Virulence

ISSN: 2150-5594 (Print) 2150-5608 (Online) Journal homepage: http://www.tandfonline.com/loi/kvir20

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To cite this article: Charlene W.J. Africa (2011) Oral colonization of Gram-negative anaerobes as a risk factor for preterm delivery, Virulence, 2:6, 498-508, DOI: 10.4161/viru.2.6.17719

To link to this article: http://dx.doi.org/10.4161/viru.2.6.17719

Virulence 2:6, 498-508; November/December 2011; © 2011 Landes Bioscience

Oral colonization of Gram-negative anaerobes as a risk factor for preterm birth

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Key words: periodontal disease, Red complex, Orange complex, preterm birth

Background: Preterm birth significantly impacts on neonatal morbidity and mortality and is apparently increasing worldwide. Several studies have attempted to define a causative role for periodontal disease in adverse pregnancy outcomes but few have focused on the microbiology of periodontal disease in relation to these outcomes.

Results: The evidence for a positive correlation is strong, supported by microbiological and immunological findings. Conflicting results are often associated with uncontrolled confounding factors.

Materials and Methods: A literature search was conducted in order to establish whether or not a role exists for oral Gram-negative bacteria in adverse pregnancy outcomes. Association and intervention studies are summarized along with pathogenic potential of the Gram-negative bacteria most frequently implicated in periodontal disease.

Introduction

Adverse pregnancy outcomes are reported to cause up to 70% of all perinatal deaths[1,2] with 30% due to clinical or subclinical infection.[3] Between 25–50% occur without any known etiology[4] and in the absence of confounding factors.[5] Life-long complications due to preterm birth (PTB) such as abnormalities in sight and hearing, developmental retardation and neurological complications have been reported,[6,7] resulting in health care costs exceeding 10 times the costs of infants born full-term (FTB).

The term "adverse pregnancy outcomes" includes many different categorizations. Preterm labor (PTL) and preterm birth (PTB) are defined as labor before 37 weeks gestation and in 30% of cases, is preceded by premature (<37 weeks gestation) rupture of membranes (PPROM). Previously, all infants born weighing <2,500 g were considered to be premature but now, with the advent of better gestational dating, it was found that not all infants born of low birth-weight (LBW) are in fact born prematurely. LBW does not distinguish between PTB and FTB infants, nor does it provide information on gestational age, yet the term premature low birth-weight (PTLBW) is often used to include infants born "small for gestational age" (SGA). SGA is a categorization for infants of all gestational ages achieved by the establishment of growth curves and detailed statistics for infants falling within the lowest 10th percentile at a given date of delivery. Their small size is attributed to intrauterine growth restriction (IUGR).[8] Sources of infection linked to adverse pregnancy outcomes include intrauterine, lower genital tract and systemic infections. Periodontal disease has also been implicated as a risk factor for adverse pregnancy outcomes and refers to a group of endogenous polymicrobial infections that cause inflammation and destruction of the gingiva (gingivitis) and other supporting structures of the tooth such as the periodontal ligament and alveolar bone (periodontitis).[9] No overt pathogen has been identified, but its etiology is strongly associated with anaerobic Gram-negative bacilli.[10,11] This review will focus on the association of the anaerobic Gram-negative bacteria implicated in periodontal disease and examine their potential to cause adverse pregnancy outcomes.

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Submitted: 04/01/11; Revised: 08/10/11; Accepted: 08/11/11
http://dx.doi.org/10.4161/viru.2.6.17719

Results

Studies displayed heterogeneity concerning diagnostic criteria of periodontal disease and types of pregnancy outcome, so no meta-analysis was performed.

Risk factors for adverse pregnancy outcomes. There are several risk factors associated with adverse pregnancy outcomes, including prenatal care,[12-14] age,[15,16] smoking,[17-21] a history of previous preterm delivery,[13,15,22-27] educational level,[13,28] poor nutrition,[27,29] maternal weight and height,[26,27,30-33] first pregnancy,[34] depression,[35] social stress factors,[16,36] antibiotic usage,[37,38] alcohol and drug abuse[39,40] and infection and inflammation.[41-43] Recently, a genetic predisposition for PTB has been proposed, either due to racial disparities that are independent of socio-economic factors,[25] or as part of the gene-environmental interaction associated with either hypo-or hyper-immune responses.[44]

In order to establish whether periodontal disease and/or other endogenous infections may pose a risk for PTB and other pregnancy outcomes, it is important to understand how the dental plaque bacterial biofilm forms and why pregnant mothers are at risk for developing periodontal disease.

