy of prenatal care index and a proposed adequacy of prenatal care utilization index. *Am J Public Health* 84:1414-20.

Leal Mdo C, da Gama SG, Ratto KMN, Cunha CB (2004). Use of the modified Kotelchuck index in the evaluation of prenatal care and its relationship to maternal characteristics and birth weight in Rio de Janeiro, Brazil. *Cad Saude Publica* 20 (Sup1):S63-S72. .

Leal Mdo C, da Gama SG, Campos MR, Cavalini LT, Garbayo LS, Brasil CLP, et al. (2004) Fatores associados à morbi-mortalidade perinatal em uma amostra de maternidades públicas e privadas do Município do Rio de Janeiro, 1999-2001. *Cad Saude Publica* 20 (1 Suppl):S20-S33.

López NJ, Smith PC, Gutierrez J (2002). Higher risk of preterm birth and low birth weight in women with periodontal disease. *J Dent Res* 81:58-63.

Lunardelli AN, Peres MA (2005). Is there an association between periodontal disease, prematurity and low birth weight? A population-based study. *J Clin Periodontol* 32:938-946.

Marin C, Segura-Egea JJ, Martinez-Sahuquillo A, Bullon P (2005). Correlation between infant birth weight and mother's periodontal status. *J Clin Periodontol* 32:299-304.

Moliterno LF, Monteiro B, Figueredo CM, Fischer RG (2005). Association between periodontitis and low birth weight: a case-control study. *J Clin Periodontol* 32:886-90.

Moore S, Ide M, Coward PY, Randhawa M, Borkowska E, Baylis R, *et al*. (2004). A prospective study to investigate the relationship between periodontal disease and adverse pregnancy outcome. *Br Dent J* 197:251-8.

Offenbacher S, Katz V, Fertik G, Collins J, Boyd D, Maynor G, *et al*. (1996). Periodontal infections as a risk factor for preterm low birth weight. *J Periodontol* 67(10 Suppl):1103-1113.

Offenbacher S, Lieff S, Boggess KA, Murtha AP, Madianos PN, Champagne CM, et al. (2001). Maternal periodontitis and prematurity. Part I: Obstetric outcome of prematurity and growth restriction. *Ann Periodontol* 6:164-74.

Radnai M, Gorzó I, Nagy E, Urbán E, Novák T, Pál A (2004). A possible association between preterm birth and early periodontitis. *J Clin Periodontol* 31:736-741.

Ramfjord SP, Ash MM. (1979) *Periodontology and periodontics*. Philadelphia, WB Saunders Company.

Rajapakse PS, Nagarathne M, Chandrasekra KB, Dasanayake AP (2005). Periodontal disease and prematurity among non-smoking Sri Lankan women. *J Dent Res* 84:274-7.

Sokol RJ, Martier SS, Ager JW (1989). The T-ACE questions: Practical prenatal detection of risk-drinking. *Am J Obstet Gynecol* 160:863-871.

Vasconcelos MTL, Anjos LA (2003). A simplified method for assessing physical activity level values for a country or study population. *Eur J Clin Nutr* 57:1025-33.

Vettore MV, Lamarca GA, Leão AT, Thomaz FB, Sheiham A, Leal MC Periodontal infection and undesirable pregnancy outcomes: a systematic review of the epidemiologic studies. *Cad Saude Publica* unpublished observations.

World Health Organization (1977) International classification of diseases: manual of the international statistical classification of diseases, injuries, and causes of death. *Ninth Revision*, Geneva, Switzerland.

Apêndice 2. Results

Of the 2,561 puerperal women invited to take part in this study, 172 (6.7%) refused to take part. Of the 2,389 subjects that agreed, 1,847 were excluded for different reasons. The reasons for exclusion were chronic hypertension (24.0%), antibiotics use during the last week (23.7%), less than 15 teeth (22.3%), professional tooth cleaning in the last 6 months (20.9%), have dentures (15.3%), chronic diabetes (5.1%), need for prophylactic antibiotics for periodontal examination (4.8%), HIV infection (1.1%), more than one child in the current delivery (0.3%). As many women have more than one item for exclusion, the sum of percentages for exclusion exceeds 100%. Figure 1 shows the flow chart for selecting study subjects. Data were collected from 542 subjects, analyzed in four pairs of case-control groups: preterm (n=110) versus non-preterm (n=422), low birth weight (LBW) (n=96) versus non-LBW (n=446), preterm and LBW (n=63) versus non-preterm and/or non-LBW (n=475), preterm and/or LBW (n=143) versus non-preterm and non-LBW (n=393). Newborn weight at birth and gestational age were available in 98% and 100% of the medical records included in the study, respectively. So, group comparisons that used weight at birth for case definitions had fewer subjects than those using gestational age.

Pregnancy variables of the 542 women examined in the study are presented in Table 1A. All variables showed significant differences between the groups, with the exception of the type of birth and neonate's sex.

Table 2A shows the distribution of social and demographic characteristics. The mean age of the sample population was 34.1 ± 3.6 years. The age range of 73% of the sample was between 30 and 36 years. There were no differences in the mean age between groups.

Corporal mass index and whether living with spouse were significantly lower in LBW group compared to non-LBW. Women who delivered preterm babies were more likely to be unemployed whereas housewife status was more frequent in the non-preterm group. Length of schooling tended to be higher in the preterm group; there was a borderline non-significant difference. In addition, women in all case groups were more likely to live in inadequate housing conditions and stopped work nearer to the delivery than controls.

The frequency of alcohol intake, alcoholism, drugs intake, physical violence, depression and use of contraceptive methods as well as physical activities and depression levels during pregnancy were similar between cases and controls (Table 3A). The LBW group had a significantly higher proportion of women smoking during the

gestational period. The number of cigarettes smoked per day was also greater in LBW mothers. No significant differences were found between cases and controls for parents' satisfaction with current pregnancy. Trait anxiety scores were significantly associated with a LBW outcome.

Table 4A presents the obstetric history variables, prenatal care attendance and systemic diseases during pregnancy. Although parity was similar between cases and controls, cases were more likely to have previous preterm and LBW babies. Inadequate prenatal care was more common in LBW and preterm mothers (P<0.005), and controls have on average 1 more prenatal appointment than cases. There was no difference in the proportion of women experiencing pre-eclampsia, anemia, urinary infection and other infections in both group comparisons, although the preterm subjects had a higher proportion who had gestational diabetes and gestational hypertension.

Periodontal clinical parameters

Table 2 shows the comparisons of periodontal clinical parameters between all pairs of cases and control groups. Number of teeth, bleeding on probing and calculus were similar in all comparisons between groups. Visible dental plaque scores were significantly higher in non-preterm compared to preterm cases (P=0.039). All other control groups had higher dental plaque scores than cases, but the difference was of marginal statistical significance.

Other comparisons are presented according to the outcome.

LBW mothers**:** The mean PPD and CAL, mean and frequency of PPD □ 4 mm, CAL □ 3 mm, and PPD □ 4 mm with CAL □ 3 mm were significantly higher in non-LBW compared to LBW (P≤0.05). The mean and frequency of CAL □ 4 mm tended to be higher in non-LBW but the statistical significance was borderline.

Preterm mothers**:** The mean and frequency of CAL □ 3 mm were lower in women who had preterm births. Although not significant, the PPD mean was higher in non-preterm (P=0.051).

Preterm and LBW mothers**:** The PPD mean and the mean and frequency of CAL □ 3 mm was significantly lower in women who had preterm births and LBW babies. The mean CAL tended to be lower in preterm and LBW mothers (P=0.056).

Preterm and/or LBW mothers**:** The mean of PPD and CAL, the mean of PPD □ 4 mm and PPD □ 4 mm with CAL □ 3 mm, and the mean and frequency of CAL □ 3 mm were higher in controls.

The unadjusted risk estimates [odds ratio (OR)] were calculated between periodontal disease and preterm LBW outcomes (Table 3). Periodontitis was more

prevalent in non-LBW, non-preterm, and non-preterm and non-LBW controls compared with respective cases according to previous methods of defining periodontal disease (Table 3). The chance of being exposed to periodontal disease was higher in non-LBW controls according to case definitions Numbers 3, 7, 8 and 9 (Table 3). The frequency of periodontal disease was higher in non-preterm than preterm mothers based on case definition Number 7. Preterm and/or LBW cases had less periodontal disease than non-preterm and non-LBW mothers according to case definitions Numbers 3, 7, 8, 9 and 12.

The relationship between periodontal disease and preterm LBW outcomes were analyzed using different levels of the distribution of percentiles of PPD □ 4 mm. The frequency of periodontal sites with PPD □ 4 mm in women who had LBW, preterm, and preterm and/or LBW was significantly lower than controls (Table 3).Periodontal inflammatory load was assessed through the percentiles of sums of all PPD ≥ 4 mm with CAL ≥ 4 mm. Table 3 also shows that the case subjects tended to have less periodontal inflammatory load than controls.

6.7 – Figuras e Tabelas

OBS: O periódico Journal of Dental Research estabelece um limite máximo de 4 tabelas e/ou figuras por artigo. Assim, as tabelas 1A, 2A, 3A e 4A foram submetidas como anexos e serão publicadas somente na versão eletrônica do artigo.

Figure 1. Flow chart for how study subjects were selected.

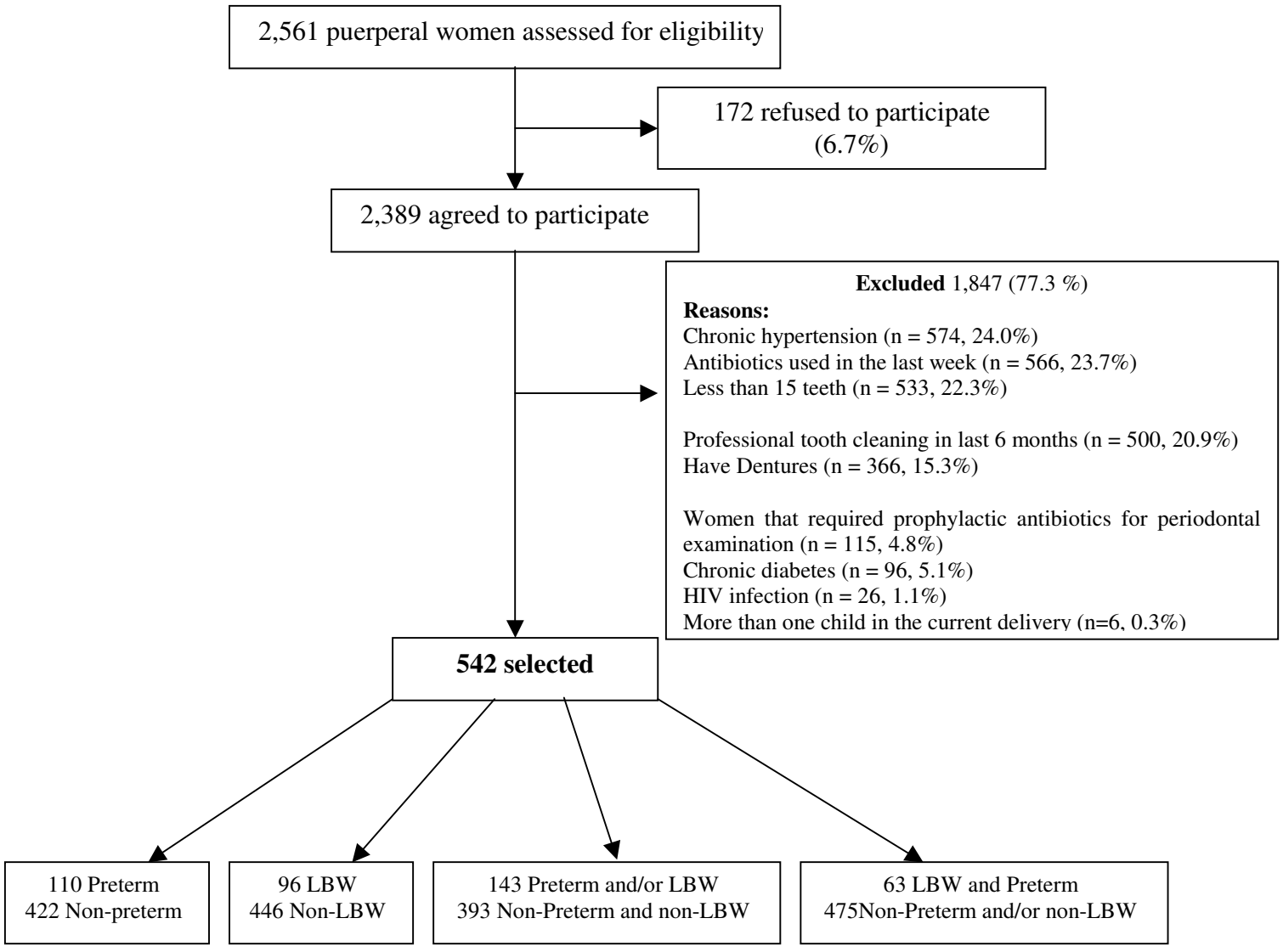


Table 1. Covariate variables of the 542 women examined in the study.

	All subjects (n=542)	Preterm (n=110)	Non-preterm (n=422)	*P*	LBW (n=96)	Non-LBW (n=446)	*P*	PB and/or LBW (n=143)	Non-PB and non-LBW (n=393)	*P*	PB and LBW (n=63)	Non-PB and/or non-LBW (n=475)	*P*
GA, weeks, mean ± SD §	38.3 ± 3.1	33.8 ± 2.6	39.5 ± 1.8	< 0.001*	34.72 ± 3.8	39.07 ± 2.4	< 0.001*	34.8 ± 3.2	39.6 ± 1.8	< 0.001*	32.9 ± 2.9	39.1 ± 2.2	< 0.001*
Birth weight (g), mean ± SD	3031.9 ± 680.0	2342.7 ± 762.0	3217.7 ± 519.7	< 0.001*	1935.5 ± 496.3	3267.9 ± 442.4	< 0.001*	2297.3 ± 692.6	3295.2 ± 442.1	< 0.001*	1825.2 ± 528.6	3200.7 ± 509.0	< 0.001*
Type of birth, n (%) §				0.171			0.322			0.485			0.061
Normal birth	284 (52.5)	51 (46.4)	226 (53.7)		46 (47.9)	238 (53.5)		71 (49.7)	208 (53.1)		26 (41.3)	256 (54.0)	
Caesarian	257 (47.5)	59 (53.6)	195 (46.3)		50 (52.1)	207 (46.5)		72 (50.3)	184 (46.9)		37 (58.7)	218 (46.0)	
Neonate sex, n (%) Male	263 (48.5)	54 (49.1)	202 (47.9)	0.819	47 (49.0)	216 (48.4)	0.925	72 (50.3)	187 (47.6)	0.571	29 (46.0)	231 (48.6)	0.698
Age, mean ± SD	34.1 ± 3.6	34.2 ± 3.7	34.1 ± 3.5	0.794*	34.2 ± 3.7	34.0 ± 3.5	0.901*	34.2 ± 3.7	34.0 ± 3.5	0.949*	34.3 ± 3.7	34.0 ± 3.6	0.695*
Corporal Mass Index §				0.084			< 0.001			0.004			0.005
≤ 21.6	114 (29.7)	30 (34.5)	82 (27.9)		31 (40.8)	83 (26.9)		42 (37.5)	70 (25.9)		19 (37.3)	95 (28.6)	
21.7 – 24.6	116 (30.2)	31 (35.6)	85 (28.9)		30 (39.5)	86 (28.0)		39 (34.8)	77 (28.5)		22 (43.1)	94 (28.3)	
≥ 24.7	154 (40.1)	26 (29.9)	127 (43.2)		15 (19.7)	139 (45.1)		31 (27.7)	123 (45.6)		10 (19.6)	143 (43.1)	
Ethnicity, n (%) §				0.959			0.983			0.875			0.804
White	172 (32.2)	36 (33.3)	134 (32.2)		30 (31.6)	142 (32.2)		48 (34.0)	123 (31.8)		18 (29.0)	153 (32.7)	
Brown	254 (47.6)	50 (46.3)	199 (47.8)		46 (48.4)	208 (47.5)		66 (46.8)	185 (47.8)		30 (48.4)	222 (47.4)	
Black	108 (20.2)	22 (20.4)	83 (20.0)		19 (20.0)	89 (20.3)		27 (19.2)	79 (20.4)		14 (22.6)	93 (19.9)	
Marital Status, n (%) §				0.070			0.087			0.063			0.070
Married/ partner	435 (80.8)	83 (75.4)	343 (82.1)		73 (76.0)	362 (81.9)		110 (76.9)	320 (82.2)		46 (73.0)	385 (81.8)	
Young	80 (14.9)	18 (16.4)	61 (14.6)		15 (15.7)	65 (14.7)		22 (15.4)	57 (14.7)		11 (17.5)	69 (14.6)	
Divorced / Widow	23 (4.3)	9 (8.2)	14 (3.3)		8 (8.3)	15 (3.4)		11 (7.7)	12 (3.1)		6 (9.5)	17 (3.6)	
Previous preterm, n (%) §	76 (14.1)	27 (30.7)	46 (13.3)	< 0.001	29 (30.5)	47 (10.6)	< 0.001	38 (33.6)	37 (11.1)	< 0.001	18 (36.0)	57 (14.4)	< 0.001
Previous LBW, n (%) §	72 (13.6)	23 (26.1)	47 (13.8)	0.005	29 (30.5)	43 (9.9)	< 0.001	34 (30.1)	37 (11.6)	< 0.001	18 (36.0)	53 (13.8)	< 0.001
PN care, n (%) §				< 0.001			0.004			< 0.001			0.015
More than adequate/Adequate	102 (22.5)	7 (7.6)	95 (26.2)		8 (10.8)	94 (24.7)		9 (7.8)	93 (27.5)		6 (12.0)	96 (23.8)	
Intermediate	203 (44.7)	41 (44.6)	162 (44.8)		31 (41.9)	172 (45.3)		53 (45.7)	150 (44.4)		19 (38.0)	184 (45.5)	
Inadequate	149 (32.8)	44 (47.8)	105 (29.0)		35 (47.3)	114 (30.0)		54 (46.5)	95 (28.1)		25 (50.0)	124 (30.7)	
Alcohol intake during pregnancy, n (%)	91 (16.8)	21 (19.1)	69 (16.4)	0.495	20 (20.8)	71 (15.9)	0.291	28 (19.5)	62 (15.8)	0.297	13 (20.6)	78 (16.4)	0.402
Number of cigarettes smoked, n (%)				0.689			0.039			0.509			0.064
Did not smoke	476 (87.9)	95 (86.4)	373 (88.4)		77 (80.2)	399 (89.5)		122 (85.3)	349 (88.8)		50 (79.4)	423 (89.1)	
< 5	30 (5.5)	6 (5.4)	24 (5.7)		8 (8.3)	22 (4.9)		9 (6.3)	21 (5.3)		5 (7.9)	25 (5.3)	
≥ 5	36 (6.6)	9 (8.2)	25 (5.9)		11 (11.5)	25 (5.6)		12 (8.4)	23 (5.9)		8 (12.7)	27 (5.6)	
Trait anxiety (α = 0.84), mean ± SD §	42 ± 8.7	42.7 ± 9.6	41.7 ± 8.4	0.286†	43.9 ± 10.4	41.6 ± 8.2	0.048†	43.1 ± 9.9	41.5 ± 8.1	0.086†	43.5 ± 10.0	41.8 ± 8.5	0.133†
Depression (α = 0.64), mean ± SD	10.2 ± 3.9	9.7 ± 4.3	10.3 ± 3.8	0.167*	9.9 ± 3.9	10.2 ± 3.9	0.693*	9.8 ± 4.1	10.3 ± 3.8	0.191*	9.9 ± 4.0	10.2 ± 3.9	0.701*
Hypertension, n (%) §	119 (22.3)	36 (34.0)	82 (19.5)	0.001	27 (28.4)	92 (21.0)	0.113	42 (30.2)	76 (19.4)	0.009	21 (33.9)	98 (20.9)	0.022
Pre-eclampsia, n (%) §	12 (2.3)	4 (3.8)	8 (1.9)	0.274‡	4 (4.2)	8 (1.8)	0.241‡	4 (2.9)	8 (2.1)	0.525‡	4 (6.5)	8 (1.7)	0.041‡
Gestational diabetes, n (%) §	28 (5.3)	10 (9.4)	17 (4.1)	0.026†	6 (6.3)	22 (5.0)	0.612†	11 (7.9)	17 (4.4)	0.124†	5 (8.1)	22 (4.7)	0.231‡
Urinary infection, n (%) §	132 (24.7)	24 (22.4)	107 (25.5)	0.507	27 (28.4)	105 (23.9)	0.356	33 (23.6)	98 (25.1)	0.714	18 (29.0)	114 (24.4)	0.424

LBW: Low Birth Weight

§: missing data (n): GA (10), Type of birth (2), Corporal Mass Index (154), Ethnicity (8), Marital status (4), Previous preterm (92), Previou
LBW (105), PN care (61), Trait anxiety (2), Hypertension (8), Pre-eclampsia (10), Gestational diabetes (10), Urinary infection (8)

p values by χ^2 test except , * Mann-Whitney test, † Test t, ‡ Exact's Fisher test

Table 2. Periodontal clinical parameters in the case-control groups.

		LBW (n = 96)	Non-LBW (n=446)	*P**	Preterm (n = 110)	Non-Preterm (n = 422)	*P**	LBW and Preterm (n = 63)	Non-LBW or non-Preterm (n = 475)	*P**	LBW and/or Preterm (n = 143)	Non-LBW and Non-Preterm (n = 393)	*P**
N of teeth		23.2 ± 4.0	23.3 ± 3.8	0.908	22.8 ± 4.2	23.4 ± 3.7	0.234	23.2 ± 4.0	23.3 ± 3.8	0.923	22.9 ± 4.2	23.4 ± 3.7	0.358
VPI	X	42.6 ± 40.6	49.0 ± 39.7	0.179	42.4 ± 41.2	50.0 ± 39.6	0.039	41.0 ± 40.5	48.9 ± 40.0	0.099	43.1 ± 41.1	50.2 ± 39.4	0.062
	%	46.6 ± 43.6	53.6 ± 42.4	0.215	46.9 ± 44.3	54.6 ± 42.2	0.079	45.4 ± 4.3	53.5 ± 42.4	0.174	47.4 ± 43.7	54.9 ± 42.1	0.088
BOP	X	18.3 ± 27.6	17.7 ± 23.9	0.828	20.1 ± 27.5	17.3 ± 24.0	0.631	20.4 ± 29.9	17.4 ± 23.9	0.722	18.7 ± 26.5	17.5 ± 24.1	0.986
	%	20.0 ± 29.1	19.9 ± 27.5	0.868	22.6 ± 30.4	19.4 ± 27.3	0.558	22.4 ± 31.6	19.6 ± 27.4	0.674	20.9 ± 29.0	19.7 ± 27.5	0.940
Calculus	X	9.2 ± 17.8	8.3 ± 18.0	0.350	8.8 ± 18.2	8.6 ± 18.2	0.898	10.7 ± 19.4	8.3 ± 17.8	0.223	8.3 ± 16.8	8.7 ± 18.5	0.963
	%	10.9 ± 22.6	10.0 ± 22.2	0.374	10.5 ± 21.7	10.3 ± 22.7	0.859	12.2 ± 23.5	10.0 ± 22.19	0.235	10.0 ± 21.4	10.4 ± 22.8	0.941
PPD	X	2.3 ± 0.5	2.5 ± 0.6	0.001	2.3 ± 0.6	2.5 ± 0.5	0.051	2.3 ± 0.5	2.5 ± 0.6	0.012	2.3 ± 0.6	2.5 ± 0.5	0.005
□ 4 mm	X	11.7 ± 13.8	17.2 ± 18.7	0.006	16.2 ± 20.9	16.4 ± 17.3	0.316	12.5 ± 14.9	16.8 ± 18.4	0.073	14.8 ± 19.3	16.8 ±17.6	0.047
	%	8.4 ± 9.7	12.7 ± 14.0	0.007	12.1 ± 15.7	12.0 ± 13.0	0.328	9.0 ± 10.3	12.4 ± 13.8	0.081	11.0 ± 14.5	12.3 ± 13.2	0.062
□ 5 mm	X	2.7 ± 4.8	4.4 ± 10.3	0.718	6.0 ± 14.6	3.7 ± 7.9	0.113	2.9 ± 4.9	4.3 ± 10.1	0.898	5.2 ± 13.1	3.8 ± 8.0	0.293
	%	1.9 ± 3.3	3.3 ± 7.8	0.665	4.6 ± 11.0	2.7 ± 6.0	0.101	2.1 ± 3.6	3.2 ± 7.6	0.938	3.9 ± 9.8	2.8 ± 6.1	0.283
□ 6 mm	X	0.2 ± 0.7	0.7 ± 2.5	0.193	1.0 ± 3.6	0.5 ± 1.9	0.252	0.2 ± 0.7	0.7 ± 2.5	0.154	0.8 ± 3.2	0.6 ± 1.9	0.329
	%	0.2 ± 0.5	0.5 ± 1.9	0.183	0.7 ± 2.6	0.4 ± 1.5	0.226	0.2 ± 0.5	0.5 ± 1.9	0.153	0.6 ± 2.3	0.4 ± 1.5	0.213
CAL	X	2.4 ± 0.6	2.6 ± 0.7	0.008	2.4 ± 0.7	2.5 ± 0.6	0.111	2.4 ± 0.6	2.5 ± 0.7	0.056	2.4 ± 0.7	2.6 ± 0.6	0.018
□ 3 mm	X	56.9 ± 36.8	68.0 ± 35.1	0.005	59.5 ± 35.0	68.3 ± 35.7	0.018	57.6 ± 36.2	67.3 ± 35.5	0.031	58.6 ± 35.8	69.1 ± 35.3	0.002
	%	41.5 ± 26.4	49.4 ± 25.5	0.006	43.7 ± 25.2	49.5± 25.9	0.042	41.8 ± 25.4	49.0 ± 25.8	0.041	43.1 ± 25.9	50.1 ± 25.6	0.006
□ 4 mm	X	16.5 ± 18.3	20.7 ± 22.3	0.057	19.9 ± 22.7	20. 2 ± 21.7	0.484	17.2 ± 18.3	20.4 ± 22.2	0.257	18.8 ± 21.8	20.5 ± 21.8	0.146
	%	12.5 ± 15.1	15.5 ± 17.2	0.061	15.1 ± 17.7	15.0 ± 16.8	0.585	12.9 ± 14.6	15.3 ± 17.1	0.261	14.4 ± 17.4	15.2 ± 16.7	0.194
□ 5 mm	X	4.7 ± 8.2	6.4 ± 14.5	0.811	7.6 ± 16.1	5.8 ± 13.1	0.176	5.0 ± 8.9	6.3 ±14.2	0.980	6.8 ± 14.5	5.9 ± 13.4	0.153
	%	3.7 ±7.2	4.9 ± 11.0	0.807	5.9 ± 12.4	4.4 ± 10.0	0.153	4.0 ± 7.8	4.8 ±10.8	0.997	5.3 ± 11.3	4.5 ± 10.2	0.129
□ 6 mm	X	0.8 ± 2.4	1.9 ± 7.4	0.216	1.9 ± 8.2	1.6 ± 6.6	0.251	1.0 ± 2.8	1.8 ± 7.2	0.238	1.6 ± 7.2	1.7 ± 6.8	0.394
	%	0.6 ± 1.7	1.4 ± 5.6	0.199	1.4 ± 5.9	1.3 ± 5.0	0.231	0.7 ± 2.0	1.4 ± 5.4	0.244	1.2 ± 5.2	1.3 ± 5.2	0.398
PPD □ 4 mm and CAL □ 3 mm	X	8.4 ± 9.7	12.6 ± 14.0	0.006	16.2 ± 20.9	16.3 ±17.3	0.315	12.5 ± 14.8	16.7 ± 18.4	0.073	14.7 ± 19.3	16.7 ± 17.6	0.048
	%	2.4 ± 0.6	2.6 ± 0.7	0.007	12.1 ± 15.7	12.0 ±12.9	0.376	8.9 ± 10.3	12.3 ± 13.8	0.080	11.0 ± 14.5	12.2 ± 13.1	0.062

*P** refers to Mann-Whitney test
LBW: low birth weight, VPI: Visible Plaque Index, BOP: Bleeding on Probing,
PPD: Periodontal Pocket depth, CAL: Clinical Attachment Level

Table 3. Unadjusted Odds Ratio between periodontal disease in preterm low birth weight mothers and controls.

