CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

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Oral Health and Adverse Pregnancy Outcomes – What's Next?

ABSTRACT

Studies on the link between periodontal disease and adverse pregnancy outcome have gone through several phases. The epidemiological studies predominantly support a positive association between these wide-affecting diseases. During the intervention phase, a few small-scale, single-center studies reported improvement of birth outcome following periodontal treatment, whereas the large-scale multicenter studies did not demonstrate efficacy. Many questions arise with regard to patient population, disease type, and therapy. In addressing these questions, it is crucial that one understands the mechanism underlying the link between these diseases. Two non-mutually exclusive hypotheses exist. In the first, periodontal disease is believed to affect the maternal and fetal immune responses systemically, leading to premature labor. Alternatively, evidence is accumulating that oral bacteria may translocate directly into the pregnant uterus, causing localized inflammation and adverse pregnancy outcome in the presence or absence of clinical periodontitis. The oral-uterine transmission is not limited to the well-recognized periodontal pathogens, but instead may also involve the commensal species. Future studies should investigate these mechanisms, to understand the host susceptibility to oral-uterine transmission. Only when a thorough understanding of the mechanism is achieved can meaningful intervention studies be designed utilizing effective therapies, targeting appropriate populations, and measuring relevant outcomes.

KEY WORDS: bacteria, inflammation, infectious disease(s), microbiology, periodontal disease(s)/periodontitis.

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INTRODUCTION

Periodontal disease is a wide-affecting infectious disease consisting of various forms. Based on its severity, periodontal disease can be broadly classified into two stages: gingivitis, a mild and reversible form characterized by inflammation without tissue damage; and periodontitis, a more advanced and severe form characterized by attachment and bone loss. The potential connection between periodontal disease and other systemic conditions, such as diabetes, cardiovascular disease, and preterm birth, has attracted much research attention in recent decades.

Preterm birth, defined as birth before 37 weeks of gestation, is the leading cause of neonatal mortality, infant morbidity, and long-term sequelae. The preterm birth rate in the US has risen 20% in the past two decades to 12.8% in 2006 (Martin *et al*., 2009). Thus, it is a major medical, social, and economic concern. A significant portion of preterm birth happens without known causes. Approximately 20% of preterm deliveries are the result of a physician's decision based on maternal and fetal indications, such as pre-eclampsia (hypertension during pregnancy) and fetal distress. The remainder (and the majority) of preterm births result from spontaneous onset of preterm labor or preterm premature rupture of membranes (PPROM) (Goldenberg *et al*., 2000). The risk factors for spontaneous preterm birth can be broadly categorized into 3 classes: social stress and race, infection and inflammation (both systemic and localized), and genetics (Muglia and Katz, 2010). Among the various risk factors, intra-uterine infection has long been recognized to play an important role in spontaneous preterm birth, especially in those occurring at fewer than 30 to 32 weeks (Goldenberg *et al*., 2000). These infections involve some or all of the following placental microenvironments: between uterus and fetal membranes (deciduitis), within fetal membranes (chorioamnionitis), within the placenta (villitis), within the amniotic fluid (amniotic fluid infection), within the umbilical cord (funisitis), or within the fetus (sepsis). The infection rate is inversely related to gestational age. It is rare in late preterm birth (34 to 36 weeks), but is present in most cases of preterm birth before 30 to 32 weeks. For instance, the rate of positive chorioamnion cultures in women with a spontaneous birth prior to 30 weeks was reported to be 73%, as compared with 40% in those delivered between 31 and 33 weeks (Goldenberg *et al*., 2000).

It was first reported in 1996 that periodontal disease was a potential risk factor for preterm birth (Offenbacher *et al*., 1996). Since then, the link between periodontal infections and preterm birth has been one of the frontiers in dental research. The potential correlation has been expanded from periodontitis and preterm birth to various forms of periodontal infections and adverse pregnancy outcomes, including preterm birth, low birthweight, stillbirth, miscarriage, intrauterine growth retardation, and pre-eclampsia. This

manuscript reviews the current status of epidemiological, mechanistic, and intervention studies on oral health and adverse pregnancy outcomes, with an emphasis on studies published in recent years, since earlier studies have been reviewed elsewhere (Bobetsis *et al*., 2006).