The bacterial plaque biofilm in pregnancy-associated periodontal disease. The tooth surface harbors a microbial population that lives in harmony with the host tissues and protects by occupying an ecological niche that would otherwise be colonized by potentially pathogenic bacteria.[45] The relatively aerobic environment of the healthy gingival sulcus tends to preclude the

growth of obligate anaerobes and the predominant cultivable flora includes facultative anaerobes, predominantly Gram-positive rods and cocci. If plaque is allowed to accumulate, demonstrable inflammation of the gingiva will occur in 2–4 days due to the production of various noxious metabolites such as endotoxins, lipoteichoic acids, mucopeptides, metabolic end products and proteolytic agents as well as a host of other enzymes such as hyaluronidase and chondroitinase, which may penetrate the gingival tissues causing their destruction by direct injury or by stimulation of inflammatory immune responses which, in turn, results in their further destruction.[46] In addition, plaque accumulation may be facilitated by the increased production of gingival crevicular fluid which contains growth-promoting factors for a wide range of bacteria including Gram-negative anaerobes such as *P. gingivalis, Prevotella intermedia* and *Fusobacterium nucleatum,* among the bacteria known to be increased in pregnant women and women taking oral contraceptives.[47-50]

Hormonal and vascular changes associated with pregnancy may exacerbate the gingival response to bacterial plaque, resulting in an inflammatory periodontal disease. Pregnancy gingivitis is the most common oral manifestation of pregnancy and comes about as a result of the hormones estrogen and progesterone, and not necessarily because of an increase in plaque bacteria.[49] It is reported to affect between 30–100% of pregnant women.[51-55] Pregnancy gingivitis usually occurs in the second month of gestation and progresses with time, reaching a peak in the eighth month of gestation. It usually subsides thereafter and immediately postpartum may revert to what was previously observed in the mouth.[51,56]

Several factors are thought to play a role in the establishment of pregnancy gingivitis. The increased hormonal levels enhance localized production of inflammatory mediators such as prostaglandin E_2 (PGE_2),[57] while progesterone also reduces fibroblast proliferation, alters collagen production and reduces the level of plasminogen activator inhibitor type-2 (PAI-2), an important inhibitor of tissue proteolysis.[48,58]

Repeated vomiting during pregnancy results in acid-induced erosion, and coupled with the inability to tolerate toothbrushing because of tooth sensitivity and nausea, the oral hygiene of the pregnant woman is severely compromised.[56,59,60] This, along with estrogen-enhanced proliferation and desquamation of the oral mucosa and nutrients provided by the bleeding gingiva creates a suitable environment for an overgrowth of Gram-negative anaerobes in the plaque.[47-50,61,62]

Association of periodontal disease with PTB. Among the first studies to associate a periodontopathogen with pregnancy outcomes was the study by Collins and colleagues,[63] who injected pregnant hamsters with *Porphyromonas gingivalis* (an oral anaerobe commonly associated with periodontal disease), and proved that oral bacteria and the inflammatory mediators produced in response to the antigens they present, could disseminate via the bloodstream to the maternal-fetal interface and induce adverse pregnancy outcomes. A human association study followed,[64] which demonstrated that mothers with periodontal disease were more likely to deliver PTLBW infants than mothers with good oral health. This sparked off a series of association studies around the world with some researchers reporting a significant association between periodontal disease and adverse pregnancy outcomes in, among others, Thailand,[65,66] Saudi Arabia,[67] Turkey,[68] Brazil,[14,69] Venezuela,[70] Chile,[71] Senegal,[72] South Africa,[73] Hungary,[74,75] Croatia,[76] Finland,[32] USA,[24,77-80] Austria,[81] Taiwan[13] and Japan.[82] No association and/or contradictory outcomes were reported from countries such as Sri Lanka,[83] Pakistan,[84] Turkey,[85] England,[86-89] Germany,[15] Iceland,[90] Tanzania,[16] Rwanda,[26] Brazil[91-93] or Chile.[94] These include both case-control and cohort studies. In case-control studies, mothers who experience adverse pregnancy outcomes such as PTB, LBW or PTLBW are examined for periodontal disease, while in cohort studies, mothers are investigated over a period of time in order to establish whether those with periodontal disease will demonstrate a higher incidence of adverse pregnancy outcomes than those without periodontal disease. However, very few of these studies examined for a microbial etiology and associations were largely based on clinical evaluations of periodontal disease. Differences in diagnosis, clinical measurements, stage of gestation, disease activity and severity, treatment programmes, along with the confounding factors listed above may affect disease progression and pregnancy outcome. These factors may explain different outcomes in populations from the same geographical area as demonstrated in the Brazilian studies.[14,69,91-93]

Microbial complexes in subgingival plaque and their association with PTB. In order to study a relationship between two events, it is imperative to establish an association first, bearing in mind that the association could easily be due to chance, bias, and/or confounding factors. This is especially relevant in attempting to determine a relationship between the complex communities of periodontopathogens and pregnancy outcomes. The following selection of some of the most frequently cited studies examining for the relationship between periodontal disease and PTB clearly demonstrates this.