Case Definitions		LBW			Preterm			LBW and preterm			LBW and/or preterm		
		OR	CI	*P**	OR	CI	*P**	OR	CI	*P**	OR	CI	*P**
1		0.67	0.25-1.75	0.540	1.21	0.55-2.63	0.789	0.61	0.18-2.04	0.582	1.13	0.55-2.34	0.891
2		0.84	0.43-1.62	0.714	1.31	0.74-2.31	0.433	1.15	0.56-2.37	0.853	1.04	0.60-1.78	0.990
3		0.57	0.36-0.90	0.021	0.66	0.43-1.03	0.084	0.66	0.39-1.15	0.186	0.57	0.38-0.86	0.009
4		0.77	0.45-1.33	0.427	0.76	0.45-1.27	0.346	0.92	0.49-1.72	0.907	0.68	0.42-1.10	0.143
5		0.86	0.18-4.11	0.821	0.91	0.19-4.45	0.765	♦	-	-	0.54	0.15-1.94	0.549
6		0.86	0.24-3.10	0.914	0.64	0.20-2.09	0.686	1.75	0.22-13.57	0.907	0.54	0.19-1.53	0.375
7		0.59	0.36-0.97	0.038	0.58	0.36-0.93	0.022	0.64	0.36-1.15	0.133	0.55	0.36-0.84	0.005
8		0.57	0.36-0.90	0.021	0.66	0.43-1.03	0.084	0.67	0.39-1.15	0.186	0.57	0.38-0.59	0.009
9		0.60	0.38-0.94	0.036	0.71	0.46-1.10	0.151	0.66	0.39-1.14	0.174	0.64	0.43-0.95	0.033
10		0.57	0.30-1.08	0.109	1.53	0.93-2.53	0.126	0.69	0.33-1.44	0.404	1.20	0.75-1.94	0.524
11		0.48	0.11-2.09	0.477	1.57	0.59-4.13	0.525	0.79	0.18-3.46	0.977	1.10	0.42-2.90	0.959
12	PH	1	-	-	1	-	-	1	-	-	1	-	-
	MP	0.71	0.39-1.28	0.251	0.59	0.33-1.05	0.073	0.82	0.40-1.70	0.596	0.55	0.33-0.93	0.027
	MSP	0.78	0.40-1.50	0.452	1.17	0.64-2.16	0.612	1.03	0.46-2.28	0.946	0.94	0.54-1.66	0.838
13	ND	1	-	-	1	-	-	-	-	-	1	-	-
	P	0.85	0.17-4.17	0.840	0.96	0.19-4.86	0.965	♦	-	-	0.54	0.14-2.04	0.360
	GPD	0.52	0.10-2.69	0.522	0.40	0.07-2.12	0.280	♦	-	-	0.27	0.07-1.06	0.060
14	1	1	-	-	1	-	-	1	-	-	1	-	-
	2	0.88	0.49-1.58	0.668	0.68	0.38 -1.23	0.203	0.88	0.44 - 1.77	0.722	0.71	0.42 – 1.23	0.204
	3	0.57	0.30-1.06	0.075	0.53	0.29 - 0.97	0.041	0.57	0.27 – 1.21	0.141	0.52	0.30 – 0.90	0.020
	4	0.47	0.24-0.90	0.023	0.79	0.44 - 1.40	0.415	0.59	0.28 – 1.26	0.171	0.63	0.37 – 1.08	0.092
15	1	1	-	-	1	-	-	1	-	-	1	-	-
	2	0.85	0.48-1.51	0.579	0.69	0.39-1.23	0.207	0.87	0.44-1.73	0.691	0.70	0.41-1.18	0.179
	3	0.60	0.32-1.13	0.111	0.53	0.28-0.98	0.043	0.60	0.28-1.28	0.185	0.53	0.30-0.92	0.024
	4	0.44	0.23-0.87	0.017	0.78	0.43-1.39	0.390	0.55	0.25-1.19	0.128	0.62	0.36-1.07	0.087

**P* refers to Mantel-Hansel Odds Ratio

LBW: low birth weight, OR: odds ratio, CI: confidence interval (95%)

♦ Odds ratio could not be performed

Exposure definitions

1: □ 1 site with a PPD □ 5 mm in each quadrant not accounting for the PPD at the distal aspects of the most posterior tooth in the quadrant (Dörtbudak *et al*. 2005).

2: □ 1 site with PPD □ 4 mm and □ 50% BOP (Radnai *et al*. 2004; Lunardelli and Perez 2005).

3: □ 4 sites with PPD □ 3.5 mm (Lunardelli and Perez 2005).

4: Mean PPD, PI and BOP > than the median (Rajapakse *et al*. 2005).

5: > 3 sites with CAL □ 3 mm (Jeffcoat *et al*. 2003).

6: □ 5 sites with CAL □ 3 mm (Jarjoura *et al*. 2005).

7: □ 60% sites with CAL □ 3 mm (Offenbacher *et al*. 1996).

8: □ 4 sites with CAL □ 3 mm and PPD □ 4 mm (Moliterno *et al*. 2005).

9: □ 4 teeth with □1 sites with CAL □ 3 mm and PPD □ 4 mm in the same site (Lopez *et al*. 2002).

10: > 5% with PPD □ 5mm and > 5% sites with CAL □ 3mm (Moore *et al*. 2004).

11: □ 1 site with PPD □ 5 mm and □ 2 sites with CAL > 6 mm and BOP > 5% (Marin *et al*. 2005).

12: PH (Periodontal health): absence of any PPD > 3 mm and no sites with CAL > 2 mm, MP (Mild periodontitis): had less disease than the moderate to severe group and had more disease than the healthy group, MSP (Moderate to severe periodontitis): □ 4 sites with PPD □ 5 mm and CAL □ 2 mm (Offenbacher *et al*. 2001).

13: ND (No disease): < 3 sites with CAL □ 3 mm, P (Periodontitis): □ 3 sites with CAL □ 3 mm, GPD (Generalized periodontal disease): □ 90% of sites with CAL □ 3 mm (Jeffcoat *et al*. 2001).

14: Different groups by percentiles of periodontal sites with PPD ≥ 4 mm. Percentiles: $P_{25} = 2$, $P_{50} = 11$, $P_{75} = 24$. Level 1: 0 to 1 sites with PPD ≥ 4 mm, Level 2: 2 to 10 sites with PPD ≥ 4 mm, Level 3: 11 to 23 sites with PPD ≥ 4 mm, Level 4: 24 or more sites with PPD ≥ 4 mm.

15: Different groups by percentiles of sum of all PPD ≥ 4 mm of sites with loss of attachment (LA) (Periodontal inflammatory load). Percentiles: $P_{25} = 8$, $P_{50} = 47$, $P_{75} = 100$. Level 1: 0 to 7 mm, the sum of all PPD ≥ 4 mm of sites with LA, Level 2: 8 to 46 mm, the sum of all PPD ≥ 4 mm of sites with LA, Level 3: 47 to 99 mm, the sum of all PPD ≥ 4 mm of sites with LA, Level 4: 100 mm and over, the sum of all PPD ≥ 4 mm of sites with LA.

Table 1A. Pregnancy variables of the 542 women examined in the study

	All subjects (n=542)	LBW (n=96)	Non-LBW (n=446)	*P*	Preterm (n=110)	Non-preterm (n=422)	*P*	PB and LBW (n=63)	Non-PB and/or non-LBW (n=475)	*P*	PB and/or LBW (n=143)	Non-PB and non-LBW (n=393)	*P*
GA, weeks, mean ± SD §	38.3 ± 3.1	34.72 ± 3.8	39.07 ± 2.4	< 0.001*	33.8 ± 2.6	39.5 ± 1.8	< 0.001*	32.9 ± 2.9	39.1 ± 2.2	< 0.001*	34.8 ± 3.2	39.6 ± 1.8	< 0.001*
Preterm babies, n (%) §	110 (20.3)	63 (68.5)	47 (10.7)	< 0.001	110 (100.0)	0 (0.0)	< 0.001‡	63 (100.0)	47 (10.0)	< 0.001‡	110 (79.1)	0 (0.0)	< 0.001‡
Birth weight(g), mean ± SD	3031.9 ± 680.0	1935.5 ± 496.3	3267.9 ± 442.4	< 0.001*	2342.7 ± 762.0	3217.7 ± 519.7	< 0.001*	1825.2 ± 528.6	3200.7 ± 509.0	< 0.001*	2297.3 ± 692.6	3295.2 ± 442.1	< 0.001*
LBW, n (%)	96 (17.7)	96 (100.0)	0 (0.0)	< 0.001‡	63 (57.3)	29 (6.9)	< 0.001	63 (100)	29 (6.1)	< 0.001	96 (67.1)	0 (0.0)	< 0.001
Categories of LBW, n (%)				< 0.001‡			< 0.001‡			< 0.001‡			< 0.001‡
Very LBW (<1500 g)	20 (3.7)	20 (20.8)	0 (0.0)		17 (15.5)	2 (0.5)		17 (27.0)	2 (0.4)		20 (14.0)	0 (0.0)	
LBW (1500-2499 g)	76 (14.0)	76 (79.2)	0 (0.0)		46 (41.8)	27 (6.4)		46 (73.0)	27 (5.7)		76 (53.1)	0 (0.0)	
Normal BW (□ 2500 g)	446 (82.3)	0 (0)	446 (100.0)		47 (42.7)	393 (93.1)		0 (0)	446 (93.9)		47 (32.9)	393 (100.0)	
Type of birth, n (%) §				0.322			0.171			0.061			0.485
Normal birth	284 (52.5)	46 (47.9)	238 (53.5)		51 (46.4)	226 (53.7)		26 (41.3)	256 (54.0)		71 (49.7)	208 (53.1)	
Caesarian	257 (47.5)	50 (52.1)	207 (46.5)		59 (53.6)	195 (46.3)		37 (58.7)	218 (46.0)		72 (50.3)	184 (46.9)	
Neonate sex, n (%) Male	263 (48.5)	47 (49.0)	216 (48.4)	0.925	54 (49.1)	202 (47.9)	0.819	29 (46.0)	231 (48.6)	0.698	72 (50.3)	187 (47.6)	0.571
Baby length §	48.4 ± 4.7	43.8 ± 4.5	49.2 ± 4.2	< 0.001*	45.6 ± 5.0	49.1 ± 4.3	< 0.001*	43.1 ± 4.7	49.1 ± 4.2	< 0.001*	45.5 ± 4.8	49.3 ± 4.3	< 0.001*
Weight/GA §				< 0.001‡			< 0.001‡			< 0.001‡			< 0.001‡
Adequate	305 (83.8)	34 (57.6)	271 (88.9)		48 (71.6)	256 (86.5)		25 (59.5)	280 (87.0)		57 (67.9)	247 (88.5)	
Small	27 (7.4)	25 (42.4)	2 (0.7)		17 (25.4)	10 (3.4)		17 (40.5)	10 (3.1)		25 (29.7)	2 (0.7)	
Big	32 (8.8)	0 (0.0)	32 (10.4)		2 (3.0)	30 (10.1)		0 (0)	32 (9.9)		2 (2.4)	30 (10.8)	

LBW: Low Birth Weight, GA: Gestational age
§: missing data (n): GA (10), Preterm babies (10), Type of birth (2), Baby length (27), Weight/GA (178)
p values by χ^2 test except
* Mann-Whitney test
‡ Exact's Fisher test

Table 2A. Socio-demographic characteristics of the 542 women examined in the study.

	All subjects (n=542)	LBW (n=96)	Non-LBW (n=446)	*P*	Preterm (n=110)	Non-preterm (n=422)	*P*	PB and LBW (n=63)	Non-PB and/or non-LBW (n=475)	*P*	PB and/or LBW (n=143)	Non-PB and non-LBW (n=393)	*P*
Age, mean ± SD	34.1 ± 3.6	34.2 ± 3.7	34.0 ± 3.5	0.901*	34.2 ± 3.7	34.1 ± 3.5	0.794*	34.3 ± 3.7	34.0 ± 3.6	0.695*	34.2 ± 3.7	34.0 ± 3.5	0.949*
Corporal Mass Index §				< 0.001			0.084			0.005			0.004
≤ 21.6	114 (29.7)	31 (40.8)	83 (26.9)		30 (34.5)	82 (27.9)		19 (37.3)	95 (28.6)		42 (37.5)	70 (25.9)	
21.7 – 24.6	116 (30.2)	30 (39.5)	86 (28.0)		31 (35.6)	85 (28.9)		22 (43.1)	94 (28.3)		39 (34.8)	77 (28.5)	
□ 24.7	154 (40.1)	15 (19.7)	139 (45.1)		26 (29.9)	127 (43.2)		10 (19.6)	143 (43.1)		31 (27.7)	123 (45.6)	
Height, mean ± SD §	1.61 ± 0.1	1.60 ± 0.1	1.61 ± 0.1	0.276*	1.62 ± 0.1	1.61 ± 0.1	0.391*	1.62 ± 0.1	1.61 ± 0.1	0.463*	1.61 ± 0.1	1.61 ± 0.1	0.477*
Ethnicity, n (%) §				0.983			0.959			0.804			0.875
White	172 (32.2)	30 (31.6)	142 (32.2)		36 (33.3)	134 (32.2)		18 (29.0)	153 (32.7)		48 (34.0)	123 (31.8)	
Brown	254 (47.6)	46 (48.4)	208 (47.5)		50 (46.3)	199 (47.8)		30 (48.4)	222 (47.4)		66 (46.8)	185 (47.8)	
Black	108 (20.2)	19 (20.0)	89 (20.3)		22 (20.4)	83 (20.0)		14 (22.6)	93 (19.9)		27 (19.2)	79 (20.4)	
Schooling (years), §				0.393			0.056			0.036			0.337
> 8 years, n (%)	232 (43.0)	45 (46.9)	187 (42.1)		57 (51.8)	175 (41.7)		35 (55.6)	197 (41.6)		67 (46.9)	165 (42.2)	
≤ 8 years	308 (57.0)	51 (53.1)	257 (57.9)		53 (48.2)	245 (58.3)		28 (44.4)	276 (58.4)		76 (53.1)	226 (57.8)	
Marital Status, n (%) §				0.087			0.070			0.070			0.063
Married/ partner	435 (80.8)	73 (76.0)	362 (81.9)		83 (75.4)	343 (82.1)		46 (73.0)	385 (81.8)		110 (76.9)	320 (82.2)	
Young	80 (14.9)	15 (15.7)	65 (14.7)		18 (16.4)	61 (14.6)		11 (17.5)	69 (14.6)		22 (15.4)	57 (14.7)	
Divorced / Widow	23 (4.3)	8 (8.3)	15 (3.4)		9 (8.2)	14 (3.3)		6 (9.5)	17 (3.6)		11 (7.7)	12 (3.1)	
Lives with spouse, n (%) §	443 (83.3)	71 (76.3)	372 (84.7)	0.049	85 (79.4)	352 (84.6)	0.198	47 (77.0)	393 (84.2)	0.161	109 (78.4)	331 (85.3)	0.060
Housing conditions, n (%)				0.015			0.012			0.001			0.046
Adequate	353 (65.5)	52 (54.7)	301 (67.8)		61 (55.5)	286 (68.3)		30 (47.6)	322 (68.2)		83 (58.5)	265 (67.8)	
Inadequate	186 (34.5)	43 (45.3)	143 (32.2)		49 (44.5)	133 (31.7)		33 (52.4)	150 (31.8)		59 (41.5)	126 (32.2)	
Work on pregnancy, n (%)				0.460			0.040			0.362			0.343
Employee	241 (45.3)	38 (39.6)	203 (46.6)		52 (47.7)	188 (45.4)		28 (44.4)	212 (45.6)		62 (43.7)	179 (46.5)	
Housewife	247 (46.4)	49 (51.0)	198 (45.4)		43 (39.5)	200 (48.3)		27 (42.9)	218 (46.9)		65 (45.8)	180 (46.8)	
Unemployed	44 (8.3)	9 (9.4)	35 (8.0)		14 (12.8)	26 (6.3)		8 (12.7)	35 (7.5)		15 (10.5)	26 (6.7)	
Income (minimum wages) §				0.127			0.997			0.627			0.337
□ ½	247 (45.6)	37 (38.5)	210 (47.1)		51 (46.4)	195 (46.2)		27 (42.9)	219 (46.1)		61 (42.7)	186 (47.3)	
< ½	295 (54.4)	59 (61.5)	236 (52.9)		59 (53.6)	227 (53.8)		36 (57.1)	256 (53.9)		82 (57.3)	207 (52.7)	
Stop work before birth				0.025			0.001			0.006			0.005
> 30	379 (69.9)	71 (73.9)	308 (69.1)		73 (66.4)	296 (70.1)		43 (68.3)	332 (69.9)		101 (70.6)	272 (69.2)	
8-30	86 (15.9)	7 (7.3)	79 (17.7)		10 (9.1)	76 (18.1)		4 (6.3)	82 (17.3)		13 (9.1)	73 (18.6)	
< 8	77 (14.2)	18 (18.8)	59 (13.2)		27 (24.5)	50 (11.8)		16 (25.4)	61 (12.8)		29 (20.3)	48 (12.2)	

LBW: Low Birth Weight, §: missing data (n): Corporal Mass Index (154), Height (144), Ethnicity (8), Schooling (2), Marital Status (4), Lives with spouse (10), Housing conditions (3), Work during pregnancy (10), Monthly income (30), Stop work before birth (20) *p* values by χ^2 test except, * Mann-Whitney test

Table 3A. Harmful maternal habits, psychosocial characteristics and satisfaction with current pregnancy of the 542 women examined in the study.

Table 4A. Obstetric history, prenatal care and systemic diseases during pregnancy among the women in the study.