EPIDEMIOLOGICAL STUDIES

Similar to previous years, recent epidemiological studies largely support a strong association between oral health and adverse pregnancy outcomes, while some controversy still remains. In a case-control study consisting of 161 systemically healthy Caucasian women in Hungary, a significant association was found between preterm birth and initial chronic localized periodontitis (Radnai *et al*., 2006). The criteria for diagnosing periodontitis used in this study were: bleeding at ≥ 50% of the examined teeth, and at least one site of probing depth of ≥ 4 mm. Two case-control studies involving Indian and Jordanian pregnant women, respectively, reported consistent findings (Kushtagi *et al*., 2008; Khader *et al*., 2009). A separate study performed in Australia reported that periodontal disease was significantly related to perinatal death (Shub *et al*., 2009). Such a relationship was not supported by a study in the US or another involving Thai women (Durand *et al*., 2009; Lohsoonthorn *et al*., 2009). In the US study, however, although periodontal disease was not found to be associated with preterm birth, low levels of *Lactobacilli* in saliva were associated with preterm birth (Durand *et al*., 2009). An earlier study also reported that salivary *Actinomyces naeslundii* genospecies 2 and *Lactobacillus casei* levels could be used to predict birth outcome (Dasanayake *et al*., 2005). These findings represented a shift from periodontal disease to oral bacteria, which may be used for early diagnosis and prediction of birth outcome.

MECHANISTIC STUDIES

There have been two hypotheses regarding the mechanism underlying the link between oral health and adverse pregnancy outcome. One is that periodontal disease causes systemic abnormal immunological changes, leading to pregnancy complications. For example, moderate to severe periodontal disease has been associated with elevated systemic inflammation early in pregnancy in African American women, as measured by serum C-reactive protein (CRP) (Horton *et al*., 2008). Pregnant women with periodontal disease and elevated CRP levels are at increased risk for pre-eclampsia (Ruma *et al*., 2008).

The second hypothesis suggests that oral bacteria directly colonize the placenta, causing localized inflammatory responses, resulting in prematurity and other adverse outcomes. Translocation of oral bacteria into the placenta has been demonstrated in animal models of both chronic and acute infections (Lin *et al*., 2003; Han *et al*., 2004). In the chronic infection model, *Porphyromonas gingivalis* was continuously inoculated systemically through a subcutaneous chamber. The organisms were detected in liver and placenta by polymerase chain-reactions (PCR), and fetal growth retardation was observed (Lin *et al*., 2003). In the acute infection model, *Fusobacterium nucleatum* was injected into the tail vein to mimic bacteremia, which occurs during periodontal infections and dental procedures. Unlike *P. gingivalis* in the chronic infection model, translocation of *F. nucleatum* in the acute infection model is organ-specific, *i.e*., only in the placenta, but not in other organs such as the liver or spleen (Han *et al*., 2004). This is likely due to the immune suppression in the placenta, which allows the bacteria to proliferate freely, whereas the bacteria are killed by the immune cells in the liver and spleen. *F. nucleatum* is an invasive organism, capable of invading different types of human cells (Han *et al*., 2000). It has been shown that *F. nucleatum* utilizes its FadA adhesin to cross the endothelium to colonize in the placenta (Han *et al*., 2005; Xu *et al*., 2007; Nithianantham *et al*., 2009). A *fadA* deletion mutation was 1000-fold defective in colonizing the placenta when compared with its wild-type parental strain or the *fadA*-complementing strain (Ikegami *et al*., 2009). Once colonized in the placenta, *F. nucleatum* proliferates quickly and eventually spreads to the amniotic fluid (mimicking amniotic fluid infection in human), fetal membranes (mimicking chorioamnionitis), and the fetus (sepsis). *F. nucleatum* also stimulates TLR4-mediated inflammatory responses in the fetal-placental unit, without causing systemic inflammation (Liu *et al*., 2007). This localized inflammation has been shown as the direct cause of fetal death, which occurs after 2 to 3 days of hematogenous infection. In the presence of a TLR4 antagonist, the bacteria colonized the placenta to the same extent without causing inflammation, but the inflammation and necrosis were suppressed, and the pups were born unaffected (Liu *et al*., 2007). Intravenous injection of *P. gingivalis* into pregnant rats also caused strain-dependent colonization in the placenta (Belanger *et al*., 2008). Placental colonization by both *F. nucleatum* and *P. gingivalis* has been associated with intrauterine infections in humans (Han *et al*., 2009, 2010; Katz *et al*., 2009).