Gram-negative anaerobes frequently implicated in pregnancy periodontal disease include members of the Orange and Red complexes described by Socransky et al.[95,96] Using cluster analysis, Socransky and his colleagues identified six complexes of bacteria which commonly occur together, and color-coded them as Blue, Green, Yellow and Purple, Orange and Red with the latter two complexes implicated as etiological agents in periodontal disease and therefore as risk factors for adverse pregnancy outcomes.[81] The Orange complex includes species such as *Campylobacter rectus, Peptostreptococcus micros, Prevotella nigrescence, Fusobacterium nucleatum* and *Prevotella intermedia* and provides the lawn for the attachment and colonization of members of the Red complex (*Tannerella forsythia, Treponema denticola, P. gingivalis*). Although clustered within the Green complex, Capnocytophaga and *Aggregatibacter actinomycetemcomitans* (Aa) have frequently been associated with periodontal disease and with PTB and are therefore included in this review.

Orange and Red complexes have been reported in 16–18% of FTB and 80–100% of PTB.[81,97] As with the clinical associations between periodontal disease and PTB, inconsistencies occur when comparing PTB and FTB for associated bacteria. Significant differences have been reported in the association of

the Orange and Red complexes (along with Aa and *F. nucleatum*) with PTB in some studies[81,98] while in others, no association was demonstrated.[99]

The prevalence of members of the Red and Orange complexes,[81] were reported to occur in the order of *T. forsythia*,[15,26,75,81,82,98,100,101] *T. denticola*[98,100] and *P. gingivalis*.[75,98] In the Orange complex, *C. rectus*[85,98,101] *P. intermedia*,[15,26,75,98] *P. micros*[85,100] and *F. nucleaturm*[15,75,85,98,100] were most frequently reported, with Aa[75,98,100] Capnocytophaga[75,100] *S. intermedius*,[85,100] *E. corrodens* and Selenomonas[85] being significantly increased in some case studies but not in others, even though their levels were seen to increase considerably in PTB.

In other cases where periodontal disease was evident[15,26] and members of the Red and Orange complexes were detected in subgingival plaque[15,99] or gingival crevicular fluid,[26] no differences between PTB and FTB were found, nor were any Red complex bacteria detected in the absence of the Orange complex in any of these studies, confirming that colonization of the Orange complex is a prerequisite for the Red complex to become established.

A lack of association with periodontal measurements in a Danish population of PTB and FTB mothers failed to support an association between periodontal disease and PTB although an association of PTB with increased prevalence of *S. intermedius, S. sanguinis* and *S. oralis*, usually considered to be primary colonizers of the plaque biofilm and not usually associated with periodontal disease, was established.[100] Even though their role in periodontal disease has not been clearly established, streptococci have been associated with adverse pregnancy outcomes through DNA analysis of amniotic fluid.[22]

Maternal and fetal immunoglobulin levels and their association with PTB. The possibility that periodontal disease could contribute to PTB is further supported by host responses to the Gram-negative bacteria implicated in periodontal disease. Examination of serum IgG provides evidence for both systemic exposure and protection by facilitating clearance. A finding of IgM would demonstrate in utero exposure to periodontopathogens and therefore fetal exposure. Periodontal pathogens and their antibodies may be found not only in subgingival plaque samples and gingival crevicular fluid but also in maternal serum or plasma, amniotic fluid and placenta. As with the detection of subgingival colonizers above, mothers who were seropositive (Immunoglobulin G, IgG) for Red complex bacteria (98%), were also positive for Orange complex bacteria and IgG responses were found to parallel colonization of the Orange complex in advance of the Red complex.[99] Maternal IgG responses showed a significant reduction in anti-*P. gingivalis* IgG in PTB compared with FTB.[98]