	All subjects (n=542)	LBW (n=96)	Non-LBW (n=446)	*P*	Preterm (n=110)	Non-preterm (n=422)	*P*	PB and LBW (n=63)	Non-PB and/or non-LBW (n=475)	*P*	PB and/or LBW (n=143)	Non-PB and non-LBW (n=393)	*P*
Alcohol intake during pregnancy, n (%)	91 (16.8)	20 (20.8)	71 (15.9)	0.291	21 (19.1)	69 (16.4)	0.495	13 (20.6)	78 (16.4)	0.402	28 (19.5)	62 (15.8)	0.297
Alcoholism - cage (Alcoholic), n (%)	19 (5.4)	5 (5.2)	24 (5.4)	0.946‡	6 (5.5)	23 (5.5)	0.999‡	4 (6.3)	25 (5.3)	0.764‡	7 (4.9)	22 (5.6)	0.833‡
Alcoholism - T-ACE (Risk drinking), n (%)	22 (4.1)	5 (5.2)	17 (3.8)	0.567‡	4 (3.6)	18 (4.3)	1.000‡	4 (6.3)	18 (3.8)	0.311‡	5 (3.5)	17 (4.3)	0.808‡
Parity, n (%) §		0.169		0.268			0.035			0.408			
0	177 (39.7)	28 (38.4)	149 (39.9)		36 (41.9)	136 (38.6)		19 (38.0)	156 (39.7)		45 (41.3)	129 (38.9)	
1	121 (27.1)	15 (20.5)	106 (28.4)		17 (19.8)	102 (29.0)		9 (18.0)	112 (28.5)		23 (21.1)	96 (28.9)	
2	77 (17.3)	13 (17.8)	64 (17.1)		15 (17.4)	62 (17.6)		9 (18.0)	68 (17.3)		17 (15.6)	60 (18.1)	
≥ 3	71 (15.9)	17 (23.3)	54 (14.5)		18 (20.9)	53 (15.1)		13 (26.0)	58 (14.8)		24 (22.0)	47 (14.2)	
Smoked during gestation, n (%)	36 (8.1)	10 (13.7)	26 (7.0)	0.034	10 (11.6)	26 (7.4)	0.783‡	9 (18.0)	27 (6.9)	0.125‡	11 (10.1)	25 (7.5)	0.578
Did not smoke	472 (87.0)	76 (79.2)	396 (88.8)		95 (86.4)	370 (87.6)		50 (79.4)	420 (88.4)		121 (84.6)	346 (88.1)	
Stopped smoking during pregnancy	22 (4.1)	7 (7.3)	15 (3.4)		4 (3.6)	18 (4.3)		4 (6.3)	18 (3.8)		7 (4.9)	15 (3.8)	
Smoked during the entire pregnancy	48 (8.9)	13 (13.5)	35 (7.8)		11 (10.0)	34 (8.1)		9 (14.3)	37 (7.8)		15 (10.5)	32 (8.1)	
Number of cigarettes smoked, n (%)				0.039			0.689			0.064			0.509
< 5	30 (5.5)	8 (8.3)	22 (4.9)		6 (5.4)	24 (5.7)		5 (7.9)	25 (5.3)		9 (6.3)	21 (5.3)	
5	36 (6.6)	11 (11.5)	25 (5.6)		9 (8.2)	25 (5.9)		8 (12.7)	27 (5.6)		12 (8.4)	23 (5.9)	
Previous preterm, n (%) §	76 (14.1)	29 (30.5)	47 (10.6)	< 0.001	27 (30.7)	46 (13.3)	< 0.001	18 (36.0)	57 (14.4)	< 0.001	38 (33.6)	37 (11.1)	< 0.001
Previous LBW, n (%) §	72 (13.6)	29 (30.5)	43 (9.9)	< 0.001	23 (26.1)	47 (13.8)	0.005	18 (36.0)	53 (13.8)	< 0.001	34 (30.1)	37 (11.6)	< 0.001
Delivery interval, mean ± SD §	355 ± 227	334 ± 234	359 ± 226	0.343*	364 ± 228	353 ± 225	0.825*	316.2 ± 225.5	359.7 ± 227.3	0.226*	364.9 ± 239.9	352.4 ± 223.4	0.799*
Attended PN care, n (%)	515 (95.0)	87 (90.6)	428 (96.0)	0.029	102 (92.7)	408 (96.7)	0.064	57 (90.5)	455 (95.8)	0.065	132 (92.3)	381 (96.9)	0.019
Drugs use during pregnancy, n (%) §	6 (1.1)	2 (2.2)	4 (0.9)	0.285‡	1 (1.0)	5 (1.2)	1.000‡	1 (1.6)	5 (1.1)	0.525‡	2 (1.5)	4 (1.0)	0.653‡
PN care ending (month), n (%) §		< 0.001		< 0.001			< 0.001			< 0.001			
In the 9th month	307 (61.0)	28 (32.6)	279 (66.9)		30 (29.7)	275 (68.9)		9 (15.8)	296 (66.7)		49 (37.7)	258 (69.4)	
Before the 9th month	196 (39.0)	58 (67.4)	138 (33.1)		71 (70.3)	124 (31.1)		48 (84.2)	148 (33.3)		81 (62.3)	114 (30.6)	
Physical activities, mean ± SD	17.8 ± 13.1	17.4 ± 14.3	17.9 ± 12.8	0.659*	17.3 ± 13.2	18.0 ± 13.0	0.514*	19.2 ± 14.0	16.6 ± 12.9	0.426*	16.5 ± 13.5	18.4 ± 12.9	0.119*
Physical violence, n (%) §	19 (3.5)	5 (5.2)	14 (3.1)	0.355‡	4 (3.6)	15 (3.6)	1.000‡	3 (4.8)	16 (3.4)	0.479‡	6 (4.2)	13 (3.3)	0.604‡
PN appointments, mean ± SD §	6.8 ± 2.5	5.9 ± 2.1	7.0 ± 2.2	< 0.001*	5.7 ± 2.1	7.1 ± 2.2	< 0.001*	5.9 ± 2.2	7.0 ± 2.2	< 0.001*	5.7 ± 2.0	7.2 ± 2.2	< 0.001*
Trait anxiety (Cronbach's α = 0.84) §				0.039			0.134			0.128			0.055
7, n (%)	265 (55.2)	27 (32.5)	238 (59.9)	< 0.001	31 (31.6)	232 (61.2)	< 0.001	19 (35.2)	245 (57.8)	0.007	39 (30.7)	225 (63.9)	< 0.001
4-6 Score 20-37	184 (38.3)	49 (59.0)	135 (34.0)		37 (38.2)	127 (33.5)		30 (55.5)	154 (36.3)		76 (59.9)	108 (30.7)	
0-3 Score 38-44	31 (6.5)	7 (8.4)	24 (6.0)		7 (7.2)	20 (5.3)		5 (9.3)	25 (5.9)		12 (9.4)	19 (5.4)	
Score > 44	190 (35.1)	43 (44.8)	147 (33.0)		46 (41.8)	136 (32.3)		28 (44.4)	159 (33.4)		61 (42.7)	124 (31.5)	
PN care (Kotelchuck index), n (%)		0.004		< 0.001			0.015			< 0.001			
More than adequate/Adequate	102 (22.5)	8 (10.8)	94 (24.7)		7 (7.6)	95 (26.2)		6 (12.0)	96 (23.8)		9 (7.8)	93 (27.5)	
Intermediate	203 (44.7)	31 (41.9)	172 (45.3)		41 (44.6)	162 (44.8)		19 (38.0)	184 (45.5)		53 (45.7)	150 (44.4)	
Inadequate	149 (32.8)	35 (47.3)	114 (30.0)		44 (47.8)	105 (29.0)		25 (50.0)	124 (30.7)		54 (46.5)	95 (28.1)	
Depression (Cronbach's α = 0.64)				0.790			0.201			0.781			0.231
Score 0-8	210 (38.6)	40 (41.7)	170 (38.1)		51 (46.4)	157 (37.2)		27 (42.9)	182 (38.3)		64 (44.8)	145 (36.9)	
Score 9-11	141 (26.0)	23 (24.0)	118 (26.5)		24 (21.8)	115 (27.3)		15 (23.8)	125 (26.3)		32 (22.4)	108 (27.5)	
Score ≥ 11	191 (35.2)	33 (34.3)	158 (35.4)		35 (31.8)	150 (35.5)		21 (33.3)	168 (35.4)		47 (32.8)	140 (35.6)	
Hypertension, n (%) §	119 (22.3)	27 (28.4)	92 (21.0)	0.113	36 (34.0)	82 (19.5)	0.001	21 (33.9)	98 (20.9)	0.022	42 (30.2)	76 (19.4)	0.009
Mother satisfied with the pregnancy, n (%) §	490 (92.8)	81 (89.4)	406 (93.3)	0.154	99 (92.5)	381 (92.7)	0.950	56 (90.3)	430 (93.5)	0.433	127 (91.4)	357 (93.2)	0.473
Pre-eclampsia, n (%) §	12 (2.3)	4 (4.2)	8 (1.8)	0.241‡	4 (3.8)	8 (1.9)	0.274‡	4 (6.5)	8 (1.7)	0.041‡	4 (2.9)	8 (2.1)	0.525‡
Mother's feelings about pregnant, n (%) §				0.884			0.960			0.781			0.988
She was trying to get pregnancy	193 (36.1)	36 (38.3)	157 (35.9)		40 (38.1)	153 (36.4)		22 (36.1)	171 (36.5)		58 (41.7)	135 (34.8)	
She would like to wait for a while	28 (5.3)	6 (6.4)	22 (5.0)		6 (5.7)	22 (5.2)		5 (8.1)	23 (4.9)		11 (7.9)	17 (4.4)	
She did not want to get pregnant	194 (35.9)	33 (34.4)	161 (36.3)		38 (34.5)	150 (35.7)		21 (33.3)	172 (36.4)		50 (35.0)	139 (35.5)	
Anaemia, n (%) §	254 (46.7)	49 (51.0)	205 (46.0)	0.432	51 (46.4)	196 (46.5)	0.937	32 (50.8)	218 (45.9)	0.452	81 (56.6)	169 (43.0)	0.004
Gestational diabetes, n (%) §	94 (17.4)	16 (16.6)	78 (17.5)	0.812‡	17 (15.5)	74 (17.6)	0.626‡	10 (15.9)	83 (17.5)	0.781‡	25 (17.4)	69 (17.7)	1.000‡
Infection, n (%) §	146 (27.6)	30 (31.9)	116 (26.7)	0.302	29 (27.6)	116 (27.9)	0.957	20 (32.8)	126 (27.2)	0.356	39 (28.3)	106 (27.4)	0.884
Father satisfied with the pregnancy, n (%) §	479 (92.8)	80 (89.9)	399 (93.4)	0.237	94 (90.4)	375 (93.3)	0.311	51 (86.4)	424 (93.6)	0.046	123 (91.8)	350 (93.1)	0.620
Urinary infection, n (%) §	132 (24.7)	27 (28.4)	105 (23.9)	0.356	24 (22.4)	107 (25.5)	0.507	18 (29.0)	114 (24.4)	0.424	33 (23.6)	98 (25.1)	0.714
Father's desire about pregnancy, n (%) §				0.944			0.556			0.416			0.984
Other infections, n (%) §	19 (3.6)	5 (5.4)	14 (3.2)	0.315‡	3 (3.0)	10 (2.4)	0.726‡	4 (6.7)	15 (3.2)	0.258‡	8 (5.8)	11 (2.9)	0.117‡
He would like her to be pregnant	331 (61.8)	58 (62.4)	273 (61.7)		69 (63.3)	259 (62.1)		40 (64.5)	289 (61.5)		87 (62.2)	243 (62.3)	
He would like her to wait for a while	83 (15.5)	15 (16.1)	68 (15.3)		13 (11.9)	66 (15.8)		6 (9.7)	75 (16.0)		22 (15.7)	59 (15.1)	
He did not want her to get pregnant	122 (22.8)	20 (21.5)	102 (23.0)		27 (24.8)	92 (22.1)		16 (25.8)	101 (21.5)		30 (21.4)	92 (23.6)	

LBW: low birth weight, PN: pre-natal care

§: missing data (n): Parity (96), Previous preterm (92), Previous LBW (105), Delivery interval (113), PN care beginning (13), PN care ending (12), PN appointments (35), Kotelchuck index (61), Hypertension (8), Pre-eclampsia (10), Anaemia (8), Gestational diabetes (10), Infection (13), Urinary infection (8), Other infections (16)

p values by χ^2 test except, * Mann-Whitney test, ‡ Exact's Fisher test

7 - Artigo IV

The relationship between maternal periodontal microbiota and preterm low birth weight.

Mario V. Vettore*†, Magda Feres‡, Anna T. Leão§, Maria doC. Leal*, Aubrey Sheiham†

* Department of Epidemiology and Quantitative Methods in Health, National School of Public Health, Oswaldo Cruz Foundation ENSP/FIOCRUZ) – RJ, Brazil

† Department of Epidemiology and Public Health, University College London, London, England

‡ Department of Periodontology, Guarulhos University - SP, Brazil

§ Department of Dental Clinic, Federal University of Rio de Janeiro (UFRJ) – RJ, Brazil

Submetido em Janeiro de 2006 ao Journal of Periodontology.

Sources of support: This investigation was supported in part by CNPq, CAPES, FAPERJ Grant E-26/170.421/2003, Hu-friedy do Brasil and Pan American Health Organization (PAHO/WHO).

There is no financial relationships between any author and a commercial firm that may pose a conflict of interest.

Running title: Maternal periodontal microbiota and preterm low birth weight

Abstract

Background: Findings on the effect of clinical periodontal disease on preterm low birth weight (PTLBW) are inconclusive. The objective of this study was to compare periodontal clinical measures and the levels and proportions of 39 bacterial species in subgingival biofilm samples between puerperal women with PTLBW and non-PTLBW.

Methods: A case-control study with 116 post-partum women aged over 30 years of age was conducted. Four groups of PTLBW cases were compared with normal non-PTLBW controls (cases/controls): Preterm (40/75), Low birthweight (LBW) (35/81), Preterm and/or LBW (50/66), and Preterm and LBW (25/90). Periodontal clinical parameters of dental plaque, calculus, bleeding on probing, periodontal pocket depth (PPD), clinical attachment level (CAL) were recorded. Covariates included socio-demographic and anthropometric characteristics, housing conditions, maternal harmful habits, obstetric history, prenatal care and diseases during pregnancy. Two subgingival biofilm samples per women were analyzed for 39 bacterial species using Checkerboard DNA-DNA Hybridization technique.

Results: The mean PPD was significantly higher in non-PTLBW controls than PTLBW cases. Frequency of periodontal sites with CAL □6 mm in women having Preterm and LBW was lower than controls. Periodontal inflammatory load was not different between all pairs of case control groups. Groups did not differ with respect to mean proportions of different microbial complexes. The mean counts of *Treponema socranskii* were lower in Preterm, and Preterm and/or LBW compared with respective controls.

Conclusion: Maternal periodontal microbiota was not associated with having PTLBW babies.

Key words: periodontal disease, microbiota, bacteria, preterm, low birthweight, case-control

7.1 - Introduction

The incidence of preterm low birth weight babies (PTLBW) is increasing in developing countries. It remains as an important public health problem with great impact on neonatal mortality and morbidity.[1] Despite the identification of some potential risk factors for PTLBW, a considerable proportion of PTLBW is of unknown etiology. It has been suggested that puerperal genitourinary tract infections affect the normal course of the gestation by altering the levels of local cytokines resulting in growth restriction of the fetus, preterm-labor and preterm birth.[2,3] Genitourinary tract infections and periodontal disease are both caused by Gram-negative anaerobic bacteria and results in local and systemic elevations of prostaglandin-E_2 (PG-E_2) and Tumor Necrosis Factor-α (TNF-α).[4,5] The similarity in biological mechanisms between maternal infections and periodontal disease involving pro-inflammatory cytokines suggested a possible influence of periodontal disease on PTLBW and prompted dental researchers to test whether periodontal disease was a new risk factor for PTLBW.

The initiation and progression of destructive periodontal disease is attributed to the presence of elevated levels of inflammatory mediators and pathogenic bacteria within the gingival crevice.[6] Although clinical measures of periodontal disease provide information on the severity of periodontitis, they do not measure disease activity.[7] Consequently, microbiological testing and biochemical analyses of the host response have been proposed in an effort to monitor the activity of periodontal disease.[8]

There is a relationship between periodontal pathogens and PTLBW in animal models. In pregnant hamsters with localized subcutaneous infection with *Porphyromonas gingivalis,* a periodontal pathogen, there was an increase on PG-E_2 and TNF-α levels in the pelvic region. Fetal development was affected and fetal weight was significantly decreased in infected compared to non-infected hamsters.[9,10] However, there is no consensus about the influence of specific bacterial species from the periodontium on PTLBW in pregnant women.

Subgingival species of periodontal pathogens were associated with preterm birth,[11] and the red complex species, *P. gingivalis, Treponema denticola* and *Tannerella forsythia*,[12] were reported to be in higher levels in PTLBW mothers.[13] On the other hand, no differences were found in levels of periodontal pathogens between preterm and non-preterm mothers,[14,15] and preterm and LBW and non-preterm and/or non-LBW.[16]

Because of the uncertainty of a relationship between periodontal pathogens and PTLBW a case-control study was carried out to test the hypothesis that levels and proportions of microorganisms related to periodontal disease in post-partum women

who had preterm low birth weight babies was significantly higher than in those with non-preterm low births. The objective was to assess the association between certain maternal periodontal pathogens and preterm low birth weights.

7.2 - Methods

In this case-control study, a sample of 116 post-partum women aged 30 years old or over were randomly selected from a large case-control study of 542 subjects that investigated the relationship between clinical parameters of periodontal disease and preterm low birth weight.[17] Figure 1 shows the flow chart for selecting study subjects. The sample size was based on practical considerations such as how many analyses could be carried out for the available funds. The women had attended referral hospitals centres for high-risk pregnancies in Rio de Janeiro.

The inclusion criteria to take part in the study were women of at least 30 years of age who had given birth to a live child in the past 3 days. Other inclusion criteria were single birth mothers; presence of 15 or more natural teeth; absence of systemic conditions linked with periodontal disease or taking medicines related to periodontal changes or psychotropic drugs; absence of professional tooth cleaning or periodontal treatment during the last six months and of systemic antibiotic treatment during the last week. Excluded women were those with HIV infection, chronic hypertension and chronic diabetes *mellitus*. Women that required prophylactic antibiotics for a periodontal examination were also excluded.

Babies delivered before 37 complete weeks of gestation were considered as preterm. The estimation of gestational age was assessed from the last menstrual period (LMP).[18] When LMP data were missing, the Capurro score was used.[19] The reliability analysis between LMP and Capurro score was tested by Intraclass Correlation Coeficient (ICC). The ICC of agreement findings was 0.92.

Low birth weight newborns were infants weighing less than 2,500 g. at birth. All newborns were weighted immediately after the delivery using calibrated scales. The gestational age estimate and infant weights were obtained from medical records.

The study was approved by the Committee of Ethics and Research of the National School of Public Health - Oswaldo Cruz Foundation (FIOCRUZ) (protocol no. 78/02). Subjects were informed that they were free to withdraw from the study.

Pilot study

A pilot study including 30 patients with at least four sites with PPD >4.0 mm was conducted to calibrate six examiners, and to test the understanding and layout of the

questionnaires. Kappa test and ICC of agreement findings for periodontal pocket depth were respectively 0.78 and 0.72 for intra-examiner, and 0.77 and 0.72 for inter-examiner. All examiners were masked concerning the purpose of the main study.

Main study

The randomly selected 116 women were assigned to the following four pairs of groups: Preterm births (PB) *vs* Non-preterm (n= 40/75), LBW *vs* Non-LBW (n = 35/81), PB and/or LBW *vs* Non-PB and non-LBW (n = 50/66) and PB and LBW (PLBW) *vs* Non-PB and/or non-PB (n = 25/90).

Covariates

Covariate data of anthropometric and socio-demographic characteristics included age, corporal mass index, ethnicity, marital status, income, level of education. Assessment of housing conditions included information about sanitation, water supply and physical environment of home.

Cigarette consumption during pregnancy was recorded and T-ACE questionnaire for risky drinking assessment in pregnant women was used.[20] The modified Kotelchuck index, adapted for a Rio de Janeiro city population, was used to assess prenatal care.[21]

Pregnancy information including gestational age, baby weight at birth, type of birth and sex of neonate were transcribed from medical records. The occurrence of previous preterm, previous LBW, hypertension during pregnancy, gestational diabetes and urinary infection were also recorded.

Measurement of Periodontal Status

Periodontal clinical measurements including Visible Plaque Index (VPI),[22] visible calculus, Bleeding on Probing Index (BOP),[22] Periodontal Pocket Depth (PPD) and Clinical Attachment Level (CAL) were measured at 6 sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual) for all teeth excluding third molars.

Periodontal Pocket Depths (PPD) were registered in millimeters from the free gingival margin to deep of gingival sulcus or periodontal pocket.[23] CAL measurements were measured using the cemento-enamel junction as a reference point. PPD and CAL measures were recorded to the nearest higher millimetre using the North Carolina periodontal probe, 15 mm in length and 0.35 mm in diameter. Oral plain mirrors and a head light¶ were used to facilitate the periodontal examinations.

 Hu-Friedy®, Chicago, IL, USA
¶ Trilhas & Rumos® model 8720, Rio de Janeiro, RJ, Brazil

Microbiological assessment

Counts of 39 subgingival species were determined in each biofilm sample using the checkerboard DNA-DNA hybridization technique.[24] Subgingival biofilm samples were taken from the 2 deepest periodontal disease sites collected from different teeth per subject before clinical examination. When the mother had no periodontal pockets, biofilm samples were collected from two random sites in different quadrants of the mouth.

After removal of supragingival biofilm, subgingival biofilm samples were taken with individual sterile Gracey curettes and placed in separate Eppendorf tubes containing 0.15 ml TE (10mM Tris-HCl, 1 mM EDTA, pH 7.6). 0.15 ml of 0.5 M NaOH was added to each tube and the samples were dispersed using a vortex mixer.

Eppdendorf tubes containing biofilm samples were stored in -2^0C and transported to the laboratory of Oral Microbiology, Guarulhos University, São Paulo, Brazil.

First, the samples were boiled for 10 min and neutralized using 0.8 ml of 5 M ammonium acetate. The released DNA was then placed into the extended slots of a Minislot-30 apparatus[#], concentrated onto a 15x15 cm positively charged nylon membrane[**] and fixed to the membrane by baking at 120º C for 20 min. The membrane was placed in a Miniblotter 45[#] with the lanes of DNA at 90º to the lanes of the device. Digoxigenin-labeled whole genomic DNA probes to 39 subgingival species were hybridized in individual lanes of the Miniblotter. After hybridization, the membranes were washed at high stringency and the DNA probes detected using antibody to digoxigenin conjugated with alkaline phosphatase and chemiluminescence detection.

The 39 reference strains employed for the development of DNA probes are shown in Table 1. Two lanes in each run contained standards at concentrations of 10^5 and 10^6 cells of each species. The sensitivity of the assay was adjusted to permit detection of 10^4 cells of a given species by adjusting the concentration of each DNA probe.

Statistical analysis

All statistical analyses were carried out using SPSS 10.0[†]. The significance level established for all analysis was 5% ($P \leq 0.05$). Comparisons between groups for covariates were tested by Chi-Square test and Exact's Fisher test for variables expressed in proportions and Mann-Whitney test and *t*-test for continuous variables.

Hu-Friedy®, Chicago, IL, USA
[#] Immunetics, Cambridge, MA, USA
[**] Boehringer Mannheim, Indianapolis, IN, USA
[†] SPSS Inc., Chicago, IL, USA

Clinical parameters including number and percentage of sites with visible plaque, visible calculus, bleeding on probing as well as the average of PPD and CAL were computed for each subject and then averaged across subjects in the groups.

Differences among clinical parameters were examined in the subset of sites according to their PPD (□ 4 mm, □ 5 mm and □ 6 mm), CAL (□ 3 mm, □ 4 mm, □ 5 mm and □ 6 mm) and using a combination of both (PPD □ 4 mm and CAL □ 3 mm). The statistical significance of differences between the groups was checked by Mann-Whitney tests.

Periodontal disease was considered in terms of load of periodontal infection according percentiles of the sum of all PPD ≥ 4 mm of sites with loss of attachment (LA). In the sample: P_{25}=12, P_{50}=56 and P_{75}=114. For each subject all sites with PPD < 3 mm were not included. Periodontal pocket depths ≥ 4 mm of sites with LA were summed giving a continuous measure of periodontal disease load. Level 1: 0 to 11 mm, the sum of all PPD ≥ 4 mm of sites with LA, Level 2: 12 to 55 mm, the sum of all PPD ≥ 4 mm of sites with LA, Level 3: 56 to 113 mm, the sum of all PPD ≥ 4 mm of sites with LA, Level 4: 114 mm and over, the sum of all PPD ≥ 4 mm of sites with LA. The Odds Ratios were calculated using Level 1 as the category of reference.

Microbiological data available for 116 subjects were counts of each of the 39 species from 2 subgingival biofilm samples for each subject.

A total of 232 subgingival biofilm samples were examined. Mean levels of each species evaluated were computed for each subject and then averaged across subjects within the different groups. The total DNA probe count was also computed at each sampled site in each subject and the proportion that individual species comprised of that count was determined and averaged across subjects in every experimental groups.

Significance of differences between pairs of groups in mean counts of bacterial species and in mean proportions of different microbial complexes[12] (Table 1) was analyzed using Mann-Whitney test.

7.3 - Results

Covariate variables of the 116 women examined in the study are presented in Table 2. The gestational ages and birth weights were significantly different between all pairs of groups.

There were no differences in the anthropometric and socio-demographic characteristics between groups. Inadequate prenatal care was associated with Preterm, LBW, and Preterm and/or LBW ($P < 0.05$).

The frequency of previous preterm and previous LBW was higher in LBW, Preterm and/or LBW, and Preterm and LBW (PLBW) groups. Previous preterm was lower in non-preterm group (Table 2). Hypertension during pregnancy was more common in preterm and/or LBW, and preterm mothers ($P < 0.05$).

Periodontal clinical parameters

Table 3 shows the comparisons of periodontal clinical parameters between all pairs of cases and control groups. Numbers of teeth, bleeding on probing and visible calculus was similar in all comparisons between groups. Visible dental plaque scores were significantly higher in non-preterm compared to preterm cases.

Mean PPD was significantly higher in controls than in cases. Frequency of periodontal sites with CAL 6 mm in women having Preterm and LBW (PLBW) was lower than in controls (Table 3). Periodontal inflammatory load was not different between all pairs of case control groups (Table 4).

Microbiological results

The comparisons of the mean counts of all species evaluated in the subgingival biofilm samples of cases and controls are shown in Figure 2. The profile of colonization was similar among the groups studied. Overall, the species found in highest levels were *Actinomyces gerencseriae, Actinomyces naeslundii genospecies 2, Veillonella parvula, Streptococcus gordonii, Streptococcus sanguinis, Campylobacter gracilis* and *Neisseria mucosa.* Few differences in the microbial composition of subgingival biofilm were observed when the pairs of groups were compared. The mean counts of *Streptococcus mitis* and *Treponema socranskii* were statistically higher in mothers with non-preterm babies compared with those with preterm newborns. When cases and controls were analyzed according to LBW status, there were no differences in mean counts of all species investigated. The mean counts of *T. socranskii* were lower in mothers with preterm and/or LBW babies compared to controls. Subjects in Preterm and LBW (PLBW) group had lower mean counts of *Propionybacterium acnes* compared to controls. The other species evaluated did not differ significantly between cases and controls.

Figure 3 presents the mean proportions of the microbial complexes described by Socransky *et al.* and Socransky & Haffajee.[12,25] The different groups of oral microorganisms are determined by distinct colours (Table 1). Some bacterial species not associated with any complex and DNA probes for new species were compiled in the same group, represented by the gray colour. The proportions of the microbial complexes were similar among different groups of subjects. In Overall, the blue complex was

found in highest mean proportions, followed by the yellow and orange complexes. There were no statistically significant differences between all pairs of case/control groups with regard to the mean proportion of these microbial complexes ($P > 0.05$).

7.4 - Discussion

The hypothesis that the numbers and proportions of periodontal bacterial pathogens in post-partum women who had preterm LBW babies were significantly higher than in those with non-preterm LBW was rejected. No association was found between specific periodontal pathogens and undesirable pregnancy outcomes. This study showed that the microbiological findings from subgingival biofilm in the selected women corresponded to the periodontal clinical findings. The evidence of no association between clinical measures of periodontal disease and preterm LBW observed in the present study accords with our previous results obtained from a population of 542 women.[17]

The checkerboard DNA-DNA hybridization technique[24] was used to compare the bacterial species as well as the oral microbial complexes between case and control groups in this study. The set of genomic probes used included 39 different bacterial species (Table 1). These species were catalogued and stratified into groups or complexes, representing bacterial consortia that appear to occur together and that are associated with periodontal health and disease.[12,26]

Minors differences were observed in the composition of the subgingival microbiota between cases and controls. Of the 39 microorganisms evaluated the levels of only 3 species differed significantly between the 4 pairs of groups evaluated. *S. mitis, T. socranskii and P. acnes* were found in lower levels in cases then in the controls. It is interesting to observe that 2 of these species, *P. acnes* and, specially, *T. socranskii* are considered to be associated with periodontal disease. A direct relationship between the frequency of detection of *T. socranskii* and the severity of periodontal disease has been reported.[27] In addition, the identification of this species was much higher in diseased than healthy sites in subjects with rapidly progressive periodontitis.[8] The counts of *T. socranskii* in the present study were significantly lower in preterm and preterm and/or LBW groups compared with the respective controls.