In a serparate study, a diverse group of oral bacteria were found to translocate to the mouse placenta following intravenous injection with pooled human saliva collected from healthy volunteers and pooled subgingival plaque samples collected from the deep pockets of periodontitis patients, respectively (Fardini *et al*., 2010). Many of the translocated species have been associated with adverse pregnancy outcome in humans, although their sources of infection were previously unknown (Fardini *et al*., 2010). These results indicate that the oral microbiome may be a previously overlooked source of intrauterine infection.

Thus, the two mechanisms differ in two aspects: the direct invasion of oral bacteria into the intrauterine cavity, and the extent of inflammation (localized or systemic). While they are not mutually exclusive, the localized placental inflammation is consistent with intrauterine infection observed in humans, a major cause of adverse pregnancy outcomes. However, the source of the origin of the infectious organisms in the intrauterine cavity deserves re-evaluation. The current paradigm in obstetrics indicates that intrauterine infection originates predominantly from the vaginal microflora, with the micro-organisms ascending into the otherwise sterile uterus. With the advancement of technology, microbial species have been identified from human intrauterine infections that do not belong to the normal vaginal flora (Han *et al*., 2009). *F. nucleatum*, a species ubiquitous to the oral cavity and associated with various forms of periodontal diseases, including gingivitis, is an example of such species. It is one of the most prevalent species associated with intrauterine infections, yet it is

not recognized as a part of the normal vaginal microflora (Hill, 1998; Han *et al*., 2004, 2009). Our recent report of a term stillbirth caused by oral *F. nucleatum* provided the first human evidence that the bacteria originated from the mother's subgingival plaque and translocated to the placenta and fetus, causing acute inflammation leading to the fetal demise (Han *et al*., 2010). No fusobacteria were detected in the mother's vaginal or rectal microflora. The pattern and duration of infection and the results of histopathological analysis of the patient's placenta corroborated those observed in animals described above.

Another example is uncultivated *Bergeyella*, which was repeatedly detected in intrauterine infections only recently (Han *et al*., 2006, 2009). Similarly, in one case analysis, the clone identified in the amniotic fluid associated with preterm birth was detected only in the mother's subgingival plaque and was absent from her vaginal flora (Han *et al*., 2006). *Bergeyella* became a "new" species associated with intrauterine infection due to the use of 16S rRNA-based culture-independent technology. Unfortunately, the hospital laboratories still use routine culturing methods for bacterial identification. Thus, a significant portion of intrauterine infection may be underestimated (Han *et al*., 2009). This notion is also confirmed in the animal study described above in which both cultivated and uncultivated oral species were found to translocate to the mouse placenta following hematogenous infection (Fardini *et al*., 2010). Interestingly, a significant portion of the translocated species are commensal or opportunistic commensal oral species, such as *Campylobacter, Capnocytophaga, Eikenella, Erysipelothrix, F. nucleatum, Leptotrichia, Neisseria, Parvimonas, Selenomonas, Streptococcus*, and *Veillonella*. The recently identified uncultivated pathogenic group of TM7 was among the translocated species. This accumulating evidence indicates that oral bacteria, both cultivated and uncultivated, play a previously unrecognized role in intrauterine infection associated with adverse pregnancy outcome. Results from these biological mechanism studies strongly support a causal relationship between poor oral health and adverse pregnancy outcome.