Another case-control study[24] showed no differences between PTB and FTB in maternal IgG for Red and Orange complexes, although a clinical correlation with PTB was established. A lack of maternal IgG response to members of the Red complex and other oral flora implicated in PTB may result in fetal exposure thus suggesting that maternal IgG may protect against both fetal exposure and PTB, while increased cord blood IgM may suggest in utero exposure of the fetus to maternal oral organisms. The literature suggests that colonization of suspected periodontopathogens in non-susceptible controls resulted in elevated antibody levels considered to prevent infection in controls while challenge with increased numbers of pathogens may overwhelm acquired immunity resulting in reduced antibody levels with a reduced protection against infection, thus allowing for hematogenous spread of Gram-negative species to the fetal-maternal interface.[102]

Intrauterine infections as a risk factor for PTB. PTB due to intrauterine infection was reported to be rare beyond 34 weeks gestation with 45% occurring between 23 and 26 weeks gestation, 16% occurring at 27–30 weeks gestation and 11% at 31–34 weeks gestation.[103] The demonstration of any bacteria in the normally sterile amniotic cavity would be indicative of bacterial invasion, and because bacteria are more frequently isolated from the chorioamniotic space than from amniotic fluid, it would suggest that microbial invasion of the amniotic cavity results from advancing disease from the extra-amniotic to the intra-amniotic space.[104-106]

Proposed pathways of intrauterine infections include the ascending route (following genital endogenous or exogenous infections) and hematogenous spread and translocation of bacteria from distant body sites to the fetal-placental unit.[107]

Infection via the ascending route. Romero et al.[106] proposed the following ascending pathway for infection. It starts with vaginal colonization of exogenous pathogens or an overgrowth of endogenous opportunistic microflora, followed by infection of either the intra- or extra-amniotic space, depending on the location of the infective agent. Extra-amniotic infection occurs when bacteria reside in the deciduas resulting in a localized inflammatory reaction which leads to deciduitis and extending to chorionitis. Fetal vessels may be invaded or the infection may proceed through the amnion into the amniotic cavity, resulting in an intra-amniotic infection. Entry of microorganisms into the intra-amniotic space is not dependent on rupture of membranes as microbes have been able to cross intact membranes.[108] Once bacteria have gained access to the amniotic cavity and infected the amniotic fluid, they have direct access to the fetus who is then infected by aspiration of the infected fluid leading to congenital pneumonia, or the microbes in the infected fluid may directly invade the fetus causing localized infections such otitis or conjunctivitis. Bacteremia and/or septicemia may occur following any seeding from these localized infections to the fetal circulation.

Goldberg et al.[109] reported that up to 80% of women who deliver preterm produced evidence of ascending bacterial infection in the amniotic fluid or membranes, with predominant organisms being those associated with bacterial vaginosis (BV), a condition brought about by an imbalance and shift of the normal vaginal flora in favor of anaerobic bacteria. When comparing women with BV and without gingivitis with those who had BV plus gingivitis, increased vaginal counts were observed in the latter.[110] This could indicate an association between BV and periodontal disease with PTB since both conditions occur frequently during pregnancy, both come about as a result of a shift in endogenous microflora from predominantly facultative organisms to predominantly anaerobic organisms and they share common

bacterial co-colonizers such as Prevotella, Porphyromonas, Bacteroides, Peptostreptococcus and Fusobacterium.[111]

Infection via translocation of bacteria. Other ways by which bacteria may gain access to the amniotic cavity include hematogenous spread and translocation of bacteria to the feto-placental unit.[107]

The focal infection theory of Miller[112] states that oral microrganisms and/or their end products are able to gain entrance to, and infect, neighboring or distant parts of the body. Because not all women who experience PTB have genital infections, and not all with genital infections deliver preterm, the possibility exists that the oral cavity may be a source of infection for adverse pregnancy outcomes[113] especially since a common pathophysiology exists due to microbial similarities between the female genital tract and the oral cavity.[44]

F. nucleatum has been associated with PLBW[75,114] and is one of the most common isolates from amniotic fluid of patients with PTB and intact membranes[115-117] and in one study it was demonstrated that the *F. nucleatum* isolate clearly matched species isolated from the mouth than from the genital tract.[118] Although it may be conceded that *F. nucleatum* enters the amniotic space as a result of ascending infection following oral-genital transfer, further studies demonstrated hematogenous spread.[118,119] Pregnant mice were injected with *F. nucleatum,* inducing infection that was restricted to the uterus causing Toll-like receptor 4-mediated localized inflammatory responses which resulted in PTB, stillbirths and non-sustained live births without spreading systemically.[98,119] This same group later demonstrated similar findings in a human case study of a stillbirth from a mother with pregnancy gingivitis, who developed a respiratory infection at term.[120] Examination of oral, vaginal and rectal flora, showed that the clone in her subgingival plaque matched the clone found in the placenta and stillborn infant but differed from that found in her supragingival plaque, vagina or rectum. It was then speculated that because of the weakening of the maternal immune system during the respiratory infection, it allowed for translocation of *F. nucleatum* from the mother's mouth to the uterus. Its ability to colonize the placenta and chorioamniotic membranes in PTB is facilitated by its most powerful virulence mechanism, namely, the FadA adhesin.[121]