We found similar loads in all pairs of cases and control groups in mean proportions of the microbial complexes, including those that harbour most of the species considered to be beneficial (blue, purple, green and yellow) and the orange and red complexes, mainly consisted of periodontal pathogens. There is strong evidence in

the literature that the red complex species (*P. gingivalis*, *T. denticola* and *T. forsythia)* are true periodontal pathogens.[28] These microorganisms and to a certain extent some orange complex species are found more frequently and in higher levels in periodontal disease than in health.[26,29-33]

Therefore, the lack of difference in counts of *T. socranskii* and in counts and proportions of microorganisms from the red complex between all pairs of case/control groups in the present study suggests no association between periodontal pathogens from dental biofilms and preterm LBW. This finding is in accordance with a study by Jarjoura *et al.*[14] and Noack *et al*.[16] who found no significant differences for the counts of red complex microorganisms between preterm and non-preterm groups, and preterm and LBW (PLBW) and controls, respectively.

The absence of differences in the prevalence of pathogens of the orange and red complexes in the periodontal biofilm of preterm mothers compared to full-mothers at delivery was also reported by Madianos *et al*.[15] However, they reported a significant increase in the prevalence of maternal IgGM for species of the orange complex, such as *Campylobacter rectus* and *Prevotella intermedia.* Similarly, Dasanayake *et al*. found a relationship between serum IgG levels against *P. gingivalis* and LBW.[34] As suggested by Jarjoura *et al*.[14] the association between such antibodies titers and pregnancy complications remains speculative due to a broad range of antibody responses to periodontal pathogens observed in patients with various forms of periodontal disease.[35]

Some studies found a positive relationship between subgingival biofilm bacterial samples counts and preterm births.[11,36,37] The average of microbial load in periodontal sites of *T. forsythia* was associated with preterm births.[11,37] Offenbacher *et al*. found that preterm LBW mothers had higher levels of *T. forsythia*, *P. gingivalis*, *Actinobacillus actinomycetencomitans* and *T. denticola*.[13] These associations were not adjusted for confounders such as smoking and other known risk factors for preterm LBW.[38] That might partly explain the difference between those studies and ours.

Intra-amniotic levels of prostaglandins E_2 (PG-E_2) and tumoral necrosis factor alpha (TNF-α) increase constantly during pregnancy and a critical threshold is achieved to induce labor, cervical dilatation and newborn. Those molecules are considered physiological mediators in normal childbirth. Anaerobic microorganisms usually produce PG-E_2, TNF-α and other cytokines. So, they act by triggering early labor in pregnant women with genitourinary tract infection.[39,40] In addition, such cytokines

might interfere with fetal growth by inducing hypertension and secondary uterine vascular changes resulting in low birth weights.

The clinical and microbiological results of the present investigation suggest that periodontal disease is not a risk factor for preterm LBW and casts doubts on the biological model suggesting such an association. In summary, that model considers that, in the same way as occurs in genitourinary tract infection, periodontal diseases can provide bacterial components (PG-E_2 and TNF-α) that affect the course of normal gestation. The model was developed based on animal studies and has shortcomings.[9,10]

Although there is evidence which correlates PG-E_2 gingival crevicular fluid levels with clinical parameters of the severity of periodontal disease,[41] a trend for an increasing gingival crevicular fluid with PG-E_2 concentrations in the absence of any clinical signs of disease progression was reported in a group of chronic periodontitis patients monitored longitudinally.[42] Even considering PG-E_2 and TNF-α as potential biological markers of periodontal disease activity, such cytokines produced in the mouth need to migrate and achieve sufficient levels in the placental region to accelerate labor and restrict nutrients to the fetus.

In summary, we conclude, within the limits of the work, that periodontal disease was not associated with preterm, low birth weight, preterm and low birth weight, and preterm and/or low birth weight.

Acknowledgments

The authors thanks to Izilvania Barreto (Universidade de Guarulhos) for laboratory analysis. This investigation was supported in part by CNPq, CAPES, FAPERJ Grant E-26/170.421/2003, Hu-friedy do Brasil and Pan American Health Organization (PAHO/WHO).

7.5 - References

1. Creasy RK, Merkatz IR. Prevention of preterm birth: clinical option. *Obstet Gynecol* 1990;76(Suppl):2-4.

2. Evaldson G, Lagrelius A, Winiarski J. Premature rupture of the membranes. *Acta Obstet Gynecol Scand* 1980;59:385-391.

3. Romero R, Baumann P, Gomez R. The relationship between spontaneous rupture of membranes, labor, and microbial invasion of the amniotic cavity and amniotic fluid concentrations of prostaglandins, and thromboxane B2 in term pregnancy. *Am J Obstet Gynecol* 1994;168:1654-1658.

4. Germain M, Krohn MA, Hillier SL, Eschenbach DA. Genital flora in pregnancy and its association with intrauterine growth retardation. *J Clin Microbiol* 1994;32(9):2162–2168.

5. Page RC. The role of inflammatory mediators in the pathogenesis of periodontal disease. *J Periodontal Res* 1991;26:230-242.

6. Socransky SS, Haffajee AD. Microbial mechanisms in the pathogenesis of destructive periodontal diseases: a critical assessment. *J Period Res* 1991;26:195-209.

7. Sahingur SE, Cohen RE. Analysis of host responses and risk for disease progression. *Periodontol 2000* 2004;34:57-83.

8. Moter AC, Hoening BK, Choi BK, Riep B, Göbel UB. (1998). Molecular epidemiology of oral treponemes associated with periodontal disease. *J Clin Microbiol* 1998;36:1399-1403.

9. Collins JG, Smith MA, Arnold RR, Offenbacher S. Effects of *Escherichia coli* and *Porphyromonas gingivalis* lipopolysaccharide on pregnancy outcome in the golden hamster. *Infect Immun* 1994;62:4652-4655.

10. Collins JG, Windley HW 3rd, Arnold RR, Offenbacher S. Effects of a *Porphyromonas gingivalis* infection on inflammatory mediator response and pregnancy outcome in hamsters. *Infect Immun* 1994;62:4356-4361.

11. Mitchell-Lewis D, Engebretson SP, Chen J, Lamster IB, Papapanou PN. Periodontal infections and pre-term birth: early findings from a cohort of young minority women in New York. *Eur J Oral Sci* 2001;109:34-39.

12. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent Jr RL. Microbial complexes in subgingival plaque. *J Clin Periodontol* 1998;25:134-144.

13. Offenbacher S, Jared HL, O´Reilly PG, et al. Potential pathogenic mechanisms of periodontitis-associated pregnancy complications. *Ann Periodontol* 1998;3:233-250.

14. Jarjoura K, Devine PC, Perez-Delboy A, Herrera-Abreu M, D'alton M, Papapanou PN. Markers of periodontal infection and preterm birth. *Am J Obstet Gynecol* 2005;2:513-519.

15. Madianos PN, Lieff S, Murtha AP, et al. Maternal periodontitis and prematurity. Part II: Maternal infection and fetal exposure. *Ann Periodontol* 2001;6:175-182.

16. Noack B, Klingenberg J, Weigelt J, Hoffmann T. Periodontal status and preterm low birth weight: a case control study. *J Periodontal Res* 2005;40:339-45.

17. Vettore MV, Leão A, Leal Mdo C, Monteiro da Silva AM, Lamarca GA, Sheiham A. The relationship between periodontitis and preterm low birth weight. *J Dent Res* Unpublished observations.

18. Alexander GR, Tompkins ME, Petersen DJ, Hulsey TC, Mor J. Discordance between LMP-based and clinically estimated gestational age: implications for research, programs, and policy. *Public Health Reports* 1995;110:395-402.

19. Capurro H, Konichezky S, Fonseca D, Caldeyro-Barcia R. A simplified method for diagnosis of gestational age in newborn infant. *J Pediat* 1978;93:120-122.

20. Sokol RJ, Martier SS, Ager JW. The T-ACE questions: Practical prenatal detection of risk-drinking. *Am J Obstet Gynecol* 1989;160:863-871.

21. Leal Mdo C, da Gama SG, Ratto KMN, Cunha CB. Use of the modified Kotelchuck index in the evaluation of prenatal care and its relationship to maternal characteristics and birth weight in Rio de Janeiro, Brazil. *Cad Saude Publica* 2004;20(Sup1):S63-S72.

22. Ainamo J, Bay I. (1975) Problems and proposals for recording gingivitis and plaque. *Int Dent J* 1975;25:229-235.

23. Armitage GC. (1995) Clinical evaluation of periodontal diseases. *Periodontol 2000* 1995;7:39-49.

24. Socransky SS, Smith CM, Martin L, Paster BJ, Dewhirst FE, Levin AE. "Checkerboard" DNA-DNA hybridization. *Biotechniques* 1994;17:788-792.

25. Socransky SS, Haffajee AD. Dental biofilms: difficult therapeutic targets. *Periodontol 2000* 2002;28:12-55.

26. Haffajee AD, Socransky SS. Microbial etiologic agents of destructive periodontal diseases. *Periodontol 2000* 1994;5:78-111.

27. Socransky SS, Haffajee AD, Smith CM, Dibart S. Relation of counts of microbial species to clinical status at the site. *J Clin Periodontol* 1991;18:766-775.

28. Socransky SS, Haffajee AD. Peridontal microbial ecology. *Peridontol 2000* 2005;38:135-187.

29. Ashimoto A, Chen C, Bakker I, Slots J. Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. *Oral Microbiol Immunol* 1996;11:266-273.

30. Choi BK, Park SH, Yoo YJ, et al.Detection of major putative periodontopathogens in Korean advanced periodontitis patients using a nucleic acid-based approach. *J Periodontol* 2000;71:1387-1394.

31. Gmur R, Thurnheer T. Direct quantitative differentiation between *Prevotella intermedia* and *Prevotella nigrescens* in clinical specimens. *Microbiology* 2002;148:1379-1387.

32. Kuboniwa M, Amano A, Kimura KR, et al. Quantitative detection of periodontal pathogens using realtime polymerase chain reaction with TaqMan probes. *Oral Microbiol Immunol* 2004;19:168-176.

33. Mayanagi G, Sato T, Shimauchi H, Takahashi N. Detection frequency of periodontitis-associated bacteria by polymerase chain reaction in subgingival and supragingival plaque of periodontitis and healthy subjects. *Oral Microbiol Immunol* 2004;19:379-385.

34. Dasanayake AP, Boyd D, Madianos PN, Offenbacher S, Hills E. The association between Porphyromonas gingivalis-specific maternal serum IgG and low birth weight. *J Periodontol* 2001;72:1491-1497.

35. Haffajee AD, Socransky SS, Taubman MA, Sioson J, Smith DJ. Patterns of antibody response in subjects with periodontitis. *Oral Microbiol Immunol* 1995;10:129-137.

36. Dörtbudak O, Eberhardt R, Ulm M, Persson GR. Periodontitis, a marker of risk in pregnancy for preterm birth. *J Clin Periodontol* 2005;32:45-52.

37. Hasegawa K, Furuichi Y, Shimotsu A, et al. Associations between systemic status, periodontal status, serum cytokine levels, and delivery outcomes in pregnant women with a diagnosis of threatened premature labor. *J Periodontol* 2003;74:1764-1770.

38. Hujoel PP, Drangsholt M, Spiekerman C, DeRoven TA. Periodnotitis-systemic disease associations in the presence of smoking-cusal or coincidental? *Periodontol 2000* 2002;30: 51-60.

39. Gibbs RS, Romero R, Hillier S, Eschenbach DA, Sweet RL. A review of premature birth and subclinical infections. *Am J Obstet Gynecol* 1992;166:1515-1528.

40. Williams CE, Davenport ES, Sterne JA, Sivapathasundaram V, Fearne JM, Curtis MA. Mechanisms of risk in preterm low-birthweight infants. *Periodontol 2000* 2000;23:142-50.

41. Offenbacher S, Heasman PA, Collins JG. Modulation of host PGE_2 secretation as a determinant of periodontal disease expression. *J Periodontol* 1993;64:432-444.

42. Preshaw PM, Heasman PA. Prostaglandin E_2 concentrations in gingival crevicular fluid: observations in untreated chronic periodontitis. *J Clin Periodontol* 2002;29:15-20.

7.6 – Figuras e Tabelas

Figure 1. Flow chart for selecting study subjects

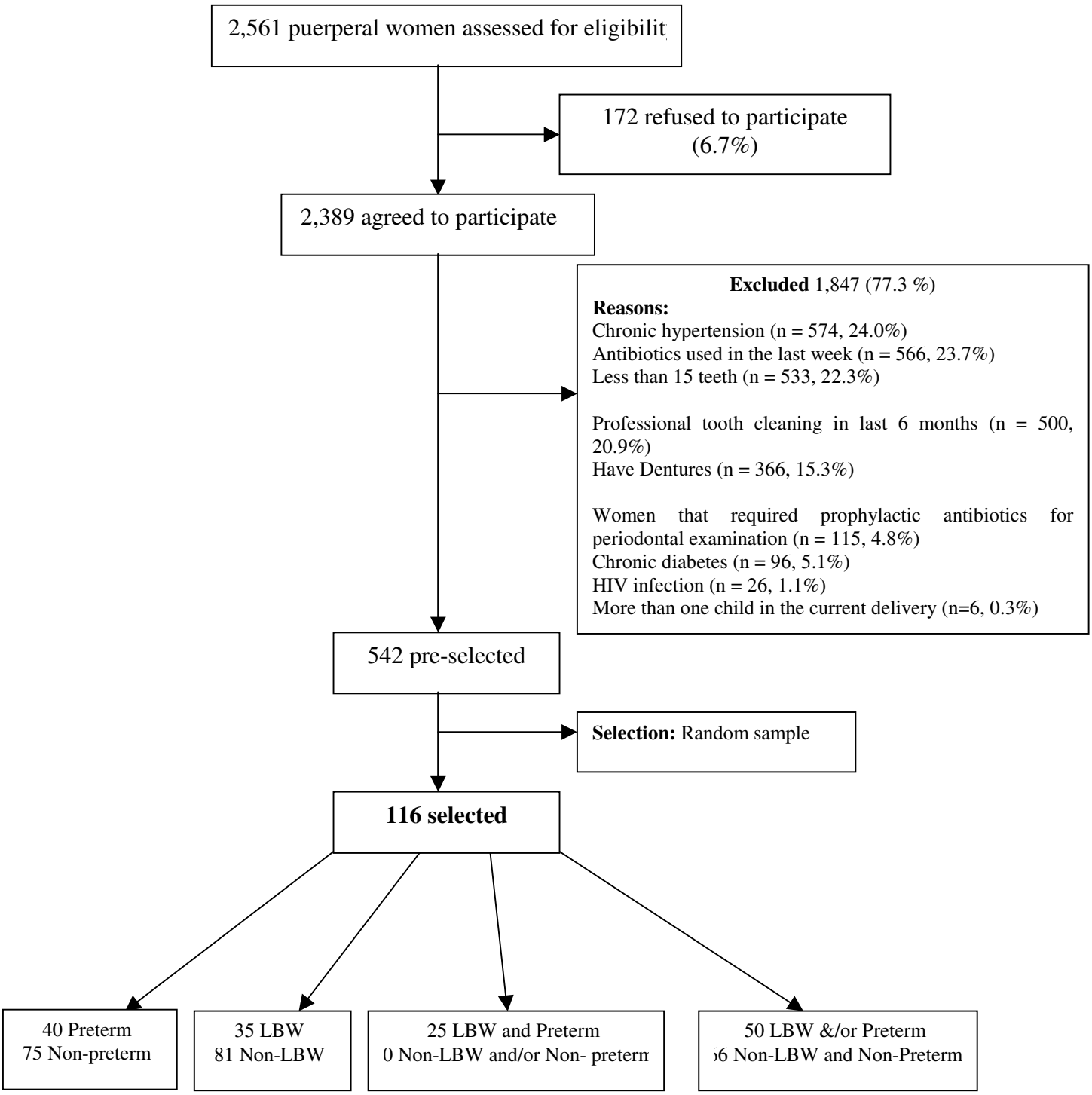
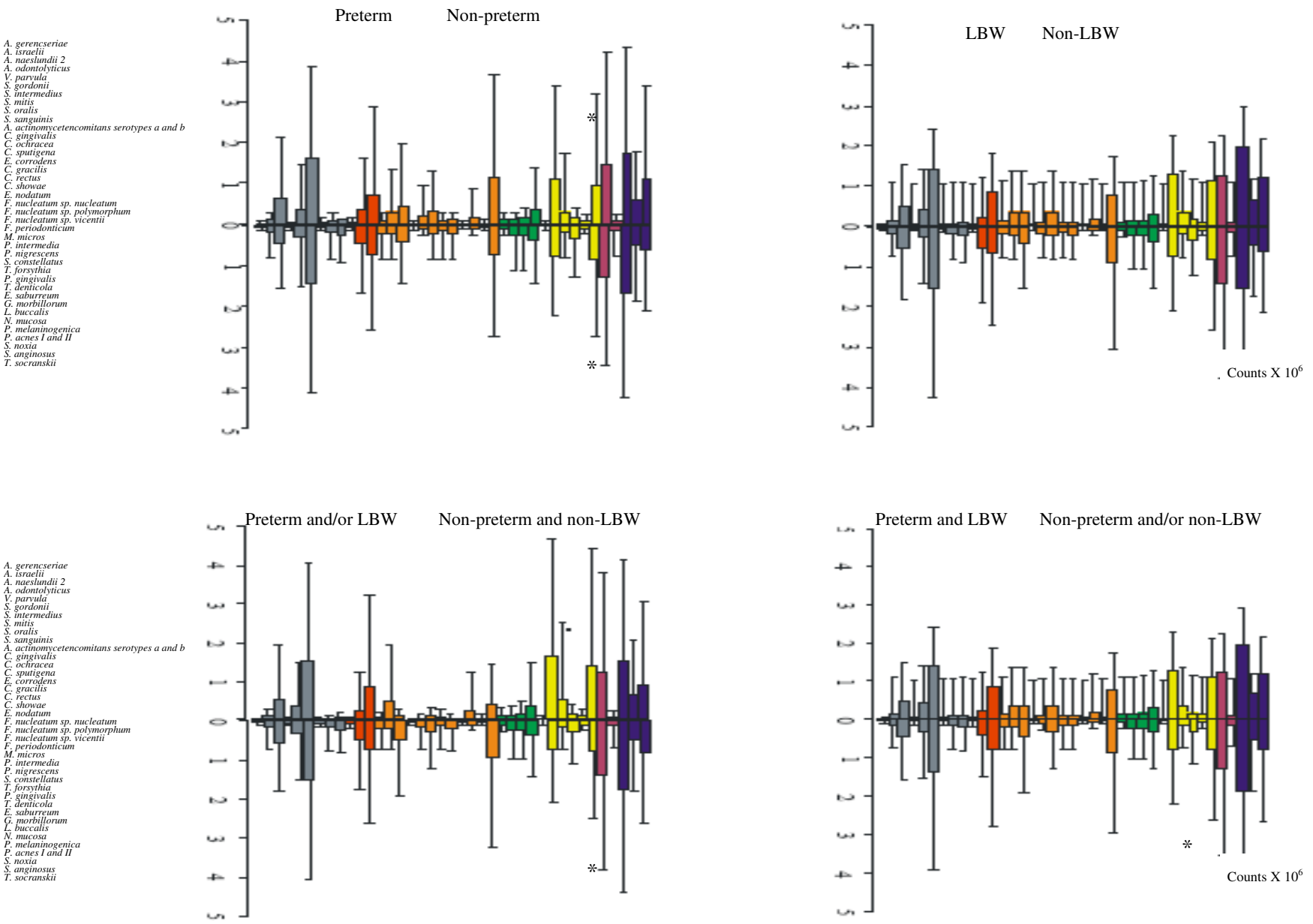
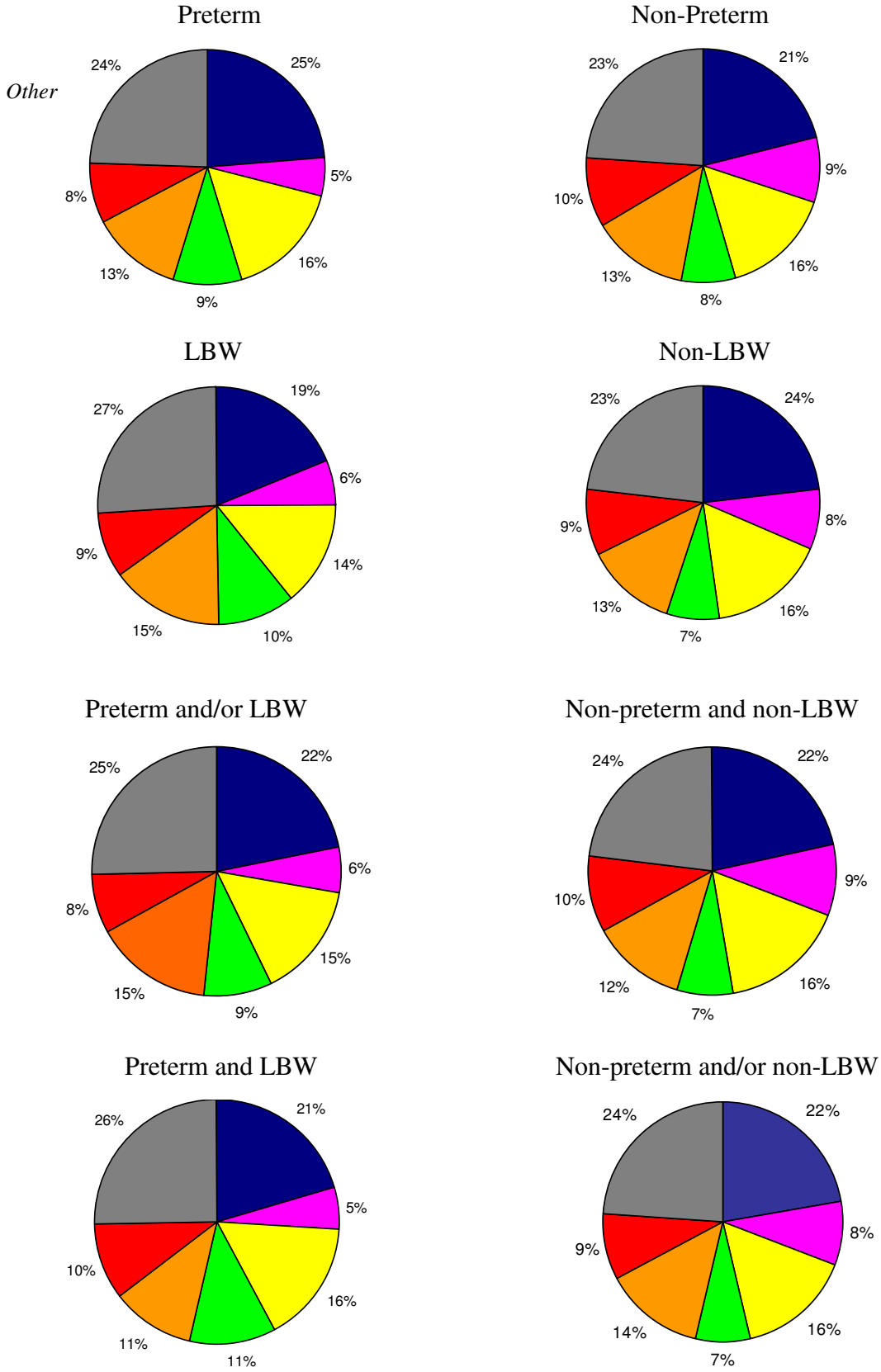


Figure 2. Bar chart of mean counts (X 10^6, ±SD) of individual species in subgingival plaque samples between preterm *vs* non-preterm, LBW *vs* non-LBW, preterm and/or LBW *vs* non-preterm and non-LBW, preterm and LBW *vs* non-preterm and/or non-LBW post-partum women.



The bars represent the mean counts and the whiskers indicate the standard deviation of the mean. Mean counts for each species were computed for a subject and then averaged across subjects in the groups. Significance of differences among groups was sought using Mann-Whitney. *$P < 0.05$. The species were ordered and grouped according to the complexes described by Socransky *et al.* [12]

Figure 3. Pie charts of the mean proportions of each microbial complex in subgingival plaque samples from subjects in the groups



LBW, Low birth weight. The colours in the pie diagrams fit with colours of microbial complexes described by Socransky *et al.*[12] (Table 1). Significance of differences in mean proportions between groups mean values for each complex was tested using the Mann-Whitney test.

Table 1. Bacterial Strains employed for the development of DNA probes.