INTERVENTION STUDIES

Similar to the epidemiological study, the clinical intervention trials using different periodontal disease criteria and therapies and involving different populations have produced inconsistent results. A meta-analysis published in 2008 examined 7 eligible controlled randomized trials and concluded that scaling and/or root planing during pregnancy significantly lowered preterm birth rates (Polyzos *et al*., 2009). Furthermore, the analysis showed a significantly favorable obstetric effect from treatment of less severe periodontal disease.

Since then, three additional intervention studies have been published, again with contradicting outcomes (Offenbacher *et al*., 2009; Radnai *et al*., 2009; Macones *et al*., 2010). The trial conducted by Radnai *et al*. focused on a specific group of pregnant women, *i.e*., those with threatening preterm birth and initial localized chronic periodontitis. Patients with a prior history of preterm birth or miscarriage were excluded, as were those who smoked, consumed alcohol in great amounts, used drugs, or were malnourished. After screening 429 patients admitted to the hospital because of threatened preterm birth, the investigators selected for trial a treatment group of 41 patients and a control group of 42. The periodontal treatment was carried out in the 3rd trimester, around 32 weeks of gestation, and consisted of scaling and polishing. The treatment group had significantly lower preterm birth rates and higher birth weight, compared with those of the control group (Radnai *et al*., 2009).

The MOTOR (for Maternal Oral Therapy to Reduce Obstetric Risk) study enrolled a large number of patients (n = 1760) from 3 different sites (Alabama, North Carolina, Texas), with 882 patients receiving scaling and root planing treatment during the second trimester and 878 receiving delayed periodontal care after delivery (Offenbacher *et al*., 2009). No difference was reported between the two groups with regard to birth outcome, including preterm birth rate, birthweight, size for gestational age, Apgar score, admission to neonatal intensive care unit, and pre-eclampsia. The periodontal health status in the treatment group improved slightly compared with that of the control group, but disease progression occurred in approximately 30% of the treated mothers. Periodontal health was not restored by the treatment provided, as reflected in the high probing depths and bleeding scores at delivery.

The PIPS (for Periodontal Infection and Prematurity Study) is another multi-center randomized trial enrolling more than 300 patients in each of the treatment and control groups. Similarly, no difference was detected in spontaneous preterm delivery between the two groups. Instead, an increase of indicated spontaneous delivery at < 35 weeks of gestation was suggested in patients receiving active treatment (Macones *et al*., 2010).

From a review of these various trials, several patterns seem to emerge. First, trials involving a single patient enrollment site and a defined sub-population tend to produce results with a positive effect of periodontal treatment (Jeffcoat *et al*., 2003; Lopez *et al*., 2005; Radnai *et al*., 2009), whereas trials involving multiple centers and a large general population fail to demonstrate efficacy (Michalowicz *et al*., 2006; Offenbacher *et al*., 2009; Macones *et al*., 2010). This could be due to several reasons: (1) Periodontal health may affect the birth outcome in a sub-population rather than in the general population, and (2) enrolling patients at multiple sites makes it more challenging to manage and produce consistent results. Inconsistent results from different sites have been reported in multi-center studies. Such inconsistency may be attributable to the differences in patient population, as well as in the practice management of patient care.