An association between microbial DNA and complications during pregnancy, including a history of miscarriage, intrauterine and neonatal death, PTB and premature rupture of membranes have been associated with the presence of *F. nucleatum* and streptococci in amniotic fluid.[22]

Capnocytophaga has been isolated in pure culture from amniotic fluid,[122] infant blood[123,124] placenta[125] maternal blood[126] uterus,[123,127] infant gastric aspirate[124,127] and endometrium.[124] Its isolation from mothers with ruptured membranes was often associated with infant respiratory failure. Capnocytophaga may be implicated in occult causes of chorioamnionitis or PTB with the prevalence being much higher than was previously reported.[128–130].

Direct evidence of oral-utero microbial transmission was demonstrated in a patient with an intrauterine infection caused by a hitherto uncultivated Bergeyella species.[131] The same strain was detected in her subgingival plaque but not in her vagina, suggesting that the infection originated from her oral cavity and was hematogenously spread to the uterus rather than via the ascending route from the vagina following oral-genital transmission.

Other oral bacteria associated with human intrauterine infections include Aa,[129] Campylobacter,[99,130,132] Eikenella[133,134] and Leptotrichia.[133]

Virulence properties of subgingival plaque colonizers. It has been proposed that microbial studies should identify potential virulence factors of oral bacterial colonizers which may relate to placental targeting fetal exposure and growth restriction before any finite conclusion can be drawn regarding the association between PTB and periodontal disease.[99]

Virulence properties of *F. nucleatum* include co-aggregative and synergistic mechanisms.[135] This is an important factor in biofilm formation as it creates an environment which favors the colonization of other anaerobes such as the Red complex (*T. forsythia, P. gingivalis, T. denticola*), Prevotella and Aa.[136-138] Its FadA adhesin and invasive mechanism allows it to colonize and infect the placenta.[119,121] Other pathogenic mechanisms include phospholipase and endotoxin activity which contribute to PTB.

Campylobacter species are known to cross the placenta and induce adverse pregnancy outcomes in sheep[139] and IUGR in mice.[140] *C. rectus, P. gingivalis,* Prevotella, Aa and their bacterial products are among the periodontopathogens reported to disseminate from the oral cavity through the circulatory system to the uterus thus inducing systemic and inflammatory responses which bring about PTB.[99,141-143] They also produce proteases which reduce chorioamniotic membrane strength and very high bacterial loads may weaken fetal membrane strength resulting in preterm rupture of membranes.[144]

Phospholipase A2, a precursor of prostaglandin synthesis, is produced by Bacteroides, Pepstostreptococcus, Fusobacterium and *Gardnerella vaginalis*.[144,145] Lysosomes in fetal membrane cells contain high concentrations of phospholipase A2 and their destruction within decidual or chorioamnion cells may trigger prostaglandin synthesis resulting in uterine contractions.

Treponema have the ability to invade, cross the placenta and infect the fetus[146] and present a risk for both PTB and still birth, especially in low income societies.[147] These findings demonstrate the virulence mechanisms of bacteria implicated in periodontal disease and along with the host response to these mechanisms in the section that follows, may suggest a role for the Orange and Red microbial complexes in the pregnancy outcomes of a susceptible host, particularly for those species showing clonal heterogeneity.