		Campylobacter showae	51146
Blue complex			
Actinomyces gerencseriae	23860	*Eubacterium nodatum*	33099
Actinomyces israelii	12102	*Fusobacterium nucleatum sp. nucleatum*	25586
Actinomyces naeslundii genospecies 2	43146	*Fusobacterium nucleatum sp. polymorphum*	10953
		Fusobacterium nucleatum sp. vicentii	49256
Purple complex		*Fusobacterium periodonticum*	33693
Actinomyces odontolyticus	17929	*Micromonas micros*	33270
Veillonella parvula	10790	*Prevotella intermedia*	25611
		Prevotella nigrescens	33563
Yellow complex		*Streptococcus constellatus*	12104
Streptococcus gordonii	10558		
Streptococcus intermedius	27335	**Red complex**	
Streptococcus mitis	49456	*Tannerella forsythia*	43037
Streptococcus oralis	35037	*Porphyromonas gingivalis*	33277
Streptococcus sanguinis	10556	*Treponema denticola*	B1
Green complex		**Other species and new DNA probes**	
Actinobacillus actinomycetencomitans	43718		
serotypes a and *b*	29523	*Eubacterium saburreum*	33271
Capnocytophaga gingivalis	33624	*Gemella morbillorum*	27824
Capnocytophaga ochracea	33598	*Leptotrichia buccalis*	14201
Capnocytophaga sputigena	33612	*Neisseria mucosa*	19696
Eikenella corrodens	23834	*Prevotella melaninogenica*	25845
			11827/1182
		Propionybacterium acnes I and II	8
Orange complex		*Selenomonas noxia*	43541
Campylobacter gracilis	33236	*Streptococcus anginosus*	33397
Campylobacter rectus	33238	*Treponema socranskii*	S1

All strains were obtained from the American Type Culture Collection (ATCC), (Rockville, MD), except *Treponema denticola* B1 and *Treponema socranskii* S1 were obtained from the Forsyth Institute, (Boston, MA). Microbial "complexes" were described by Socransky *et al.*[12, 25]

Table 2. Covariate variables of the 116 women examined in the study

	All subjects (n=116)	Preterm (n=40)	Non-preterm (n=75)	*P*	LBW (n=35)	Non-LBW (n=81)	*P*	PB and/or LBW (n=50)	Non-PB and non-LBW (n=66)	*P*	PB and LBW (n=25)	Non-PB and/or non-LBW (n=90)	*P*
GA, weeks, mean ± SD	37.5 ± 3.4	33.6 ± 2.2	39.6 ± 1.6	<0.05 †	34.2 ± 3.0	38.9 ± 2.5	<0.05 †	34.4 ± 2.7	39.8 ± 1.6	<0.05 †	32.8 ± 2.2	38.8 ± 2.4	<0.05 †
Birth weight (g), mean ± SD	2862.0 ± 768.2	2271.4 ± 692.8	3186.6 ± 601.5	<0.05 ‡	1949.3 ± 479.8	3256.3 ± 478.1	<0.05 ‡	2250.4 ± 628.2	3325.3 ± 490.9	<0.05 ‡	1862.5 ± 526.0	3147.6 ± 564.7	<0.05 ‡
Type of birth, %				NS			NS			NS			NS
Normal birth	50.0	37.5	56.0		48.6	50.6		46.0	53.0		36.0	53.3	
Caesarian	50.0	62.5	44.0		51.4	49.4		54.0	47.0		64.0	46.7	
Neonate sex, % Male	51.7	42.5	56.0	NS	48.6	53.1	NS	46.0	56.1	NS	44.0	53.3	NS
Age, mean ± SD	34.4 ± 4.2	34.5 ± 3.8	34.3 ± 4.5	NS †	34.0 ± 3.8	34.6 ± 4.4	NS †	34.6 ± 4.0	34.2 ± 4.5	NS †	33.6 ± 3.4	34.6 ± 4.5	NS †
Corporal Mass Index, mean ± SD *	23.7 ± 4.7	24.0 ± 5.6	23.5 ± 4.0	NS ‡	23.0 ± 5.4	24.1 ± 4.3	NS ‡	23.3 ± 5.5	24.1 ± 3.8	NS ‡	23.9 ± 5.6	23.6 ± 4.4	NS ‡
Ethnicity, %				NS			NS			NS			NS
White	31.9	25.0	34.7		34.3	30.9		32.0	31.8		24.0	33.3	
Brown	48.3	50.0	48.0		42.8	50.6		44.0	51.5		52.0	47.8	
Black	19.8	25.0	17.3		22.9	18.5		24.0	16.7		24.0	18.9	
Marital Status, %				NS			NS			NS			NS
Married/ partner	86.2	82.5	88.0		80.0	88.9		82.0	89.4		80.0	87.8	
Young	8.6	10.0	8.0		8.6	8.6		10.0	7.6		8.0	8.9	
Divorced / Widow	5.2	7.5	4.0		11.4	2.5		8.0	3.0		12.0	3.3	
Income (minimum wages), %				NS			NS			NS			NS
≥ ½	48.3	50.0	48.0		40.0	51.9		46.0	50.0		44.0	50.0	
<½	51.7	50.0	52.0		60.0	48.1		54.0	50.0		56.0	50.0	
Schooling (years), %				NS			NS			NS			NS
> 8 years	43.1	50.0	40.0		42.9	43.2		46.0	40.9		48.0	42.2	
≤ 8 years	56.9	50.0	60.0		57.1	56.8		54.0	59.1		52.0	57.8	
Housing conditions, % *				NS			NS			NS			NS
Adequate	61.7	52.5	67.6		50.0	66.7		53.1	68.2		48.0	66.3	
Inadequate	38.3	47.5	32.4		50.0	33.3		46.9	31.8		52.0	33.7	
PN care, % *				<0.05			<0.05			<0.05			NS
More than adequate/Adequate	15.0	5.4	20.0		9.7	17.1		6.7	21.0		8.7	16.7	
Intermediate	45.8	37.8	50.0		32.3	51.3		35.6	53.2		34.8	48.8	
Inadequate	39.2	56.8	30.0		58.0	31.6		57.7	25.8		56.5	34.5	
Risk-drinking, %	5.2	7.5	4.0	NS	11.4	2.5	NS	8.0	3.0	NS	12.0	3.3	NS
Number of cigarettes smoked, %				NS			NS			NS			NS
Did not smoke	88.8	87.5	89.4		80.0	92.6		86.0	91.0		80.0	91.2	
< 5	6.0	7.5	5.3		11.4	3.7		8.0	4.5		12.0	4.4	
≥ 5	5.2	5.0	5.3		8.6	3.7		6.0	4.5		8.0	4.4	
Previous preterm, % *	24.5	43.3	15.9	<0.05	46.4	15.2	<0.05	45.0	9.3	<0.05	44.4	20.0	<0.05
Previous LBW, % *	21.3	30.0	17.5	NS	42.9	12.1	<0.05	35.0	11.1	<0.05	38.9	17.3	<0.05
Hypertension, % *	25.2	38.5	18.7	<0.05	34.3	21.3	NS	34.7	18.2	<0.05	40.0	21.3	NS
Gestational diabetes, % *	5.3	10.5	2.7	NS §	5.7	5.1	NS §	8.3	3.0	NS §	8.0	4.5	NS §
Urinary infection, % *	26.1	23.1	28.0	NS	22.9	27.5	NS	20.4	30.3	NS	28.0	25.8	NS

LBW: Low Birth Weight

*: missing data (n): Corporal Mass Index (33), Housing conditions (1), PN care (9), Previous preterm (22), Previous LBW (22), Hypertension (1), Pre-eclampsia (1), Gestational diabetes (2), Urinary infection (1)

P values by χ^2 test except , † Mann-Whitney test, ‡ Test t, § Exact's Fisher test NS: not significant at the level of $p > 0.05$

Table 3. Periodontal clinical parameters between cases and control groups of women.

		LBW (n = 35)	Non-LBW (n = 81)		Preterm (n = 40)	Non-Preterm (n = 75)		LBW and Preterm (n = 25)	Non-LBW or non-Preterm (n = 90)		LBW and/or Preterm (n = 50)	Non-LBW and Non-Preterm (n = 66)	
				*P**			*P**			*P**			*P**
N of teeth		23.7 ± 3.6	23.1 ± 3.5	0.262	23.3 ± 3.5	23.2 ± 3.5	0.918	23.9 ± 3.6	23.1 ± 3.5	0.279	23.3 ± 3.5	23.2 ± 3.5	0.816
VPI	X	47.4 ± 43.4	56.3 ± 38.6	0.396	42.1 ± 42.8	59.7 ± 37.8	0.013	41.2 ± 43.4	57.0 ± 38.9	0.091	46.3 ± 43.0	59.1 ± 37.1	0.080
	%	49.6 ± 44.3	62.0 ± 41.4	0.282	45.3 ± 45.8	65.1 ± 39.5	0.017	43.4 ± 45.4	62.3 ± 41.2	0.067	49.3 ± 44.9	65.1 ± 39.6	0.079
BOP	X	17.7 ± 28.8	16.7 ± 21.8	0.878	14.3 ± 24.2	18.4 ± 24.0	0.117	16.4 ± 27.2	17.1 ± 23.3	0.741	15.6 ± 26.1	18.0 ± 22.4	0.169
	%	18.9 ± 30.0	18.3 ± 24.6	0.868	15.3 ± 26.1	20.1 ± 26.5	0.112	17.8 ± 29.7	18.6 ± 25.5	0.726	16.6 ± 27.1	19.9 ± 25.6	0.163
Calculus	X	7.5 ± 21.5	4.1 ± 11.8	0.943	7.2 ± 21.2	4.1 ± 11.2	0.323	9.5 ± 24.9	4.0 ± 11.5	0.996	6.3 ± 19.3	4.2 ± 11.6	0.314
	%	8.5 ± 25.0	5.1 ± 15.8	0.917	8.4 ± 25.2	5.0 ± 14.9	0.325	10.9 ± 29.1	4.9 ± 15.2	1.000	7.3 ± 22.8	5.3 ± 15.7	0.303
PPD	X	2.3 ± 0.5	2.5 ± 0.6	0.050	2.3 ± 0.5	2.5 ± 0.5	0.032	2.3 ± 0.5	2.5 ± 0.5	0.048	2.3 ± 0.5	2.5 ± 0.5	0.027
□ 4 mm	X	14.4 ± 15.9	16.5 ± 13.9	0.243	15.0 ± 16.0	16.3 ± 13.8	0.358	14.3 ± 16.9	16.3 ± 13.9	0.280	14.9 ± 15.5	16.5 ±13.8	0.284
	%	9.6 ± 10.3	12.0 ± 10.5	0.163	10.6 ± 10.9	11.7 ± 10.3	0.337	9.7 ± 11.2	11.7 ± 10.3	0.230	10.3 ± 10.4	12.1 ± 10.5	0.222
□ 5 mm	X	4.3 ± 6.7	4.0 ± 6.4	0.683	4.9 ± 7.2	3.6 ± 6.1	0.652	3.8 ± 6.6	4.1 ± 6.5	0.904	5.0 ± 7.1	3.3 ± 5.9	0.367
	%	2.9 ± 4.6	3.0 ± 5.1	0.806	3.6 ± 5.3	2.7 ± 4.8	0.637	2.6 ± 4.7	3.1 ± 5.1	0.840	3.6 ± 5.1	2.5 ± 4.8	0.402
□ 6 mm	X	0.1 ± 0.6	0.3 ± 1.1	0.678	0.3 ± 1.4	0.2 ± 0.7	0.465	0.0 ± 0.0	0.3 ± 1.1	0.055	0.3 ± 1.4	0.2 ± 0.6	0.605
	%	0.1 ± 0.4	0.2 ± 0.8	0.670	0.2 ± 0.9	0.1 ± 0.5	0.500	0.0 ± 0.0	0.2 ± 0.7	0.055	0.2 ± 0.9	0.1 ± 0.4	0.576
CAL	X	2.4 ± 0.6	2.6 ± 0.5	0.138	2.4 ± 0.6	2.6 ± 0.6	0.112	2.4 ± 0.6	2.6 ± 0.5	0.110	2.5 ± 0.6	2.6 ± 0.5	0.120
□ 3 mm	X	66.1 ± 41.9	73.6 ± 33.9	0.262	63.7 ± 38.1	75.5 ± 35.4	0.077	63.7 ± 41.1	73.5 ± 35.2	0.196	65.4 ± 39.3	75.8 ± 33.8	0.095
	%	45.6 ± 27.1	53.6 ± 24.0	0.140	45.5 ± 25.7	54.3± 24.6	0.083	44.3 ± 27.2	53.1 ± 24.5	0.140	46.2 ± 25.9	54.9 ± 24.00	0.072
□ 4 mm	X	19.1 ± 18.8	20.4 ± 16.3	0.452	19.1 ± 17.1	20. 5 ± 17.1	0.526	18.2 ± 18.3	20.5 ± 16.8	0.361	19.6 ± 17.7	20.4 ± 16.6	0.585
	%	13.3 ± 12.9	15.4 ± 13.6	0.375	14.0 ± 12.6	15.3 ± 13.8	0.630	13.0 ± 13.0	15.4 ± 13.5	0.332	14.1 ± 12.6	15.4 ± 13.9	0.632
□ 5 mm	X	5.1 ± 7.7	5.5 ± 9.0	0.605	5.7 ± 8.0	5.3 ± 9.0	0.615	4.4 ± 7.7	5.7 ± 8.9	0.603	5.9 ± 7.9	5.0 ± 9.1	0.167
	%	3.6 ±5.3	4.4 ± 7.7	0.652	4.2 ± 6.0	4.1 ± 7.6	0.583	3.1 ± 5.5	4.4 ± 7.4	0.571	4.3 ± 5.7	4.0 ± 7.9	0.161
□ 6 mm	X	0.6 ± 2.1	1.0 ± 2.8	0.172	0.7 ± 2.4	1.0 ± 2.8	0.558	0.5 ± 2.4	1.0 ± 2.7	0.018	0.7 ± 2.2	1.0 ± 2.9	0.873
	%	0.4 ± 1.4	0.9 ± 2.6	0.164	0.5 ± 1.6	0.9 ± 2.6	0.558	0.3 ± 1.6	0.9 ± 2.5	0.017	0.5 ± 1.4	0.9 ± 2.8	0.888
PPD □ 4 mm and CAL □ 3 mm	X	14.3 ± 15.8	16.4 ± 13.9	0.241	15.0 ± 16.0	16.2 ±13.8	0.356	14.2 ± 16.9	16.2 ± 13.9	0.277	14.9 ± 15.4	16.5 ± 13.8	0.284
	%	9.6 ± 10.2	12.0 ± 10.4	0.156	10.5 ± 10.9	11.7 ± 10.3	0.326	9.7 ± 11.1	11.7 ± 10.3	0.217	10.3 ± 10.4	12.1 ± 10.5	0.217

*P** refers to Mann-Whitney test
LBW: low birth weight, VPI: Visible Plaque Index, BOP: Bleeding on Probing,
PPD: Periodontal Pocket depth, CAL: Clinical Attachment Level

Table 4. Unadjusted Odds Ratio of relationship between periodontal disease in PTLBW mothers and controls in different pregnancy outcome groups by percentiles of Periodontal Inflammatory Load (PIL)

	LBW			Preterm			LBW and preterm			LBW and/or preterm		
Levels	OR	CI	*P**	OR	CI	*P**	OR	CI	*P**	OR	CI	*P**
1	1	-	-	1	-	-	1	-	-	1	-	-
2	0.76	0.26-2.27	0.625	0.86	0.30-2.49	0.783	1.11	0.35-3.55	0.860	0.62	0.22-1.77	0.371
3	0.58	0.19-1.80	0.348	0.43	0.14-1.37	0.156	0.44	0.11-1.70	0.232	0.50	0.17-1.45	0.200
4	0.51	0.16-1.63	0.254	0.55	0.24-2.14	0.551	0.57	0.16-2.07	0.389	0.61	0.21-1.76	0.356

*P** refers to Odds Ratio
PIL: sum of all periodontal pocket depths measurements over three millimetres of sites with loss of periodontal attachment
Percentiles: P_{25} = 12, P_{50} = 56, P_{75} = 114 of sum of all PPD ≥ 4 mm of sites with loss of attachment
Level 1: 0 to 11 mm, the sum of all PPD ≥ 4 mm
Level 2: 12 to 55 mm, the sum of all PPD ≥ 4 mm
Level 3: 56 to 113 mm, the sum of all PPD ≥ 4 mm
Level 4: 114 mm and over, the sum of all PPD ≥ 4 mm.

8 – Conclusões da tese

A revisão sistemática sobre a associação entre a doença periodontal e a prematuridade e/ou baixo peso ao nascer (P/BPN) revelou uma inconsistência de achados. A heterogeneidade entre os estudos em relação ao método de mensuração da doença periodontal e dos desfechos indesejáveis da gestação, e falta de controle adequado de variáveis de confusão na maioria dos estudos não permitem adequadas conclusões sobre o real efeito da doença periodontal sobre os desfechos da gestação.

O método proposto para mensurar a doença periodontal, *Periodontal Inflammatory Load* – (PIL) mostrou-se válido. A associação da medida PIL com a carga de patógenos periodontais e a ausência de correlação com as espécies não relacionadas com a doença periodontal sugerem que a medida PIL é um método clínico adequado para avaliar a doença periodontal.

Medidas clínicas da doença periodontal destrutiva observadas em uma amostra de 542 mulheres não foram mais severas nos grupos de puérperas que tiveram bebês prematuros, com baixo peso ao nascer (BPN), prematuros e/ou com BPN, e prematuros e com BPN, em relação aos respectivos grupos controles. A doença periodontal não esteve associada à P/BPN utilizando 15 diferentes pontos de corte para a definição de periodontite. Observou-se uma redução nas estimativas de associação entre a doença periodontal e a P/BPN entre os grupos de mulheres com mais doença periodontal destrutiva. As puérperas dos grupos controles apresentaram medidas clínicas mais severas de doença periodontal destrutiva em relação àquelas presentes nos grupos de casos.

Os resultados microbiológicos corroboraram com os achados clínicos. Não se encontrou associação entre os níveis e proporções de patógenos periodontais identificados em áreas subgengivais das puérperas com os desfechos de prematuridade, baixo peso ao nascer (BPN), prematuridade e/ou BPN, e prematuridade e/ou BPN. Os grupos de casos e controles não diferiram em relação à média de proporções dos complexos microbianos. Os níveis de *Treponema socranskii* estiveram menores nas puérperas com prematuridade, e com prematuridade e BPN.

A doença periodontal não foi um fator de risco para a prematuridade, baixo peso ao nascer, prematuridade e/ou baixo peso ao nascer, e prematuridade e baixo peso ao nascer em mulheres com 30 anos de idade ou mais. A hipótese de associação entre a doença periodontal e a P/BPN foi rejeitada.

9 - Referências bibliográficas gerais

1. Arafat AH. Periodontal status during pregnancy. J Periodontol 1970; 45:641-3.

2. Lopatin DE, Kornman KS, Loesche WJ. Modulation of immunoreactivity to periodontal disease-associated microorganisms during pregnancy. Infect Immun 1980; 28:170-5.

3. Lyon LZ, Wishan MS. Management of pregnant dental patients. Dent Clin North Am 1965; Nov:623-34.

4. Miyazaki H, Yamashita Y, Shirahama R, Goto-Kimura K, Shimada N, Sogame A, et al. Periodontal condition of pregnant women assessed by CPITN. J Clin Periodontol 1991; 18:751-4.

5. Muramatsu Y, Takaesu Y. Oral health status related to subgingival bacterial flora and sex hormones in saliva during pregnancy. Bull Tokyo Dent Coll 1994; 35:139–51.

6. Samant A, Malik CP, Chabra SK, Devi PK. Gingivitis and periodontal disease in pregnancy. J Periodontol 1976; 47:415-8.

7. Silness J, Löe H. Periodontal disease in pregnancy. 3. Response to local treatment. Acta Odontol Scand 1966; 24:747-59.

8. Ziskin D, Blackberg S, Stout A. The gingivae during pregnancy. Surg Gynecol Obstet 1933; 57:719–26.

9. Collins JG, Smith MA, Arnold RR, Offenbacher S. Effects of *Escherichia coli* and *Porphyromonas gingivalis* lipopolysaccharide on pregnancy outcome in the golden hamster. Infect Immun 1994; 62:4652-5.

10. Collins JG, Windley HW 3rd, Arnold RR, Offenbacher S. Effects of a *Porphyromonas gingivalis* infection on inflammatory mediator response and pregnancy outcome in hamsters. Infect Immun 1994; 62:4356-61.

11. Offenbacher S, Katz V, Fertik G, Collins J, Boyd D, Maynor G, et al. Periodontal infections as a risk factor for preterm low birth weight. J Periodontol 1996; 67:1103-13.

12. Maier A, Orban B. Gingivitis in pregnancy. Oral Surgery 1949; 2:334–73.

13. Hilming F. Gingivitis of pregnancy; clinical and etiological study with emphasis on the importance of vitamin. C Tandlaegebladet 1950; 54:809-11.

14. Raynal MT. Gingivitis in pregnancy, the co-called gingivitis of pregnant women. Revue Stomatol 1950; 51:64-80.

15. Tilakaratne A, Soory 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; 27:787–92.

16. Löe H, Silness J. Periodontal disease in pregnancy I. Prevalence and severity. Acta Odontol Scand 1963; 21:533-51.

17. Löe H. Periodontal changes in pregancy. J Periodontol 1965; 36:209-17.

18. Jensen J, Liljemark W, Bloomquist C. The effect of female sex hormones on subgingival plaque. J Periodontol 1981; 52:599-602.

19. Raber-Durlacher JE, van Steenbergen TJ, Vander Velden U, de Graaff J, Inpijn L. Experimental gingivitis during pregnancy and post-partum: clinical, endocrinological, and microbiological aspects. J Clin Periodontol 1994; 21:549-58.

20. World Health Organization. International classification of diseases: manual of the international statistical classification of diseases, injuries, and causes of death. Geneva: World Health Organization; 1977.

21. Orban B. Classification and nomenclature of periodontal diseases (Based on pathology, etiology, and clinical picture). J Periodontol 1942; 13:88-91.

22. Socransky SS, Haffajee AD, Goodson JM, Lindhe J. New concepts of destructive periodontal disease. J Clin Periodontol 1984; 11:21-32.

23. Kornman KS, Loeshce WJ. The subgingival microbial flora during pregnancy. J Periodontol Res 1980; 15:111-22.

24. Raber-Durlacher JE, Leene W, Palmer-Bouva CC, Raber J, Abraham-Inpijn L. Experimental gingivitis during pregnancy and post-partum: immunohistochemical aspects. J Periodontol 1993; 63:211-8.

25. Arafat AH. Periodontal status during pregnancy. J Periodontol 1974; 45:641-3

26. O'Neil TC. Maternal T-lymphocyte response and gingivitis in pregnancy. J Periodontol 1979; 50:178-84.

27. Moller M, Thomsen AC, Borch K, Dinesen K, Zdravkovic M. Rupture of fetal membranes and premature delivery associated with group B streptococci in urine of pregnant women. Lancet 1984; 2:69-70.

28. Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. Bull WHO 1987; 65:663-737.

29. Romero R, Mazor M. Infection and preterm labor. Clin Obstet Gynecol 1988; 31:553-84.

30. Gibbs RS, Romero R, Hillier SL, Eschenbach DA, Sweet RL. A review of premature birth and subclinical infections. Am J Obstet Gynecol 1992; 166:1515-28.

31. Hillier SL, Nugent RP, Eschenbach DA, Krohn MA, Gibbs RS, Martin DH, et al. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. New Engl J Medicine 1995; 333:1737-42.

32. Minkoff H, Grunebaum AN, Schwartz RH. Risk factor for prematurity and premature rupture of the membranes: A prospective study of the vaginal flora in pregnancy. Am J Obstet Gynecol 1984; 150:965-72.
33. McDonald HM, O'Loughlin JA, Jolley P, Gigneswaran P, McDonald PJ. Vaginal infections and preterm labor. Br J Obstet Gynecol 1991; 98:427-35.
34. Leigh L, Stoll BJ, Rahman M, McGowan J Jr. Psudomonas aeruginosa infection in very low birth weight infants: a case-control study. Paediatr Infect Dis J 1995; 14:367-71.
35. Gibbs RS. The relationship between infections and adverse pregnancy outcomes: an overview. Ann Periodontol 2001; 6:153-63.
36. Rezende J, Montenegro CAB. Obstetrícia Fundamental. Rio de Janeiro: Guanabara Koogan; 1992.
37. Bernal AL, Hansell DJ, Soler RC, Keeling JW, Turnbull AC. Prostaglandins, chorioamnionitis and preterm labour. Eur J Obst Gynaecol 1987; 94:1156-8.
38. Romero R, Wu YK, Mazor M, Hobbins JC, Mitchell MD. Amniotic fluid prostaglandin E2 in preterm lavor. Protaglandins Leukot Essent Fatty Acids. 1988; 34:141-5.
39. Beutler B, Cerami A. The biology of cachectin/TNF--a primary mediator of the host response. Annu Rev Immunol 1989; 7:625-55.
40. Romero R, Baumann P, Gomez R. The relationship between spontaneous rupture of membranes, labor, and microbial invasion of the amniotic cavity and amniotic fluid concentrations of prostaglandins, and thromboxane B2 in term pregnancy. Am J Obstet Gynecol 1994;168:1654-8.
41. Newman MG. Current concepts of the pathogenesis of periodontal disease. Microbiology emphasis. J Periodontol 1985; 56:734-9.
42. Page RC, Kornman KS. The pathogenesis of human periodontitis: an introduction. Periodontol 2000 1997; 14:9-11.
43. Page RC. The role of inflammatory mediators in the pathogenesis of periodontal disease. J Periodontal Res 1991;26(3 Pt 2):230–242.
44. Skarnes RC, Harper MJ. Relationship between endotoxin-induced abortion and the synthesis of prostaglandin F. Prostaglandins 1972; 1:191-203
45. Laning JC, Hilbelink DR, Chen LT. Teratogenic effects of endotoxin on the golden hamster. Teratog Carcing Mutagen 1983; 3:145-9.
46. Coid CR. Bacteial endotoxin and impaired fetal development. Experimentia 1976; 32:735-6.