One question arising is what kind of sub-population is relevant for intervention. With regard to periodontal disease, it appears that treatment of less severe diseases, such as pregnancy-associated gingivitis or initial localized chronic periodontitis, tends to be effective in improving birth outcome (Lopez *et al*., 2005; Radnai *et al*., 2009). This conclusion was indicated in the meta-analysis and was confirmed by the Radnai trial which was not included in the analysis (Polyzos *et al*., 2009; Radnai *et al*., 2009). This does not necessarily contradict earlier reports that the preterm birth rate is affected by the severity of periodontitis (Jeffcoat *et al*., 2001). Rather, it could be due to the responsiveness to treatment. Less severe periodontitis is easier to reverse and improve with the current therapy of scaling and root planing; thus treatment of such diseases may produce a measurable improvement in birth outcome.

Supporting such notions are the two case studies described above in which oral bacteria were found to cause stillbirth and preterm birth, respectively (Han *et al*., 2006, 2010). In both cases, the mothers showed no signs of clinical periodontal disease at *post partum* examination, while they both reported excessive gingival bleeding during pregnancy, indicating pregnancy-associated gingivitis. Neither woman would have qualified for the intervention studies targeted only at women with periodontitis. They would, however, qualify in studies targeting pregnancy-associated gingivitis, and they might have experienced different birth outcomes with effective periodontal therapy.

Second, it appears that a single treatment of scaling and root planing during the second trimester does not produce consistent results, as opposed to therapies that aimed at resolution, *i.e*., multiple treatments throughout pregnancy until the periodontal health conditions improve (Lopez *et al*., 2005; Michalowicz *et al*., 2006; Offenbacher *et al*., 2009; Radnai *et al*., 2009). This is understandable in light of the results from the mechanistic studies. Early treatment does not preclude the periodontal conditions from worsening later in gestation. It has been documented that the gingival conditions change throughout pregnancy and improve following delivery (Raber-Durlacher *et al*., 1994). Thus, a single treatment during the 2nd trimester may not be sufficient to prevent gingival inflammation later in pregnancy. Transient bacteremia and hematogenous transmission may occur as a result of the treatment or when the periodontal conditions regress after the treatment stops. Thus, it may be necessary to perform multiple treatments to keep the periodontal inflammation at bay. It is also plausible that when a more aggressive treatment plan is implemented, an effect may be noted for the more severe forms of periodontal disease, such as periodontitis.

Besides the study design, patient selection, and periodontal therapies discussed above, future intervention studies should take into consideration the results obtained from the mechanistic studies. For instance, if hematogenous transmission resulting from transient bacteremia is a significant cause of adverse pregnancy outcome, prophylactic antibiotic therapy should be considered prior to periodontal treatment. Previous studies have shown that the use of macrolides (such as azithromycin) and clindamycin helped the prevention of preterm birth in high-risk populations, while metronidazole did not (Morency and Bujold, 2007). Therefore, it may be worthwhile to consider using macrolides and clindamycin prior to performing scaling and root planing on pregnant women, to prevent transient bacteremia from occurring. Such antibiotics may also be considered when pregnant women with periodontal infections experience additional underlying conditions that can weaken their immune defense, as reported in the stillbirth case described earlier (Han *et al*., 2010).

CONCLUDING REMARKS

With regard to birth outcome, two clinical trials sharing similar study designs have each reported reduced incidences of stillbirths in the treatment groups compared to the control groups (Michalowicz *et al*., 2006; Newnham *et al*., 2009). Although the differences were not statistically significant in either study alone due to small sample sizes, a meta-analysis combining these two studies indicated a significant reduction of stillbirths in the treatment groups (Newnham *et al*., 2009). This finding is intriguing and suggests that a closer examination of different types of adverse pregnancy outcome is warranted.

Evidence is mounting that oral bacteria play a previously unrecognized role in intrauterine infection, and that periodontal infection plays a causal role in adverse pregnancy outcome. The question is how we should go about finding the effective therapy and patient management strategy to improve the birth outcome. Continued studies on the mechanisms underlying the link between oral health and adverse pregnancy outcome are needed. Only when we have a thorough understanding of the mechanism can we then design meaningful intervention studies targeting the appropriate populations, utilizing effective therapies, and measuring the relevant outcomes.

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