Inflammatory responses due to microbial products. During pregnancy, innate pro-inflammatory immune responses are strictly regulated within the uterus thus preventing immunological rejection of the fetal allograph. If bacteria and their toxins enter the uterine cavity via the ascending route from the lower genital tract, or if they invaded the chorioamniotic space, thereby disrupting this delicate balance and activating fetal membranes, this could lead to the maternal immune response being triggered to produce a vast array of cytokines and growth factors at the maternal-fetal interface. This, in turn, may elicit

an inflammatory burden that may damage the placenta resulting in fetal growth restriction (FGR). Added to that, the maternal cytokine responses may lead to the stimulation of prostaglandin synthesis, particularly E_2 (PGE_2) and the release of metalloproteinases (MMPs), which bring about uterine contractions and membrane rupture, thereby prematurely activating the parturition mechanism resulting in PTB, PPROM and LBW.[148]

It was postulated that periodontal infections are able to produce local and systemic host responses, leading to transient bacteremia.[149] Lipopolysaccharide (LPS), an endotoxin released by Gram-negative bacteria implicated in the etiology of periodontal disease, along with other bacterial products gain access to gingival tissue where they initiate and perpetuate local inflammatory reactions including the production of pro-inflammatory cytokines. These maternal inflammatory responses play an important role in the pathophysiology of adverse pregnancy outcomes.[65,104,150,151] Gingival crevicular fluid (GCF) levels of PGE_2 and interleukin-1β (IL-1β) are significantly elevated in PTB.[152,153] The bacteria from subgingival plaque along with LPS and pro-inflammatory cytokines from the inflamed gingival tissue can enter the bloodstream, reach the maternal-fetal interface and trigger or exacerbate the maternal inflammatory response increasing plasma levels of prostaglandin and cytokines such as IL-1β, tumor necrosis factor-α (TNFα), IL-6 and PGE_2.[18,24,82,150,154-156] PGE_2 is responsible for mediating cervical ripening and stimulating uterine contractions and thus PTB is initiated.

Associations of elevated levels of IL-6 in maternal blood and amniotic fluid in cases of PTB have suggested a role for IL-6 as an indicator of intrauterine infection and a predictor of PTB.[3,157-161] It has also earned the role of diagnostic marker in periodontal disease.[162,163] A study of murine maternal responses to *P. gingivalis* during pregnancy[164] showed that *P. gingivalis* dissemination to the uterus has to occur for fetal growth retardation (FGR). Maternal humoral responses demonstrated elevated IgG and TNFα in FGR fetuses. *P. gingivalis* LPS challenge immunization was found to enhance effects on growth restriction rather than offer protection for the fetus, probably due to exacerbation of the inflammatory response. IL-6 and TNFα levels were elevated in the PTB group while anti-inflammatory IL-10 decreased significantly. Increased IL-6 and reduced amniotic fluid levels of IL-10 in the second trimester was also considered to be an indicator of PTB in humans.[157] IL-10 is important for regulating the balance between innate inflammatory responses and acquired humoral responses. Contradictions in the literature create confusion regarding the role of IL-10 in PTB and its apparent multifarious role at the maternal-fetal interface remains controversial.[165] Reduced levels of IL-10 in PTB[3,166] and periodontal disease[167,168] associated with elevated levels of IL-6 were reported to promote PTB, while a protective role for elevated levels of IL-10 in preventing PTB has also been proposed.[169,170]

Amniotic fluid levels of IL-6 and PGE_2 were significantly elevated in PTB and correlated well with bacterial plaque cultures in a study by Dortbudak et al.[81] while IL-8 was significantly increased in FTB. No difference was found between PTB and FTB for amniotic fluid levels of TNFα, or IL-1 in this study, while IL-1β and IL-8 were reported to be significantly increased in a Japanese population.[82]

Intervention studies. Periodontal disease is both treatable and preventable and thus it would be expected that periodontal treatment/intervention during pregnancy would improve pregnancy outcomes[171,172] thereby reducing the risk of fetal exposure[173] and in turn, strengthening the argument in favor of the role played by periodontal disease in adverse pregnancy outcomes.[163,174-176]

A 28% reduction in PLBW was associated with treatment in a study of women with predominantly low socio-economic backgrounds (60% African-American, 39% Hispanic).[101] The group who received oral prophylaxis during pregnancy had an incidence of 13.5% PLBW, while the control group who were recruited postpartum showed 18.9% PLBW. This difference was not statistically different although elevated levels of *T. forsythia* and *C. rectus* were observed in plaque from PLBW mothers.

The successful use of antibiotics for treatment to reduce the risk for adverse pregnancy outcomes in mothers with either BV or periodontal disease, yielded conflicting results[33,177-185] and in fact, one study[185] demonstrated an increase in PTB following metronidazole administration.

In a randomized control trial,[186] a sample population was divided into four groups with Group 1 = prophylaxis + placebo, Group 2 = scaling and root planing + placebo, Group 3 = scaling and root planning + metronodazole and Group 4 = controls. Results showed that scaling and root planing reduced PTB while the addition of metronidazole did nothing to improve pregnancy outcomes.