47. Ornoy A, Altshuler G. Maternal endotoxemia, fetal anomalies, and central nervous system damage: a rat model of a human problem. Am J Obst Gynecol 1976; 124:196-204.

48. Dombroski RA, Woodard DS, Harper MJ, Gibbs RS. A rabbit model for bateria-induced preterm pregnancy loss. Am J Obstet Gynecol 1990; 163 (6 Pt 1):1938-43.

49. Rivera MT, Marques de Araújo S, Lucas R, Deman J, Truyens C, Defresne MP, et al. High tumor necrosis factor alpha (TNF-□) production in *Trypanosoma cruzi*-infected pregnant mice and increased TNF-□ gene transcription in their offspring. Infec Immun 1995; 63:591-5.

50. McDuffie RS Jr, Sherman MP, Gibbs RS. Amniotic fluid tumor necrosis factor-alpha and interleukin-1 in a rabbit model of bacterially induced preterm pregnancy loss. Am J Obstet Gynecol 1992; 167:1583-8

51. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent Jr RL. Microbial complexes in subgingival plaque. J Clin Periodontol 1998; 25:134-44.

52. Williams CE, Davenport ES, Sterne JA, Sivapathasundaram V, Fearne JM, Curtis MA Mechanisms of risk in preterm low-birthweight infants. Periodontol 2000 2000; 23:142-150.

53. Lin D, Smith MA, Champagne C, Elter J, Beck J, Offenbacher S. *Porphyromonas gingivalis* Infection during pregnancy increases maternal Tumor Necrosis Factor alpha, suppresses maternal Interleukin-10, and enhances fetal growth restriction and resorption in mice. Infect Immun 2003; 71:5156–62.

54. Lin D, Smith MA, Champagne C, Elter J, Champagne C, Downey CL, Beck J, Offenbacher. *Porphyromonas gingivalis* infection in pregnant mice is associated with placental dissemination, an increase in the placental Th1/Th2 cytokine ratio, and fetal growth restriction. Infect Immun 2003: 71:5163–8.

55. Offenbacher S, Riche EL, Barros SP, Bobetsis YA, Lin D, Beck JD. Effects of maternal Campylobacter rectus infection on murine placenta, fetal and neonatal survival, and brain development. J Periodontol. 2005; 76 (11 Suppl):2133-43.

56. Breborowicz GH, Szymanjiewicz M, Anholcer A, Gadzinowsku J. Wezesniactwo problem oilozniczy i neonatologiczny. Medipress Ginekol Poloz 1999; 5:3-12.

57. Billings F. Chronic focal infection and their etiologic relations to arthritis. Arch Intern Med 1912; 9:484-498.

58. Vettore MV, Sheiham A, Peres MA. Low birth weight and periodontal diseases association. Rev Saude Publica 2006; 40:184-5.

59. Andrade CLT, Szwarcwald CL, Gama SGN, Leal Mdo C. Desigualdades sócio-econômicas do baixo peso ao nascer e da mortalidade perinatal no Município do Rio de Janeiro, Cad Saude Publica 2004; 20 (1 Suppl):S44-S51.

60. Flores-de-Jacoby L, Bruchmann S, Mengel R, Zafiropoulos GGK. Periodontal conditions in Rio de Janeiro city (Brazil) using CPITN. Community Dent Oral Epidemiol 1991; 19:127-8.

61. Fleiss JL. Statistical Methods for rates and proportions. New York: John Wiley & Sons; 1981.

62. Alexander GR, Tompkins ME, Petersen DJ, Hulsey TC, Mor J. Discordance between LMP-based and clinically estimated gestational age: implications for research, programs, and policy. Public Health Reports 1995; 110:395-402.

63. Capurro H, Konichezky S, Fonseca D, Caldeyro-Barcia R. A simplified method for diagnosis of gestational age in newborn infant. J Pediat 1978; 93:120-2.

64. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J 1975; 25:229-35.

65. Ewing JA. Detecting alcoholism. The CAGE questionnaire. JAMA 1984; 12:1905-7.

66. Sokol RJ, Martier SS, Ager JW. The T-ACE questions: Practical prenatal detection of risk-drinking. Am J Obstet Gynecol 1989; 160:863-71.

67. Vasconcelos MTL, Anjos LA. A simplified method for assessing physical activity level values for a country or study population. Eur J Clin Nutr 2003; 57:1025-33.

68. Biaggio AMB, Natalício L, Spielberger CD. Desenvolvimento da forma experimental em português do Inventário de Ansiedade Traço-Estado (IDATE). Arq Bras Psic Apl 1977; 29:31-44.

69. Hathaway SR, McKinley JC. (A. Benko & R.J.P. Simões, Trads.) Inventário Multifásico Minnesota de Personalidade – Manual. Rio de Janeiro: Centro de Psicologia Aplicada; 1971.

70. Leal Mdo C, da Gama SG, Ratto KMN, Cunha CB. Use of the modified Kotelchuck index in the evaluation of prenatal care and its relationship to maternal characteristics and birth weight in Rio de Janeiro, Brazil. Cad Saude Publica 2004; 20 (1 Suppl):S63-S72.

71. Leal Mdo C, da Gama SG, Campos MR, Cavalini LT, Garbayo LS, Brasil CLP, et al. Fatores associados à morbi-mortalidade perinatal em uma amostra de maternidades públicas e privadas do Município do Rio de Janeiro, 1999-2001. Cad Saude Publica 2004; 20 (1 Suppl):S20-S33.

72. Socransky SS, Smith CM, Martin L, Paster BJ, Dewhirst FE, Levin AE. "Checkerboard" DNA-DNA hybridization. Biotechniques 1994; 17:788-92.

73. Haffajee AD, Cugini MA, Dibart S, Smith C, Kent Jr RL, Socransky SS. The effect of SRP on the clinical and microbiological parameters of periodontal diseases. J Clin Periodontol 1997; 24:324-34.

74. Feinberg AP, Vogeilsten B. A technique for radiolabeling DNA restriction endonuclease fragments to high specificity activity. Annal Biochem 1983; 132:6.

75. Haffajee AD, Socransky SS. Microbial etiologic agents of destructive periodontal diseases. Periodontol 2000 1994; 5:78-111.

76. Moore WEC, Moore VH. The bacteria of periodontal diseases. Periodontol 2000 1994; 5:66–77.

77. Dörtbudak O, Eberhardt R, Ulm M, Persson GR. Periodontitis, a marker of risk in pregnancy for preterm birth. J Clin Periodontol 2005; 32:45-52.

78. Radnai M, Gorzó I, Nagy E, Urbán E, Novák T, Pál A. A possible association between preterm birth and early periodontitis. J Clin Periodontol 2004; 31:736-41.

79. Lunardelli AN, Peres MA. Is there an association between periodontal disease, prematurity and low birth weight? A population-based study. J Clin Periodontol 2005; 32:938-46.

80. Rajapakse PS, Nagarathne M, Chandrasekra KB, Dasanayake AP. Periodontal disease and prematurity among non-smoking Sri Lankan women. J Dent Res 2005; 84:274-7.

81. Jeffcoat MK, Hauth JC, Geurs NC, Reddy MS, Cliver SP, Hodgkins PM, et al. Periodontal disease and preterm birth: results of pilot intervention study. J Periodontol 2003; 74:1214-8.

82. Jarjoura K, Devine PC, Perez-Delboy A, Herrera-Abreu M, D'alton M, Papapanou PN. Markers of periodontal infection and preterm birth. Am J Obstet Gynecol 2005; 2:513-9.

83. Moliterno LF, Monteiro B, Figueredo CMS, Fischer RG. Association between periodontitis and low birth weight: a case-control study. J Clin Periodontol 2005; 32:886-90.

84. López NJ, Smith PC, Gutierrez J. Higher risk of preterm birth and low birth weight in women with periodontal disease. J Dent Res 2002; 81:58-63.

85. Moore S, Ide M, Coward PY, Randhawa M, Borkowska E, Baylis R, et al. A prospective study to investigate the relationship between periodontal disease and adverse pregnancy outcome. Brit Dent J 2004; 197:251-8.

86. Marin C, Segura-Egea JJ, Martinez-Sahuquillo A, Bullon P. Correlation between infant birth weight and mother's periodontal status. J Clin Periodontol 2005; 32:299-304.

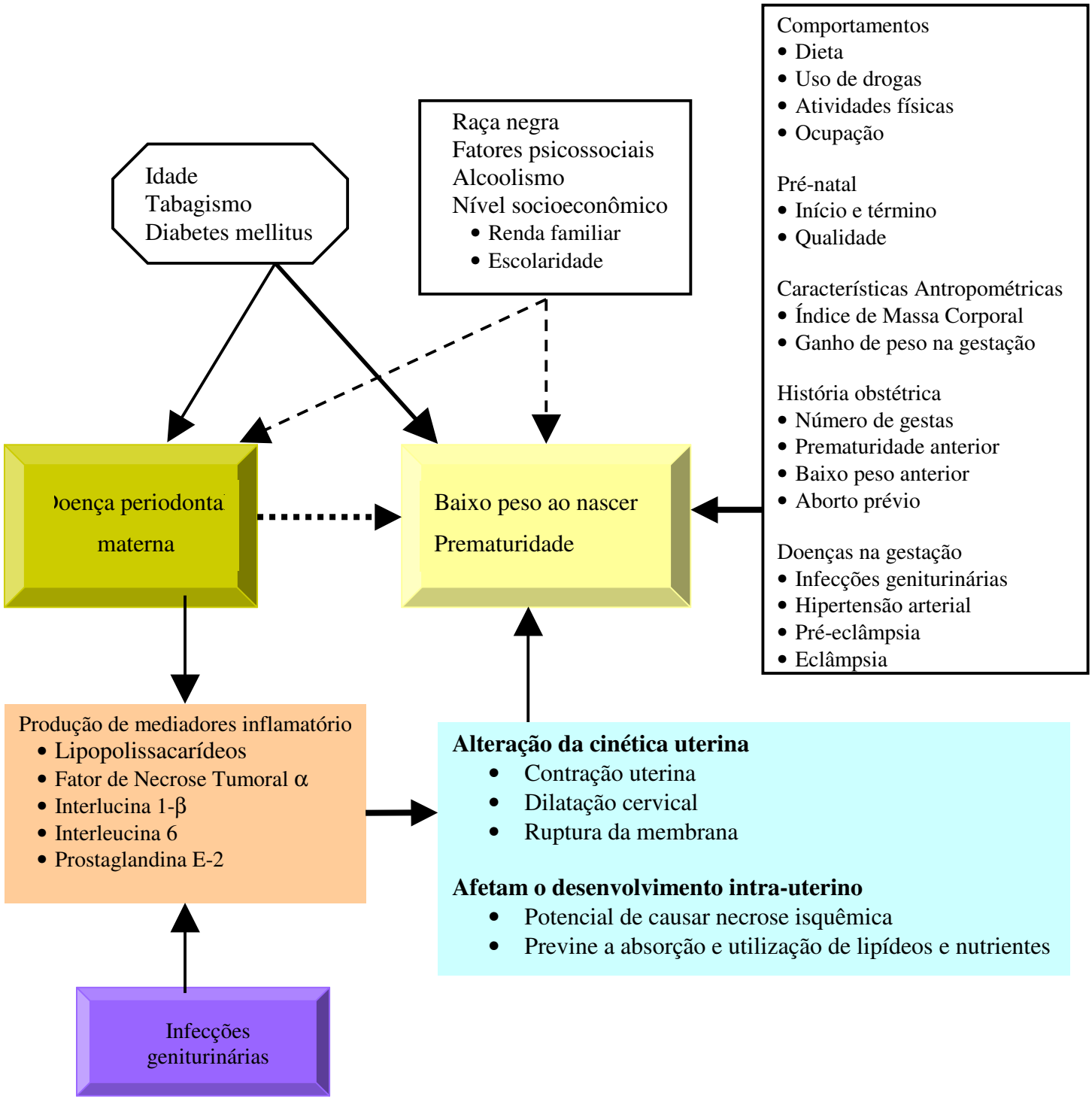
87. Offenbacher S, Lieff S, Boggess KA, Murtha AP, Madianos PN, Champagne CM, et al. Maternal periodontitis and prematurity. Part I: Obstetric outcome of prematurity and growth restriction. Ann Periodontol 2001; 6:164-74.

88. Jeffcoat MK, Geurs NC, Reddy MS, Cliver SP, Goldenberg RL, Hauth JC. Periodontal infection and preterm birth: results of a prospective study. J Am Dent Assoc 2001; 132:875-80.

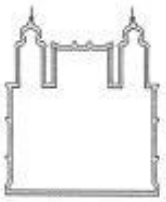
10 – Anexos

Anexo 1

Modelo estruturado de associação entre a doença periodontal e a prematuridade e/ou o baixo peso ao nascer.



Anexo 2



Ministério da Saúde
Fundação Oswaldo Cruz
Comitê de Ética em Pesquisa

ENSP

Parecer nº 78/02

Rio de Janeiro, 26 de dezembro de 2002

Projeto: A relação da infecção periodontal com o parto prematuro e/ou baixo peso ao nascimento – avaliação clínica e microbiológica
Classificação no fluxograma: Grupo III
Coordenador: Maria do Carmo Leal (ENSP/FIOCRUZ)
Colaboradores: Mario Vianna Vettore
Silvana Granado Nogueira da Gama (ENSP/FIOCRUZ)
Anna Thereza Thomé Leão (UFRJ)
Angela Maria Monteiro da Silva (UFRRJ)
Magda Gomes Féres (UNG)
Neide Pires Leal (ENSP/FIOCRUZ)

As solicitações feitas anteriormente pelo Comitê de Ética em Pesquisa foram atendidas satisfatoriamente.

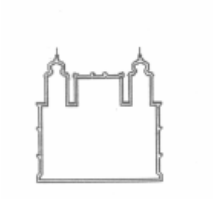
Parecer do CEP: Aprovado

Atenciosamente,

Carlos M. Lins
COORD. SUBSTITUTO

PROF. FERMIN ROLAND SCHRAMM
Coordenador do Comitê de
Ética em Pesquisa
ENSP/FIOCRUZ

Anexo 3



Fundação Oswaldo Cruz
Comitê de Ética em Pesquisa
Escola Nacional de Saúde Pública

Termo de Consentimento Livre e Esclarecido

Prezada mãe,

Este documento lhe dará informações e pedirá o seu consentimento para participar de uma pesquisa que está sendo desenvolvida pela Fundação Oswaldo Cruz.

O estudo pretende identificar a importância da saúde bucal, de fatores sociais, psicológicos, familiares e da saúde da gestante para o risco a prematuridade e o baixo peso ao nascimento. O objetivo final é ter informações que melhorem o atendimento pré-natal para que doenças infantis se reduzam, assim como a mortalidade.

A pesquisa será conduzida através de questionários que perguntarão sobre suas condições de moradia, nível de instrução, sua história reprodutiva, sobre a atenção pré-natal que você recebeu e comportamentos que influenciam na sua saúde. Além disso, um exame bucal será realizado e a placa bacteriana será coleta. O exame bucal, o tratamento psicológico e das gengivas, quando necessários, serão gratuitos. Desta forma, como benefícios você receberá a avaliação e o tratamento de uma infecção bucal que, além de poder afetar sua saúde sistêmica, causa o sangramento de suas gengivas, pode deixar seus dentes moles e levar até a perda dos mesmos.

Você tem o direito de pedir outros esclarecimentos sobre a pesquisa e pode se recusar a participar ou interromper a sua participação nela a qualquer momento, sem que isto lhe traga qualquer prejuízo.

As informações que você nos der serão mantidas em sigilo e não serão divulgadas em qualquer hipótese. Os resultados do estudo serão apresentados em conjunto, não sendo possível identificar os indivíduos que dele participaram.

Declaro estar ciente das informações deste Termo de Consentimento e concordo em participar desta pesquisa

Participante: _____

Coordenadora da Pesquisa

Drª. Maria do Carmo Leal

Rua Leopoldo Bulhões nº1408/809 Rio de Janeiro - RJ CEP: 21041-210 / Tel:0**21-25982620

Comitê de Ética em Pesquisa da Escola Nacional de Saúde Pública – CEP/ENSP

Rua Leopoldo Bulhões nº1408/321 Manguinhos - Rio de Janeiro - RJ CEP: 21041-210

Tel: 0**21-2900085 Ramal: 2054

Rio de janeiro, _____ / _____ / _____

Anexo 4

Características somáticas e neurológicas para o cálculo da idade gestacional pelo Método do Capurro.

Características somáticas e neurológicas	Características somáticas	**Formação do mamilo**	Apenas sensível 0	Auréola visível com discreta pigmentação 5	Posição da cabeça ao levantar 10	Posição da cabeça ao levantar 15	
		Textura da pele	Muito fina, gelatinosa 0	Fina e lisa 5	Algo mais grossa, discreta descamação superficial 10	Grossa, marcas superficiais, descamação nas mãos e pés 15	Grossa, enrugada, com marcas profundas 20
		Forma da orelha	Chata, disforme, pavilhão não encurvado 0	Pavilhão parcialmente encurvado na borda 8	Pavilhão parcialmente encurvado em toda a parte superior 16	Pavilhão totalmente encurvado 24	
		Glândula mamária	Não palpável 0	Palpável, menor que 5mm 5	Entre 5 e 10mm 10	Maior que 10mm 15	
		Pregas plantares	Sem pregas 0	Marcas mal definidas sobre a parte anterior da planta 5	Marcas bem definidas sobre a metade anterior e sulcos no terço anterior 10	Sulcos na metade anterior da planta 15	Sulcos em mais da metade anterior da planta 20
	Sinal do cachecol		O cotovelo alcança a linha axilar anterior do lado oposto 0	O cotovelo situado entre a linha axilar anterior do lado oposto e a linha média 8	O cotovelo situado ao nível da linha média 12	O cotovelo situado entre a linha média e a linha axilar anterior do mesmo lado 18	
	Posição da cabeça ao levantar		Cabeça totalmente deflexionada, ângulo torácico 270° 0	Ângulo cérvico-torácico entre 180 e 270° 4	Ângulo cérvico-torácico igual a 180 8	Ângulo cérvico-torácico menor que 180 12	

Anexo 5

Resultados da calibração intra e inter-examinador para medidas de profundidade de bolsa à sondagem.

Examinador	Calibração											
	Intra-examinador		Inter-examinador									
	Teste K	CCI	Teste K	CCI	Teste K	CCI	Teste K	CCI	Teste K	CCI	Teste K	CCI
1	0,78	0,72	2: 0,77	2: 0,72	3: 0,81	3: 0,78	4: 0,83	4: 0,77	5: 0,85	5: 0,80	6: 0,79	6: 0,75
2	0,92	0,86	3: 0,78	3: 0,73	4: 0,80	4: 0,76	5: 0,81	5: 0,76	6: 0,77	6: 0,72		
3	0,83	0,80	4: 0,84	4: 0,80	5: 0,82	5: 0,78	6: 0,79	6: 0,75				
4	0,79	0,76	5: 0,78	5: 0,72	6: 0,76	6: 0,72						
5	0,85	0,79	6: 0,80	6: 0,77								
6	0,81	0,77										

Teste K: teste Kappa

CCI: Coeficiente de Correlação Intra-Classe

Anexo 6

Números dos questionários das puérperas selecionadas para o estudo principal*

2	128	224	**360**	492	596	670	**790**	870	946	**1048**	1116	**1182**	1251	1329	1425
4	129	225	**362**	493	597	671	**791**	871	948	**1050**	1117	**1183**	1252	1331	1426
5	130	226	363	494	**598**	672	792	878	953	**1055**	1118	1186	1253	**1332**	1427
7	133	**227**	**364**	**501**	599	673	793	879	960	**1056**	1119	1187	1254	1335	1429
8	**141**	228	369	503	601	**674**	794	882	961	**1057**	1123	1188	1256	1336	1430
9	145	230	379	508	607	675	797	883	968	**1058**	1124	1189	1259	1338	1431
12	147	233	380	510	608	676	**798**	884	973	**1060**	**1128**	1192	1260	1340	1434
15	**149**	234	384	511	609	677	**818**	885	974	1065	1130	1193	1262	1341	1439
18	**150**	**238**	391	514	**610**	678	**819**	886	**977**	1066	1131	1195	1264	1342	1441
24	152	250	401	515	**612**	695	**820**	887	**979**	1067	**1132**	1197	1270	1343	**1446**
26	157	258	403	516	**614**	**698**	**821**	888	**981**	1068	**1134**	1198	1279	**1346**	1448
36	160	265	420	523	**617**	699	**822**	**889**	982	1069	**1137**	1204	1281	1347	1449
39	163	276	421	526	**618**	704	826	**890**	983	1070	1138	1205	1287	1349	1473
40	164	283	423	528	619	707	828	891	**985**	1072	1141	1206	1288	1350	1475
41	165	287	431	**531**	621	711	829	893	988	1073	1142	1209	1289	1351	1476
42	166	290	**434**	534	623	712	830	894	991	1074	1145	1211	1291	1354	1477
43	170	302	446	537	624	718	831	895	992	1075	1146	1212	1293	1355	1478
44	174	305	448	538	631	721	834	896	994	1076	1147	1213	1296	1356	1479
48	175	307	452	540	**632**	722	**836**	897	**996**	1079	**1149**	1214	1297	**1366**	1484
49	176	313	454	541	634	724	837	898	997	1080	**1150**	1216	**1298**	**1367**	**1485**
67	178	314	455	543	637	**725**	**838**	901	**999**	**1081**	**1151**	1217	1301	1369	1486
79	180	317	460	544	642	726	842	903	1001	**1083**	1156	1223	1303	1370	1489
89	181	321	461	546	**647**	**729**	843	**906**	1002	**1086**	1157	1224	1306	1374	1521
96	182	327	463	548	**648**	**730**	844	914	1003	1089	**1158**	**1227**	**1309**	1375	1736
97	184	331	464	549	649	**732**	846	**921**	1007	1093	1159	1228	1311	1376	1737
103	186	334	468	551	**650**	735	847	**924**	1008	1099	1161	1234	1318	1378	1999
104	197	**344**	470	552	**651**	736	**853**	925	1013	1100	1163	1235	1319	1383	**2000**
105	198	345	474	553	652	738	**855**	**926**	**1033**	**1102**	**1164**	**1236**	1321	1384	2001
118	201	346	477	556	664	741	**856**	927	**1035**	**1106**	1167	**1237**	1322	1386	2002
119	205	**347**	478	586	665	745	857	**928**	**1038**	1111	**1169**	**1240**	**1323**	1388	2003
120	212	349	481	588	666	**785**	861	**932**	**1041**	1112	**1171**	**1243**	**1324**	1390	2004
124	216	**350**	485	589	667	**786**	864	**933**	1042	1113	**1177**	**1244**	1325	1392	2005
125	217	**356**	489	590	668	788	865	**934**	1044	1114	**1179**	1245	**1326**	1395	
127	219	**357**	**490**	594	669	789	869	**937**	1046	1115	**1180**	1250	1328	1398	

***** Os números dos questionários em negrito representam a sub-amostra selecionada para o exame microbiológico

Anexo 7

QUESTIONÁRIO PARA A MÃE NA MATERNIDADE

Identificação do questionário

Questionário número: ☐☐☐☐☐ Data da entrevista: ☐☐/☐☐/☐☐ Horário ☐☐:☐☐

Código da instituição: ☐☐☐☐☐ Código do entrevistador: ☐☐

Revisado em: ☐☐/☐☐/☐☐ Código do supervisor: ☐☐

Corrigido em: ☐☐/☐☐/☐☐

Digitado em: ☐☐/☐☐/☐☐ Código do digitador: ☐☐

QUESTIONÁRIO PARA SELEÇÃO DAS MÃES

LOCAL DA ENTREVISTA: ☐ Nº DO PRONTUÁRIO _____

1. MATERNIDADE ALEXANDER FLEMING
2. MATERNIDADE CARMELA DUTRA
3. MATERNIDADE FERNANDO MAGALHÃES

"Meu nome é (nome do entrevistador). Sou entrevistador da pesquisa sobre a influência da saúde bucal na prematuridade e no baixo peso ao nascimento, e gostaria de pedir meia hora de sua atenção".

PASSAR PARA A LEITURA DO CONSENTIMENTO LIVRE E ESCLARECIDO

"Então eu gostaria começar fazendo algumas perguntas sobre a senhora".

1. Qual é o seu nome:

_____

2. Quantos anos completos a senhora tem? _____

3. A senhora usa dentadura?

1.Sim (encerrar o questionário) 2. Não ☐

4. A senhora tem 15 dentes ou mais? Se a resposta da mãe for "**não sei**", explique: ***"Às vezes a gente se confunde com algumas coisas que parecem simples. A senhora me permitiria dar uma olhada rápida e contar quantos dentes a senhora tem?"***

1.Sim **2. Não (encerrar o questionário)** ☐

"Faremos algumas perguntas sobre doenças que a senhora possa ter e remédios que a senhora possa estar usando ou ter feito uso. Para estas perguntas não existe resposta certa nem errada"

A senhora possui alguma(s) da(s) doença(s) abaixo:

1.Sim 2. Não **3. Não sei**

4. Diabetes antes da gravidez? (Sim: encerrar o quest.) ☐

5. AIDS ou infecção pelo HIV? (Sim: encerrar o quest.) ☐

6. A senhora possui alguma outra doença? ☐

7. Qual doença?_____

8. A senhora usa algum remédio?

1.Sim **2. Não (vá para a questão 11)** ☐

9. Qual é o nome do remédio?_____

10. Para que a senhora usa o remédio?

_____

11. A senhora já usou algum remédio para hipertensão, epilepsia ou transplante de algum órgão? ☐

1.Sim **2. Não**

12. Qual era o nome do remédio?

_____

13. Para que a senhora usou o remédio?

_____

14. A senhora utilizou algum antibiótico na última semana? ☐

1.Sim (encerrar o questionário) 2. Não **3. Não sei**

15. A senhora recebeu tratamento para problemas na sua gengiva nos últimos 6 meses? ☐

1.Sim (encerrar o questionário) **2. Não**

16. A senhora tem ou já teve febre reumática, endocardite bacteriana ou prolapso de válvula mitral? ☐

1.Sim (encerrar o questionário) **2. Não**

17. A PACIENTE PREENCHE OS CRITÉRIOS DE SELEÇÃO PARA PARTICIPAR DA PESQUISA? ☐

1.Sim (seguir para o próximo questionário)
2. Não. Dar a explicação abaixo

Infelizmente nem todas as mulheres que entrevistamos participarão da pesquisa devido a critérios pré-estabelecidos Em virtude da senhora ..... (EXPLICAR O MOTIVO DA EXCLUSÃO***) nossa entrevista termina aqui. Muito obrigado(a) pela sua colaboração.***

18. EM CASO DE "NÃO PARTICIPAÇÃO", PORQUE A PACIENTE SERÁ EXCLUÍDA DA PESQUISA?

_____

Anexo 8

AVALIAÇÃO SÓCIO-ECONÔMICA

Agora gostaria de fazer algumas perguntas gerais, antes de lhe perguntar sobre sua saúde.Essas informações são confidenciais e serão utilizadas apenas para a pesquisa

IDENTIFICAÇÃO MATERNA

1. Qual a sua data de nascimento: ☐☐/☐☐/☐☐

2. Qual é a sua cor (raça)?

1.Branca	2. Negra	3.Amarela Oriental	4. Parda Mulata Morena Cabocla	5.Indígena	6. Ignorada

3. Nesta escala, na qual "**1**" quer dizer **Muito Clara** e "**10**" quer dizer **Muito Escura**, qual é a cor da sua pele?

1 2 3 4 5 6 7 8 9 10

Muito Clara **Muito Escura**

4. Qual a cor da puérpera, de acordo com o examinador?

1.Branca	2. Negra	3.Amarela Oriental	4. Parda Mulata Morena Cabocla	5.Indígena	6. Ignorada

5. Qual a sua situação conjugal? ☐

1) Solteira **2) Casada/companheiro** 3) Divorciada/separada **4) Viúva**

6. Qual seu endereço de residência?

_____

7. Qual seu telefone? _____

8. A senhora poderia nos dar um nome com telefone para contato? _____

CONDIÇÕES DE MORADIA

8. Em que local a senhora mora? ☐

1) Bairro **2) Comunidade (favela)** 3) Rua

4) Loteamento (favela-bairro) 5) Outro

9. Como é o local que a senhora mora? ☐

1) Casa ou apartamento **2) Quarto ou cômodo** 3) Palafita ou barraco

10. Qual a espécie de domicílio? ☐

1) Alugado **2) Próprio** 3) Emprestada **4) Outro**

11. A senhora tem banheiro em casa? ☐

1) Sim **2) Não (vá para a questão n° 14)**

12. Seu banheiro é dentro de casa? ☐

1) Sim **2) Não**

13. Qual o tipo de banheiro que a senhora tem? ☐

1) Com descarga **2) Sem descarga**

14. Quantos quartos e salas têm na sua casa? ☐☐

15. Que tipo de parede sua casa possui? ☐

1) Cimento, tijolo, pedra ou madeira aparelhada

2) Tijolo de barro 3) Sapê, palha e madeira aproveitada

4) Plástico **5) Metal**

6) Outra: especificar_____

16. Que tipo de piso tem no interior da sua casa? ☐

1) Piso revestido (concreto/cimento, lajota, tijolo)

2) Piso de terra batida 3) Ambos

17. Qual o destino dado ao seu lixo? ☐

1) É recolhido pelo lixeiro **2) É colocado na caçamba**

3) É enterrado/queimado **4) É jogado a céu aberto**

18. Qual a fonte de abastecimento de água da sua casa? ☐

1) Água encanada dentro de casa **2) Água encanada fora de casa**

3) Outro: _____

19. Como é o esgoto na sua casa? ☐

1) Não tem **2) Rede geral** 3) Fossa rudimentar **4) Fossa séptica**

20. Na rua em que a senhora mora tem valão a céu aberto? ☐

1) Sim **2) Não**

21. Qual é o calçamento na rua em que a senhora mora? ☐

1) Asfaltada/cimentada **2) Paralelepípedo** 3) Terra

22. Quem mora com a senhora? (em relação à gestante)

1° Nome	Sexo(M/F)	Idade	Parentesco	Ocupação

ESCOLARIDADE DA MÃE E RELAÇÃO COM O PAI DA CRIANÇA

23. A senhora lê e escreve? ☐

1) Sim **2) Não (desconsidere a próxima questão)**

24. Qual a última série que a senhora concluiu com aprovação? Nº da série ☐ Nº do grau ☐ **Não sei** ☐

Se o pai morar com a puérpera vá para a questão nº 29

25. Em virtude da presença de mães adolescentes na pesquisa precisamos saber se a senhora sabe quem é o pai da criança? 1) Sim **2) Não (vá para a questão n°30)** ☐

26. A senhora vive com o pai da criança? ☐

1) Sim **2) Não**

27. O pai da criança trabalha? ☐

1) Sim **2) Não (desconsidere a próxima questão)**

28. Qual o trabalho do pai da criança?

_____

29. Qual a última série que o pai da criança concluiu com aprovação? Lê e escreve ☐ Nº da série ☐ Nº do grau ☐

"As perguntas que irei lhe fazer agora são sobre seu trabalho antes e depois da senhora engravidar"

DADOS OCUPACIONAIS

30. Qual a sua ocupação mais recente?

_____

31. Excluindo o trabalho de dona-de-casa, a senhora teve algum trabalho remunerado durante a gestação? ☐

1) Sim (Empregada) **2) Sim (Empregadora)** 3) Sim (Autônoma)
4) Não (Pensionista) **5) Não/dona-de-casa (ir para a questão 38)**
6) Não (Estudante) 7) Não (trabalho voluntário) **8) Não (Desempregada)**

32. Quanto a senhora ganha por mês? (soma total de rendimentos, incluso pensão, aposentadoria)
R$ _____

33. A senhora trabalha com carteira assinada ou tem vínculo empregatício? 1) Sim **2) Não** ☐

34. Há quanto tempo a senhora exerce esta função?

_____

35. Qual sua forma de deslocamento para o seu trabalho? ☐

1) à pé **2) Ônibus** 3) Lotação (Van) **4) Trem**
5) Metro **6)Carro** 7) Barcas

36. Quais os seus horários neste emprego?

	Horário de trabalho			Horário de intervalo
Segunda		às		
Terça		às		
Quarta		às		
Quinta		às		
Sexta		às		
Sábado		às		
Domingo		às		

37. Quantos dias antes do parto a senhora parou de trabalhar (aproximado)? ☐☐☐

ATIVIDADES FÍSICAS NO PERÍODO GESTACIONAL

"Vou fazer perguntas com o objetivo de saber o tempo que a senhora gastou fazendo suas atividades num dia típico durante os últimos 3 meses de sua gestação".

Caso seja estudante (trabalho = escola)

H: hora; Min: minutos.

Perguntar se a respiração*: 1 = Não se modifica, 2 = Fica ofegante, 3 = Fica muito ofegante

	H	Min	Resp.*		
	A		B		
1. Trabalhando (todas as atividades)					
2. Sentado ou em pé no trabalho					
3. Andando no trabalho			1	2	3
4. Levantando ou carregando objetos de até 10 Kg no trabalho (ou realizando atividade de esforço semelhante)					
5. Levantando ou Carregando objetos de mais de 10 Kg no trabalho (ou realizando atividade de esforço semelhante)					
6. Indo e voltando do trabalho					
7. Andando (Fora do trabalho, incluindo indo para o trabalho e lazer)			1	2	3
8. Realizando tarefas domésticas intensas			1	2	3
9. Realizando outras tarefas não intensas em casa			1	2	3
10. Realizando outras atividades sociais ou de lazer (participação em associações, cultos religiosos, etc)					
11. Dormindo					
12. Exercício para condicionamento físico. Qual			1	2	3
13. Outras atividades			1	2	3
14. Assistindo TV sentado, trabalhando no computador (internet) ou brincando com vídeo-games					

CARACTERÍSTICAS COMPORTAMENTAIS

"Mudando de assunto, agora podemos falar um pouco sobre alguns hábitos e coisas que a senhora e costuma fazer no seu dia-a-dia..."

SERVIÇO ODONTOLÓGICO UTILIZADO

BEBIDA ALCOÓLICA, FUMO E USO DE DROGAS

38. A senhora ingeriu bebidas alcoólicas durante a gravidez?

1) Sim **2) Não (vá a questão 49)** ☐

Que tipo e freqüência ?

1) Nunca **2) Raras vezes** 3) Finais de semana

4) Freqüentemente 5) Diariamente

39. Whisky/cachaça/vodca ☐

40. Vinho ☐

41. Cerveja ☐

42. Quantas doses são necessárias p/ deixar a senhora "alta"? ☐☐

43. As pessoas a aborreciam criticando o seu modo de beber? 1) Sim **2) Não** ☐

44. A senhora sentiu que deveria ter parado de beber durante a gravidez? 1) Sim **2) Não** ☐

45. Alguma vez precisou de uma dose de bebida para começar o dia? (1 tulipa=1 dose ou **2 latas = 3 doses**)

1) Sim **2) Não** ☐

46. A senhora teve sentimentos de culpa sobre a bebida?

1) Sim **2) Não** ☐

47. A senhora fumava antes de engravidar?

1) Sim **2) Não (vá para a questão n°52)** ☐

48. Quantos cigarros a senhora fumava por dia? ☐☐

49. Há quantos anos a senhora é fumante? ☐☐

50. A senhora fumou durante a gravidez? ☐

1) Sim **2) Não (vá para a questão n°55)**

51. Em que período? 1) Toda a gestação ☐ **2) Parou no mês** ☐

52. Quantos cigarros a senhora fumou por dia? ☐☐

53. As pessoas a aborreciam criticando o seu modo de fumar?

1) Sim **2) Não** ☐

54. Alguém fumava perto de a senhora (em casa ou no trabalho)? 1) Sim **2) Não** ☐

"Hoje em dia é muito comum que as pessoas já tenham experimentado algum tipo de droga, como a maconha e a cocaína. As duas próximas perguntas são sobre o uso dessas substâncias pela senhora. Essas questões são muito importantes pra gente. Gostaria de lembrar que, como todo o resto do questionário, essas informaçõess são confidenciais e somente serão usadas para a pesquisa"

55. A senhora usou algum tipo de droga durante a gravidez?

1) Sim **2) Não** ☐

56. Que tipo?_____

PADRÃO E ESTRUTURA DA HIGIENE BUCAL

57. Quantas vezes a senhora escova os dentes por dia? ☐

58. Quantos dias a senhora escova os dentes por semana? ☐

59. Quantas vezes a senhora usa fio dental por dia? ☐

60. Quantos dias a senhora usa fio dental por semana? ☐

61. A senhora já foi ao dentista? 1) Sim **2) Não** ☐

62. Quando foi sua última visita ao dentista? ☐☐/☐☐

mês ano

63. Quantas vezes a senhora foi ao dentista no último ano? ☐☐

64. Qual o tipo de serviço odontológico que a senhora utiliza?

1) Público **2) Convênio** 3) Particular **4) Nenhum** ☐

HISTÓRIA MÉDICA

"Agora eu gostaria de fazer perguntas sobre outras gestações, seu pré-natal possíveis e doenças e medicamentos nesta gravidez".

ANTECEDENTES OBSTÉTRICOS

65. Quantas vezes a senhora esteve **grávida**, excluindo esta gravidez? ☐

0) Nenhuma (vá para a questão 78) **1) 1 vez**

2) 2 vezes 3) 3 vezes **4) 4 vezes** 5) 5 vezes ou mais

66. Quantos filhos nasceram vivos? ☐☐

67. Quantos filhos nasceram mortos? ☐☐

68. Alguma gravidez que teve resultou em aborto? ☐

(aborto= até 500 gramas ou 22 semanas)

1) Sim **2) Não (vá para a questão71)**

69. Quantos foram espontâneos? ☐☐

70. Quantos foram provocados? ☐☐

71. Sem contar com esta gravidez, a senhora já teve algum bebê prematuro, quer dizer, que tenha nascido antes do tempo, antes dos 9 meses de gravidez?

1) Sim **2) Não (desconsidere a próxima questão)** ☐

72. Quantos? ☐☐

73. Sem contar com esta gravidez, a senhora já teve algum filho com peso menor que 2 quilos e meio?

1) Sim **2) Não (desconsidere a próxima questão)** ☐

74. Quantos? ☐☐

75. Quando foi seu último parto, excluindo este?

☐☐/☐☐/☐☐

dia mês ano

ASSISTÊNCIA PRÉ-NATAL (GESTAÇÃO ATUAL)

76. A senhora fez pré-natal? ☐

1) Sim **2) Não (vá para a questão 81)**

77. Nome do posto de saúde ou hospital?

_____

78. A partir de que mês de gestação a senhora começou o pré-natal? ☐☐

79. Até que mês de gestação a senhora fez pré-natal? ☐☐

80. A quantas consultas a senhora foi? ☐☐

A senhora teve algum dos problemas a seguir na gravidez?

1) Sim **2) Não**

81. Hipertensão (pressão alta) na gravidez ☐

82. Pré-eclâmpsia ☐

83. Hepatite B ☐

84. Anemia ☐

85. Diabetes gestacional ☐

86. Infecção urinária ☐

87. Outras infecções? ☐

Quais?_____ _____

88. Outras doenças? ☐

Quais?_____

89. Tomou algum medicamento nesta gravidez? ☐

1) Sim **2) Não (desconsidere a próxima questão)**

90. Qual: _____

INFORMAÇÕES DA GESTAÇÃO ATUAL

91. Qual o seu peso antes da gravidez? ☐☐ ,☐ Kg

92. Quando a senhora foi pesada? ☐☐/☐☐/☐☐

93. Qual o seu peso ao final da gravidez, antes de ter o bebê ☐☐ ,☐ Kg

94. Quando a senhora foi pesada? ☐☐/☐☐/☐☐

95. Qual a sua altura? ☐ ,☐☐ m

96. A senhora estava utilizando algum método para evitar gravidez? 1) Sim **2) Não** ☐

97. Quando ficou grávida, a senhora: ☐

1) Estava querendo engravidar **2) Queria esperar mais um tempo**
3) Não queria engravidar

98. E o pai do neném, quando soube que a senhora estava grávida: ☐

1) Queria que a senhora estivesse grávida **2) Queria esperar mais um tempo** 3) Não queria que a senhora estivesse grávida

99. Qual foi o seu tipo de parto? ☐

1) Parto normal 2) Cesárea **3) Fórceps** 0) Ignorado

100. Quando sua regra veio pela última vez? ☐☐/☐☐/☐☐

CARACTERÍSTICA DO RECÉM-NASCIDO

101. Qual a data de nascimento do bebê?

☐☐/☐☐/☐☐

102. Qual o sexo do seu bebê? ☐

1) Masculino **2) Feminino**

103. Qual o peso do seu bebê nascimento? ☐☐☐☐ g

104. Com quantas semanas nasceu seu bebê? ☐☐

105. Qual o comprimento ☐☐ cm

106. O recém-nascido apresentou algum problema de saúde ao nascimento? ☐

1) Sim **2) Não (desconsidere a próxima questão)**

107. Qual? _____

"As perguntas que faremos agora se referem a como a senhora se percebe na sua vida em geral, a senhora deve responder sobre elas considerando o período anterior à gravidez".

AVALIAÇÃO DA ANSIEDADE TRAÇO

"Agora vou ler algumas perguntas e a senhora deverá me mostrar neste cartão como a senhora GERALMENTE SE SENTE. Não gaste muito tempo numa única afirmação, mas tente dar a resposta que mais se aproximar de como a senhora SE SENTE GERALMENTE."

Muitíssimo ---------- 1 Um pouco ---------- 3
Bastante ---------- 2 Absolutamente não ------- 4

1. Geralmente a senhora se sente bem	1	2	3	4
2. Geralmente a senhora se cansa facilmente	1	2	3	4
3. Geralmente a senhora tem vontade de chorar	1	2	3	4
4. Geralmente a senhora gostaria de ser tão feliz como os outros parecem ser	1	2	3	4
5. Geralmente a senhora perde oportunidades porque não consegue tomar decisões rapidamente	1	2	3	4
6. Geralmente a senhora se sente descansada	1	2	3	4
7. Geralmente a senhora é calma, ponderada (equilibrada), e senhora de si mesma	1	2	3	4
8. Geralmente a senhora sente que as dificuldades estão se acumulando de tal forma que não as consegue resolver	1	2	3	4
9. Geralmente a senhora se preocupa demais com as coisas sem importância	1	2	3	4
10. Geralmente a senhora é feliz	1	2	3	4
11. Geralmente a senhora se deixa afetar muito pelas coisas	1	2	3	4
12. Geralmente a senhora não tem muita confiança em si mesma	1	2	3	4
13. Geralmente a senhora se sente segura	1	2	3	4
14. Geralmente a senhora evita ter que enfrentar crises ou problemas	1	2	3	4
15. Geralmente a senhora se sente deprimida	1	2	3	4
16. Geralmente a senhora está satisfeita	1	2	3	4
17. As vezes, as idéias sem importância entram na sua cabeça e ficam lhe preocupando	1	2	3	4
18. Geralmente a senhora leva os desapontamentos (decepções) tão à sério que não consegue tirá-los da cabeça	1	2	3	4
19. Geralmente a senhora é uma pessoa estável (seu estado emocional não muda, ex: de triste para alegre)	1	2	3	4
20. Geralmente a senhora fica tensa e perturbada quando pensa em seus problemas	1	2	3	4

INVENTÁRIO DE TRAÇO DE DEPRESSÃO - MMPI

"Nas perguntas a seguir a senhora deverá me dizer se a frase é verdadeira ou falsa". 1. Verdadeira **2. Falsa**
A opção (3) será marcada se a entrevistada desconhecer a proposição, porém esta alternativa não deve ser apresentada à ela.

1. A senhora é acordada facilmente por ruídos	1	2	3
2. Sua vida cotidiana é cheia de coisas que lhe mantém interessada	1	2	3
3. A senhora é tão capaz para trabalhar como sempre foi	1	2	3
4. Raramente a senhora tem prisão de ventre	1	2	3
5. É sujeita a ataques de náuseas e de vômitos	1	2	3
6. Tem dificuldades de se concentrar em trabalhos ou tarefas	1	2	3
7. Tem tido períodos de dias, semanas ou meses em que a senhora não tem podido cuidar das coisas por falta de ânimo	1	2	3
8. Seu sono é sobressaltado e agitado	1	2	3
9. Sua capacidade de julgar está melhor do que nunca	1	2	3
10. A senhora tem tão boa saúde como a maioria dos seus amigos	1	2	3
11. A senhora é muito sociável	1	2	3
12. A senhora desejaria ser tão feliz quanto os outros parecem ser	1	2	3
13. A senhora certamente não tem confiança em si mesma	1	2	3
14. Geralmente a senhora sente que vale a pena viver	1	2	3
15. A senhora é feliz quase sempre	1	2	3
16. Parece que a senhora é tão capaz e esperta quanto a maioria dos que te rodeiam	1	2	3
17. Certamente, às vezes, a senhora sente que é inútil	1	2	3
18. Durante os últimos anos a senhora tem passado bem a maior parte do tempo	1	2	3
19. A senhora não está ganhando nem perdendo peso	1	2	3
20. A senhora chora facilmente	1	2	3
21. Não compreende tão bem o que lê, como acontecia antes	1	2	3
22. Nunca, na sua vida, a senhora se sentiu melhor do que agora	1	2	3
23. Sua memória parece estar boa	1	2	3
24. Freqüentemente a senhora sente uma fraqueza geral	1	2	3
25. A senhora acredita que não seja mais nervosa que a maioria das pessoas	1	2	3
26. Às vezes, sem qualquer razão, ou mesmo quando as coisas correm mal, a senhora se sente excessivamente feliz, "navegando num mar de rosas"	1	2	3
27. A senhora tem dificuldades em iniciar coisas	1	2	3
28. A senhora não censura uma pessoa que se aproveite de quem se deixe explorar	1	2	3
29. Às vezes a senhora se sente cheia de energia	1	2	3

"Agora eu gostaria de conversar um pouquinho sobre a violência que, às vezes, somos vítimas e também sobre como as pessoas que moram na sua casa resolvem os desentendimentos e desavenças do dia-a-dia. Nós sabemos que algumas das próximas perguntas podem ser delicadas e pessoais e que, às vezes, parece difícil falar sobre elas. Mas é muito importante para nossa pesquisa que a senhora faça um esforço para relembrar com a gente como sua família se entendeu, quer dizer, como as pessoas se deram umas com as outras durante sua gravidez. Eu queria lembrar que tudo que será dito aqui ficará somente entre nós e que essas informações serão muito importantes para prevenir que outros recém-nascidos nasçam prematuros"

VIOLÊNCIA FAMILIAR

108. Desde que a senhora engravidou alguém lhe agrediu fisicamente? ☐

1) Sim **2) Não (desconsidere as próximas questões)**

109. Quem fez isso com a senhora? ☐

1) Marido **2) Ex-marido** 3) Namorado **4) Estranho**
5) Outro (especificar):
_____
6) Mais de um (especificar): _____

110. Quantas vezes isso aconteceu? ☐☐☐

111. Qual dessas vezes foi a mais marcante, mais importante?_____
_____

112. Quando foi? ☐☐/☐☐/☐☐
dia mês ano

113. Nessa vez, poderia me dizer quais das coisas que vou falar agora aconteceram?

Tipologia	Ações	(1)Sim (2) Não
1	Ameaça de maus-tratos ou agressão. Inclusive com arma	
2	Tapa	
2	Empurrão sem machucado, sem ferimento ou sem dor duradoura	
3	Soco	
3	Chute	
3	Machucado ou "mancha roxa"	
3	Corte ou dor contínua	
4	Espancamento	
4	Contusões graves	
4	Queimaduras	
4	Ossos quebrados	
5	Danos ou problemas na cabeça	
5	Danos ou problemas em órgãos internos	
5	Danos permanentes	
6	Uso de arma	
6	Ferimento por arma	

INFORMAÇÕES DO PRONTUÁRIO MÉDICO

GRAVIDEZ ATUAL

1. Peso anterior ☐☐
2. Estatura ☐☐☐
3. DUM: ____ / _____ / _____
4. Hospitalização na gravidez: ☐
   1) Sim 2) Não
5. Quantas ☐☐
6. Fuma? ☐
   1) Sim 2) Não
7. Número de cigarros / dia ☐☐
8. Número de consultas pré-natais? ☐☐
9. Peso na admissão_____
10. Altura _____
11. Medicamentos que usou na gestação atual?