Comparison of treatment before 28 weeks gestation was compared with treatment postpartum in a randomized study in Chile.[174] Treatment consisted of oral hygiene instruction, scaling and root planning, plus a daily dose of Chlorhexidine mouth rinse. They reported a 82% reduction in PLBW. A later study by the same group,[187] further proved that mechanical debridement on its own was not as effective as with the adjunct use of Chlorhexidine in reducing PTB. Treatment was initiated in the second trimester and continued into the third trimester. Scaling and root planning in the second trimester of pregnancy showed no success in reducing PTB,[171,172] and was instead associated with elevated proportions of the Red complex.[98]

Women with periodontal disease, hospitalized because of the threat of PTB, were randomly assigned to two groups; one group received treatment for periodontal disease by way of oral hygiene instruction, scaling and root planing plus fluoride polishing while the other group received no treatment.[188] Treatment was provided in the third trimester. A significant difference in gestational age and weight of their infants was observed with those who had been treated delivering at 37.5 weeks with a weight of 3,079 g, while the untreated mothers delivered at 36.1 weeks with a birthweight of 2,602 g. From these studies, it would appear that treatment during the third trimester was more beneficial to the patient than treatment in the second trimester.

There is the threat that mechanical debridement in the absence of a concurrent administration of an antimicrobial agent, might result in a bacteremia[192] the degree of which correlates with the severity of periodontal disease[193-195] inciting an acute

inflammatory response leading to elevated levels of IL-6, known to be a risk indicator for PTB.[194-196] It would also appear that timing of periodontal treatment dictates the outcome.[197] Evidence of periodontal treatment being deleterious during pregnancy is inconclusive, yet, there is a tendency for dentists to delay dental treatment until postpartum because of the risks associated with bacteremia, taking of radiographs and drug administration. The adverse outcomes associated with delay in treating mothers with periodontal disease far out-weigh the perceived complications which may occur through immediate action. Thus, if maternal periodontal treatment is restricted to the early part of the third trimester, with the inclusion of antimicrobial agents, such intervention therapy may reduce adverse pregnancy outcomes.[13]

However, multicenter intervention studies such as The Maternal Oral Therapy to Reduce Obstetric Risk (MOTOR),[171] the Periodontal Infection and Prematurity Study (PIPS)[189] and the Obstetrics and Periodontal Therapy (OPT) trials[172,190] did not significantly reduce the rates of adverse pregnancy outcomes although the periodontal health of the patients improved. The OPT study examined for the Orange and Red complexes along with Aa.[190] Pregnant women were examined before 21 weeks of gestation or postpartum (PTB and FTB). Subgingival plaque samples were analyzed using quantitative PCR at baseline and post treatment. Although treatment significantly reduced the proportions of the Red and Orange complex bacteria, no significant difference was observed between PTB and FTB for any of the bacteria. Aa levels were not significantly reduced post treatment. Although these studies proved that treatment of periodontal disease during pregnancy was safe and effective, the study groups were large and covered general population groups, leaving room for the effect of confounding factors. These results contradict smaller studies which showed significant differences between PTB and FTB following treatment.[186,188] Inconsistencies may arise due to population differences, severity of disease, different clonal types of bacterial species as well as differences in the management of patients.[191]

The use of BANA for the screening of mothers at risk for PTB due to periodontal disease was recommended by Africa et al.[73] and confirmed by Chan et al.[13] The BANA test is based on the principle that members of the Red complex produce a trypsin-like enzyme which hydrolyses the substrate benzoyl-DL-argenine-naphthylamide (BANA) producing a color change in the presence of 10^4 cell concentrations. Controlling for other risk factors, BANA-positive plaques in the third trimester could be associated with PTB. The authors thus concluded that BANA screening informed the need for intervention between 28–32 weeks gestation.

Since BANA has been reported to weakly detect Capnocytophaga species as well[198] BANA-positive plaques in the absence of the Red complex could also indicate a risk for Capnocytophaga-associated chorioamnionitis.[199]

Bacterial co-aggregations in biofilms such as plaque are common, and microbial interactions may modulate their pathogenic potential. Examples are *P. gingivalis*, *T. denticola, T. forsythia, F. nucleatum*, Aa and Campylobacter,[200-203] resulting in the production of excessive inflammatory responses.[204] Bacterial combinations of three or more species yielded significant correlations with PTB[26] especially when *T. forsythia,* Aa and *P. intermedia* were included in the combination. Aa appeared to be a necessary co-factor for significant associations of bacterial combinations and there is speculation that PTB may be attributed to specific genotypes of Aa. *F. nucleatum* mediates co-aggregation of bacterial species by creating an anaerobic environment which favors the colonization of the Orange and Red complexes allowing for synergistic interaction and increased virulence expression.[136]

Materials and Methods

Strategy for literature search. PubMed and Science Direct were searched for original articles using the following key words: adverse pregnancy outcomes OR preterm birth OR low birthweight OR pregnancy AND risk factors OR periodontal disease OR periodontopathogens OR Red complex OR plaque bacteria OR Orange complex.

Inclusion criteria. Studies of the association between suspected periodontopathogens and adverse pregnancy outcomes from 1990 through 2011 were included. The search included human and animal studies.

Exclusion criteria. The search was limited to reports written in English only and excluded pregnancy confounded by other systemic illnesses.

Summary and Conclusion

The ultimate aim of this review was to provide evidence for the role of periodontal disease-associated Gram-negative anaerobes and PTB and to determine whether periodontal treatment could reduce the incidence of adverse pregnancy outcomes.

When implicating any specific bacterial species in the etiology of PTB, one has to demonstrate its role in ascending infection, its ability to elicit maternal and/or fetal antibody responses and its ability to produce inflammatory responses such as the production of prostaglandins and cytokines as a result of its metabolic products or virulence properties. The microbial consortia investigated have shown an association with, and displayed virulence mechanisms for their implication in PTB.

However, a meta-analysis of 44 studies[206] found no significant association between periodontal disease and PTB, although a trend toward association was observed in low-income groups. Marked differences were observed in oral microflora between subjects from different geographical locations. These population differences are, in turn, related to race, lifestyle and socioeconomic conditions, all of which will influence therapeutic outcomes. Different microbial profiles will demonstrate differences in response to therapy thus explaining the different success rates of intervention studies.[191]

A bleeding gingivitis associated with proportional overgrowth of Gram-negative anaerobes was found to occur during the second trimester of pregnancy[47,62] while reduced levels of maternal IgG appeared to predict PTB only when assessed in the second trimester of pregnancy,[102] thus supporting the need for specific periods of gestation for meaningful microbial assessments.

However, periodontal treatment during the second trimester did not reduce the risk of adverse pregnancy outcomes.[171,172]

The strength of an association between two events increases the likelihood of a causal relationship. Evidence of the pathogenic potential of the oral Gram-negative bacilli, the frequent association of the Orange and Red complexes with both periodontal disease and PTB and their elimination after intervention treatment would suggest a role for them in adverse pregnancy outcomes. However, association studies come with their limitations. Confounding variables differ significantly among the different studies with some adjusting for their impact and others not. The perceived contradictions of these studies may largely be due to the many different definitions of adverse pregnancy outcomes[205] coupled with differences in gestational ages.[11] Some researchers sample during the second trimester, while others advocate sampling during the third trimester and yet others recommend postpartum investigations.[62,81,98] Selection of different diagnostic definitions and measurements of periodontal disease states[26,51,206-209] will also lead to different outcomes.[210] In limiting the inflammation source to periodontal disease only, the total inflammatory burden from the oral cavity may not be accurately assessed.[32] One can also argue that these studies examined for a select group of oral anaerobes only, which, bearing in mind the complexity of the etiology of periodontal diseases, may exclude many potential pathogens.

A systematic review of epidemiological studies[211] found no sound scientific justification to recommend routine screening for periodontal disease in pregnant women in an effort to prevent adverse pregnancy outcomes, while recent studies have indicated that the BANA test may be a useful screening tool in identifying mothers at risk for PTB[13,26,73] and would be worth exploring.

It is reasonable to suggest that infection of the gingiva and periodontium by Gram-negative anaerobic bacteria provide a reservoir for microbial products (e.g., LPS) and sufficiently challenge the host to produce responses which may be deleterious to both the pregnant mother and the fetus.[212] In addition, a non-causal correlation between periodontal disease and PTB has been hypothesized where hyper-inflammatory response to bacterial challenge is genetically determined.[213] As demonstrated in these studies, the association between bacterial colonization and maternal host responses remain speculative. There are too many variables to draw any finite conclusions. However, the way has been paved for future research to focus on establishing why some women develop adverse pregnancy outcomes due to an oral inflammatory burden while others do not. A standardized universal protocol is needed for the outcomes to be conclusive.

Acknowledgments

This material is based upon work supported financially by the National Research Foundation. Any opinion, findings and conclusions or recommendations expressed in this material are those of the author and therefore the NRF does not accept liability in regard thereto.

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