PARTO

12. Idade Gestacional pelo Capurro ☐☐
13. Qual o tipo de parto? ☐
    1)Normal 2) Cesáreo 3) Fórceps
14. Data do parto ☐☐ / ☐☐ / ☐☐

PATOLOGIA NA GESTAÇÃO/PARTO/PUERPÉRIO

_____

RECÉM-NASCIDO

15. Sexo ☐
    1) Masculino 2) Feminino
16. Peso ao nascer? ☐☐☐☐ g
17. Peso/IG ☐
    1) Adequado 2) Pequeno 3) Grande
18. Comprimento cm ☐☐
19. Perímetro cefálico cm ☐☐

OBSERVAÇÕES DO ENTREVISTADOR

PARA SER PREENCHIDO PELO ENTREVISTADOR NO FIM DA ENTREVISTA INDIVIDUAL

A cooperação do entrevistado foi:	1.Excelente	2.Muito boa	3.Boa	4.Adequada	5.Fraca
Grau em que as respostas eram **precisas** e **completas**	1.Muito Alta	2.Alta	3.Média	4.Baixa	5.Muito Baixa
Alguma **circunstância incomum** durante a entrevista:					
Algum **outro comentário**:					

Anexo 9

FICHA DE EXAME CLÍNICO PERIODONTAL

IP - Índice De Placa	**0** – Sem placa visível e sem placa na sonda	**1** – Com placa na sonda ou placa visível
ISS – Índice de sangramento à sondagem	**0** – Sem sangramento após sondagem do sulco ou bolsa	**1** – Com sangramento após sondagem do sulco ou bolsa
Cálculo Visível	**0** – Sem cálculo visível	**1** – Presença de cálculo visível

	17				16				15				14				13				12				11				21				22				23				24				25				26				27			
	P	D	V	M	P	D	V	M	P	D	V	M	P	D	V	M	P	D	V	M	P	D	V	M	P	D	V	M	M	V	D	P	M	V	D	P	M	V	D	P	M	V	D	P	M	V	D	P	M	V	D	P
IP																																																					
ISS																																																					
C																																																					

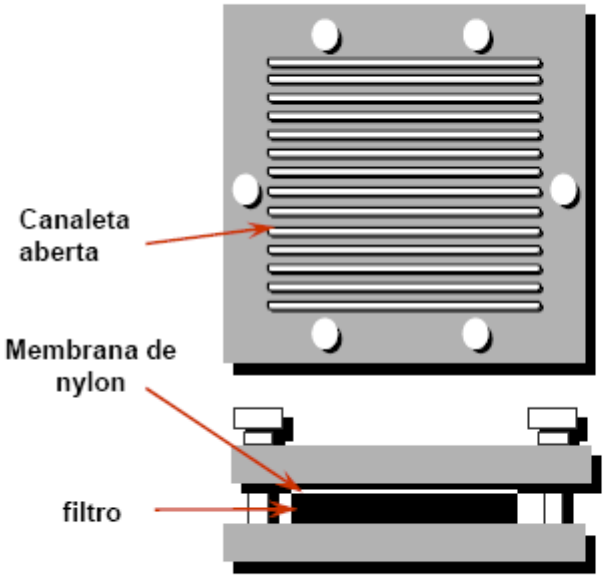
	47				46				45				44				43				42				41				31				32				33				34				35				36				37			
	L	D	V	M	L	D	V	M	L	D	V	M	L	D	V	M	L	D	V	M	L	D	V	M	L	D	V	M	M	V	D	L	M	V	D	L	M	V	D	L	M	V	D	L	M	V	D	L	M	V	D	L
IP																																																					
ISS																																																					
C																																																					

	VESTIBULAR																																																				
	17				16				15				14				13				12				11				21				22				23				24				25				26				27			
	D		MD	M	D		MD	M	D		MD	M	D		MD	M	D		MD	M	D		MD	M	D		MD	M	M		MD	D	M		MD	D	M		MD	D	M		MD	D	M		MD	D	M		MD	D
PBS																																																					
NCI																																																						
	PALATINA																																																				
	17				16				15				14				13				12				11				21				22				23				24				25				26				27			
	D		MD	M	D		MD	M	D		MD	M	D		MD	M	D		MD	M	D		MD	M	D		MD	M	M		MD	D	M		MD	D	M		MD	D	M		MD	D	M		MD	D	M		MD	D
PBS																																																					
NCI																																																						

	VESTIBULAR																																																			
	47				46				45				44				43				42				41				31				32				33				34				35				36				37			
	D		MD	M	D		MD	M	D		MD	M	D		MD	M	D		MD	M	D		MD	M	D		MD	M	M		MD	D	M		MD	D	M		MD	D	M		MD	D	M		MD	D	M		MD	D
PBS																																																					
NCI																																																						
	LINGUAL																																																				
	47				46				45				44				43				42				41				31				32				33				34				35				36				37			
	D		MD	M	D		MD	M	D		MD	M	D		MD	M	D		MD	M	D		MD	M	D		MD	M	M		MD	D	M		MD	D	M		MD	D	M		MD	D	M		MD	D	M		MD	D
PBS																																																					
NCI																																																						

Anexo 10

Esquema do aparato "Minislot 30" (Immunetics, Cambridge, MA, USA) e da preparação das amostras de placa dental.



Colocar amostra em 150µl tampão TE pH 7.6

↓

Adicionar 100 µl NaOH 0.5 M

↓

Ferver durante 10 minutos

↓

Adicionar 800 µl Acetato de Amônia 5 M

↓

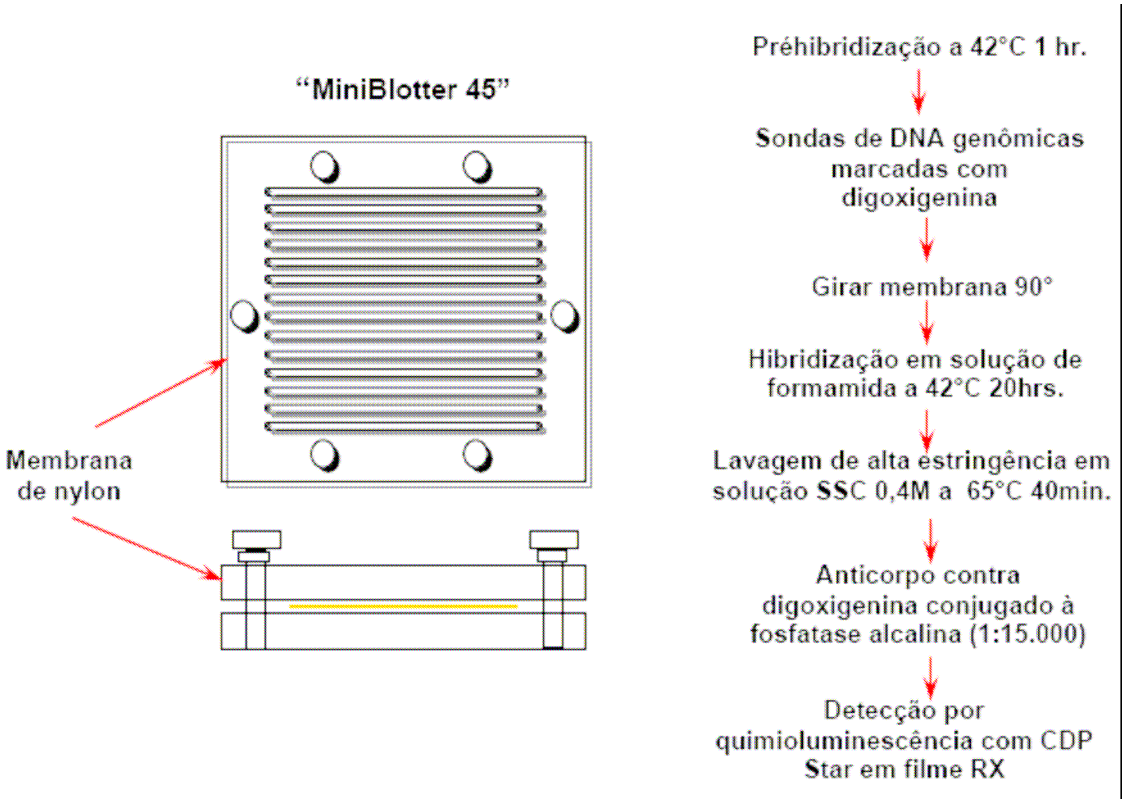
Depositar amostras nas canaletas

↓

Fixar DNA na membrana a 120°C por 20 min

Anexo 11

Esquema do aparato "Miniblotter 45" (Immunetics, Cambridge, MA, USA) e da hibridização com as sondas de DNA.



Anexo 12

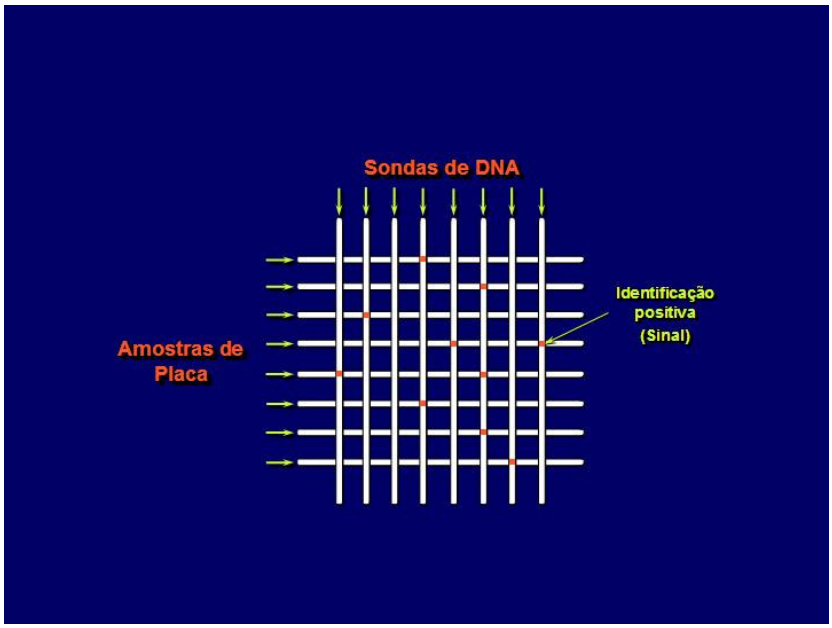
Relação das cepas empregadas na confecção das sondas de DNA.

		Campylobacter showae	51146
Blue complex			
Actinomyces gerencseriae	23860	*Eubacterium nodatum*	33099
Actinomyces israelii	12102	*Fusobacterium nucleatum sp. nucleatum*	25586
Actinomyces naeslundii genospecies 2	43146	*Fusobacterium nucleatum sp. polymorphum*	10953
		Fusobacterium nucleatum sp. vicentii	49256
Purple complex		*Fusobacterium periodonticum*	33693
Actinomyces odontolyticus	17929	*Micromonas micros*	33270
Veillonella parvula	10790	*Prevotella intermedia*	25611
		Prevotella nigrescens	33563
Yellow complex		*Streptococcus constellatus*	27823
Streptococcus gordonii	10558		
Streptococcus intermedius	27335	**Red complex**	
Streptococcus mitis	49456	*Tannerella forsythia*	43037
Streptococcus oralis	35037	*Porphyromonas gingivalis*	33277
Streptococcus sanguinis	10556	*Treponema denticola*	B1
Green complex		**Other species and new DNA probes**	
Actinobacillus actinomycetencomitans	43718		
serotypes a and *b*	29523	*Eubacterium saburreum*	33271
Capnocytophaga gingivalis	33624	*Gemella morbillorum*	27824
Capnocytophaga ochracea	33598	*Leptotrichia buccalis*	14201
Capnocytophaga sputigena	33612	*Neisseria mucosa*	19696
Eikenella corrodens	23834	*Prevotella melaninogenica*	25845
		Propionybacterium acnes I and II	11827 and 11828
Orange complex		*Selenomonas noxia*	43541
Campylobacter gracilis	33236	*Streptococcus anginosus*	33397
Campylobacter rectus	33238	*Treponema socranskii*	S1

Todas as cepas foram obtidas do American Type Culture Collection (ATCC), (Rockville, MD), exceto *Treponema denticola* B1 e *Treponema socranskii* S1 que foram obtidas do Forsyth Institute, (Boston, MA). Os "complexos" microbianos foram descritos por Socransky et al. [51].

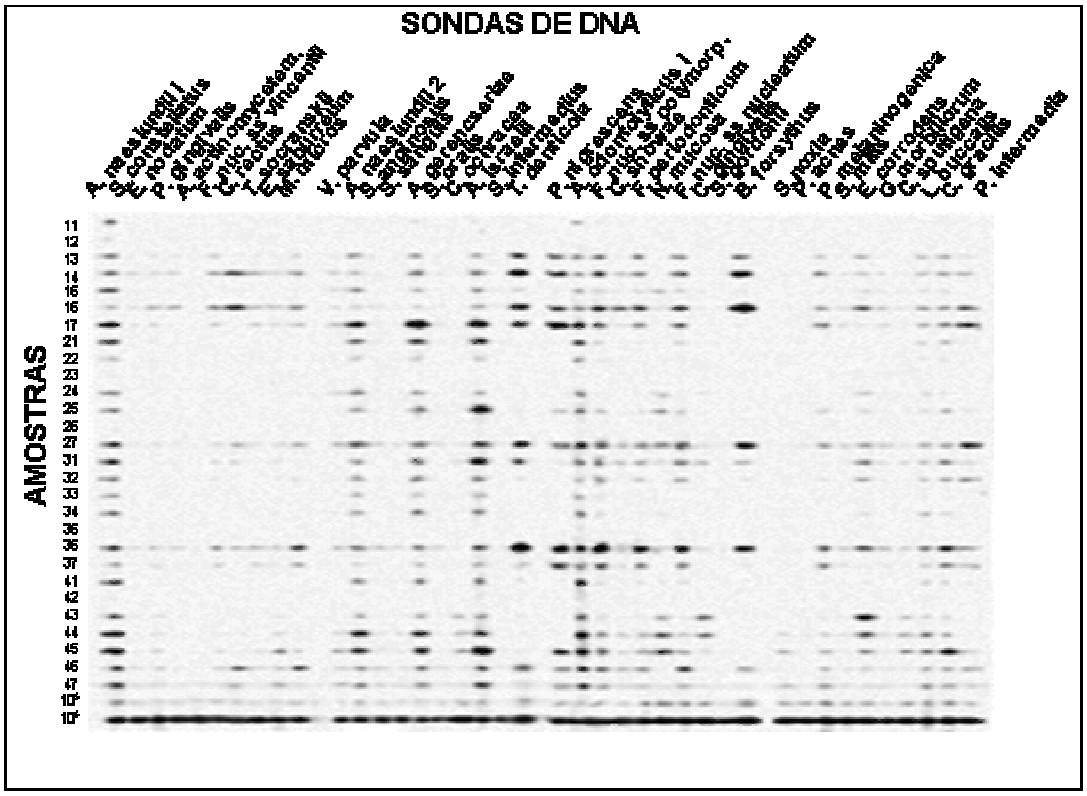
Anexo 13

Representação gráfica do padrão de hibridização entre as bactérias presentes nas amostras de placa e as sondas de DNA (técnica do *Checkerboard DNA-DNA Hybridization*).



Anexo 14

Padrão de hibridização das sondas de DNA com as amostras de placa subgengival em forma de tabuleiro de xadrez.



Anexo 15

Índice utilizado para a determinação dos níveis dos microorganismos nas amostras de biofilme supra e subgengivais.

Índice	Nível do microorganismo
0	Não detectado
1	Menos de 10^5 células
2	Aproximadamente 10^5 células
3	Entre 10^5 e 10^6 células
4	Aproximadamente 10^6 células
5	Mais de 10^6 células

Anexo 16

Resultados das análises brutas e ajustadas entre a doença periodontal e prematuridade e/ou baixo peso ao nascer

A associação entre a doença periodontal e a prematuridade e/ou baixo peso ao nascer foi conduzida utilizando-se as 15 definições de doença periodontal e os quatro defechos pré-estabelecidos. As *odds ratio* brutas e ajustadas são apresentadas no Anexo 17. Considerou-se nas análises ajustadas potenciais variáveis de confusão no estudo de associação da doença periodontal com a prematuridade e/ou o baixo peso ao nascer, incluindo: escolaridade materna, renda familiar, tabgismo na gestação, ansiedade, índice de massa corporal, qualidade do pré-natal, condições de moradia.

Apesar de se observar em algumas associações um efeito protetor da doença periodontal sobre os desfechos estudados, nas medidas ajustadas nenhuma *odds ratio* demonstrou associação significativa entre a doença periodontal e a prematuridade e/ou o baixo peso ao nascer.

Anexo 17

Odds ratios brutas e ajustadas entre doença periodontal e prematuridade e/ou baixo peso ao nascer

Doença periodontal		BPN			Prematuridade			BPN e prematuridade			BPN e/ou prematuridade		
		OR	IC	*P**	OR	IC	*P**	OR	IC	*P**	OR	IC	*P**
1†		0,67	0,25-1,75	0,540	1,21	0,55-2,63	0,789	0,61	0,18-2,04	0,582	1,13	0,55-2,34	0,891
1§		0,31	0,70-1,38	0,124	1,07	0,42-2,71	0,888	0,47	0,10-2,12	0,324	0,80	0,32-1,99	0,626
2†		0,84	0,43-1,62	0,714	1,31	0,74-2,31	0,433	1,15	0,56-2,37	0,853	1,04	0,60-1,78	0,990
2§		0,74	0,32-1,79	0,471	1,19	0,60-2,38	0,621	1,01	0,41-2,49	0,978	0,95	0,49-1,85	0,948
3†		0,57	0,36-0,90	0,021	0,66	0,43-1,03	0,084	0,66	0,39-1,15	0,186	0,57	0,38-0,86	0,009
3§		0,60	0,32-1,12	0,110	0,70	0,41-1,17	0,172	0,76	0,39-1,47	0,415	0,693	0,40-1,19	0,693
4†		0,77	0,45-1,33	0,427	0,76	0,45-1,27	0,346	0,92	0,49-1,72	0,907	0,68	0,42-1,10	0,143
4§		0,96	0,46-2,03	0,924	0,86	0,43-1,75	0,683	1,39	0,61-3,21	0,435	0,71	0,36-1,38	0,310
5†		0,86	0,18-4,11	0,821	0,91	0,19-4,45	0,765	♦	-	-	0,54	0,15-1,94	0,549
5§		0,48	0,05-4,77	0,529	0,47	0,08-2,91	0,420	♦	-	-	0,24	0,04-1,36	0,108
6†		0,86	0,24-3,10	0,914	0,64	0,20-2,09	0,686	1,75	0,22-13,57	0,907	0,54	0,19-1,53	0,375
6§		0,48	0,08-2,76	0,413	0,51	0,11-2,20	0,379	0,85	0,09-7,84	0,886	0,36	0,09-1,55	0,362
7†		0,59	0,36-0,97	0,038	0,58	0,36-0,93	0,022	0,64	0,36-1,15	0,133	0,55	0,36-0,84	0,005
7§		0,55	0,28-1,10	0,084	0,67	0,36-1,25	0,207	0,78	0,36-1,70	0,534	0,52	0,27-1,02	0,055
8†		0,57	0,36-0,90	0,021	0,66	0,43-1,03	0,084	0,67	0,39-1,15	0,186	0,57	0,38-0,59	0,009
8§		1,66	0,89-3,10	0,110	1,08	0,60-1,94	0,808	1,17	0,56-2,44	0,684	1,44	0,84-2,49	0,187
9†		0,60	0,38-0,94	0,036	0,71	0,46-1,10	0,151	0,66	0,39-1,14	0,174	0,64	0,43-0,95	0,033
9§		0,58	0,31-1,08	0,086	0,90	0,50-1,61	0,712	0,79	0,38-1,64	0,524	0,68	0,40-1,18	0,169
10†		0,57	0,30-1,08	0,109	1,53	0,93-2,53	0,126	0,69	0,33-1,44	0,404	1,20	0,75-1,94	0,524
10§		0,58	0,30-1,12	0,15	1,29	0,68-2,43	0,442	0,48	0,19-1,23	0,125	0,99	0,54-1,81	0,963
11†		0,48	0,11-2,09	0,477	1,57	0,59-4,13	0,525	0,79	0,18-3,46	0,977	1,10	0,42-2,90	0,959
11§		♦	-	-	0,58	0,11-3,18	0,533	♦	-	-	0,53	0,10-2,85	0,462
12	PH	1	-	-	1	-	-	1	-	-	1	-	-
	MP†	0,71	0,39-1,28	0,251	0,59	0,33-1,05	0,073	0,82	0,40-1,70	0,596	0,55	0,33-0,93	0,027
	MP§	0,69	0,31-1,50	0,348	0,77	0,36-1,66	0,500	0,86	0,33-2,56	0,760	0,65	0,32-1,30	0,221
	MSP†	0,78	0,40-1,50	0,452	1,17	0,64-2,16	0,612	1,03	0,46-2,28	0,946	0,94	0,54-1,66	0,838
	MSP§	0,51	0,21-1,25	0,141	1,08	0,48-2,44	0,845	0,75	0,26-2,20	0,752	0,78	0,37-1,65	0,523
13	ND	1	-	-	1	-	-	-	-	-	1	-	-
	P†	0,85	0,17-4,17	0,840	0,96	0,19-4,86	0,965	♦	-	-	0,54	0,14-2,04	0,360
	P§	0,53	0,05-5,30	0,588	0,56	0,09-3,50	0,539	♦	-	-	0,28	0,05-1,61	0,155
	GPD†	0,52	0,10-2,69	0,522	0,40	0,07-2,12	0,280	♦	-	-	0,27	0,07-1,06	0,060
	GPD§	0,34	0,03-3,64	0,337	0,24	0,04-1,65	0,148	♦	-	-	♦	-	-
14	1	1	-	-	1	-	-	1	-	-	1	-	-
	2†	0,88	0,49-1,58	0,668	0,68	0,38 -1,23	0,203	0,88	0,44 - 1,77	0,722	0,71	0,42 – 1,23	0,204
	2§	1,06	0,36-3,16	0,916	0,74	0,42-2,29	0,597	1,06	0,29-3,94	0,931	0,80	0,29-2,20	0,670
	3†	0,57	0,30-1,06	0,075	0,53	0,29 - 0,97	0,041	0,57	0,27 – 1,21	0,141	0,52	0,30 – 0,90	0,020
	3§	0,87	0,28-2,74	0,814	1,17	0,40-3,38	0,776	1,06	0,28-4,06	0,936	0,99	0,37-2,68	0,983
	4†	0,47	0,24-0,90	0,023	0,79	0,44 - 1,40	0,415	0,59	0,28 – 1,26	0,171	0,63	0,37 – 1,08	0,092
	4§	0,53	0,25-1,15	0,109	0,87	0,41-1,82	0,705	0,75	0,29-1,95	0,555	0,65	0,33-1,27	0,205
15	1	1	-	-	1	-	-	1	-	-	1	-	-
	2†	0,85	0,48-1,51	0,579	0,69	0,39-1,23	0,207	0,87	0,44-1,73	0,691	0,70	0,41-1,18	0,179
	2§	0,85	0,42-1,70	0,637	0,80	0,40-1,56	0,505	1,03	0,44-2,38	0,953	0,72	0,39-1,33	0,294
	3†	0,60	0,32-1,13	0,111	0,53	0,28-0,98	0,043	0,60	0,28-1,28	0,185	0,53	0,30-0,92	0,024
	3§	0,77	0,36-1,62	0,491	0,71	0,34-1,47	0,354	0,99	0,40-2,43	0,985	0,62	0,32-1,19	0,149
	4†	0,44	0,23-0,87	0,017	0,78	0,43-1,39	0,390	0,55	0,25-1,19	0,128	0,62	0,36-1,07	0,087
	4§	0,32	0,22-1,15	0,188	0,64	0,32-1,30	0,217	0,44	0,17-1,19	0,107	0,48	0,30-1,17	0,135

*_P_ refere-se a *odds ratio*

† refere-se a medidas de associação não ajustadas

§ refere-se a medidas de associação ajustadas para escolaridade materna, renda familiar, tabgismo na gestação, ansiedade, índice de massa corporal, qualidade do pré-natal, condições de moradia.

BPN: baixo peso ao nascer, OR: *odds ratio*, IC: intervalo de confiança (95%)

♦ *Odds ratio* não pode ser calculada

Definições de doença periodontal

Definição 1: pelo menos 1 sítio com PBS □ 5 mm em cada quadrante, não considerando as PBS nas faces distais dos dentes mais posteriores de cada quadrante,

Definição 2: pelo menos 1 sítio com PBS □ 4 mm, e pelo menos 50% dos sítios com ISS igual a 1,

Definição 3: pelo menos 4 sítios com PBS □ 3,5 mm,

Definição 4: média de PBS, índice de placa e ISS acima da mediana,

Definição 5: mais de 3 sítios com NCI □ 3 mm,

Definição 6: pelo menos 5 sítios com NCI □ 3 mm,

Definição 7: pelo menos 60% dos sítios com NCI □ 3 mm,

Definição 8: pelo menos 4 sítios com NCI □ 3 mm e PBS □ 4 mm,

Definição 9: pelo menos 4 dentes com 1 sítio ou mais com NCI □ 3 mm e PBS □ 4 mm no mesmo sítio,

Definição 10: mais de 5% dos sítios com PBS □ 5 mm e mais de 5% dos sítios com NCI □ 3 mm,

Definição 11: pelo menos 1 sítio com PBS □ 5 mm e 2 ou mais sítios com NCI > 6 mm e sangramento à sondagem > 5%,

Definição 12: Saúde periodontal: ausência de PBS > 3 mm e ausência de sítios com NCI > 2 mm, Periodontite leve: pacientes com menos doença do que o grupo moderado a severo e mais doença que o grupo saudável, Periodontite moderada a severa: pelo menos 4 sítios com PBS □ 5 mm e NCI □ 2 mm,

Definição 13: Ausência de doença: menos de 3 sítios com NCI □ 3 mm, Periodontite: pelo menos 3 sítios com NCI □ 3 mm, DP generalizada: pelo menos 90% dos sítios com NCI □ 3 mm .

Definição 14: Diferentes grupos de acordo os percentis do número de PBS ≥ 4 mm. Os quartis de distribuição de PBS ≥ 4 mm foram calculados e as puérperas agrupadas em diferentes níveis para PBS. Nível 1: 0 a 1 sítio com PBS ≥ 4 mm, Nível 2: 2 a 10 sítios com PBS ≥ 4 mm, Nível 3: 11 a 23 sítios com PBS ≥ 4 mm e Nível 4: 24 sítios ou mais com PBS ≥ 4 mm.

Definição 15: Diferentes grupos de acordo os percentis da soma de PBS ≥ 4 mm associados à NCI ≥ 4 mm. Os quartis de distribuição de PBS ≥ 4 mm associados à NCI ≥ 4 mm foram calculados e as puérperas agrupadas em diferentes níveis. Nível 1: 0 a 7 mm, Nível 2: 8 a 46 mm, Nível 3: 47 a 99 mm e Nível 4: 100 mm ou mais.