Associations Between Maternal Periodontitis, Periodontal Treatment and Pregnancy Complications – Part 1: Potential Pathogenic Mechanisms and Risk Factors

Suzanne Farrell, Mark Ide

Much attention has recently been given to possible links between maternal periodontitis and pregnancy outcome. This paper aims to explore the potential explanatory mechanisms for such links, and the evidence from both in vitro and animal studies to support these concepts. Potential mechanisms rely on the role of proinflammatory molecules involved in the normal physiological process of parturition being influenced by distant or local inflammatory processes caused by infections in maternal tissues. However, there are a number of shared risk factors that may complicate analyses of any such relationship.

Key words: periodontitis, pregnancy, complications, periodontal treatment

INTRODUCTION

Historical Context of "Periodontal Medicine"

Potential relationships between periodontal disease and systemic health have been a matter of interest for many years. It is generally accepted that a range of systemic conditions, environmental challenges and diseases may influence periodontal health as well as the response to periodontal treatment. This concept has been steadily developed over the past century and although many aspects remain to be fully understood, periodontists are aware of the many clinical manifestations and effects of changes in the systemic state.

In contrast, the possibility that periodontal disease may influence systemic health has varied in popularity over the past 100 years. This concept was widespread around 100 years ago, when a range of other conditions was ascribed to dental and periodontal diseases. Subsequently, probably as a result of the inability of dental clearances to eliminate most acute cases of arthritis, heart disease and other problems, the theory of focal sepsis was largely refuted and the idea fell from favour. Even so, it was recognised that it is possible for local infections to have adverse effects in distant parts of the body. Prime examples are the damage caused by distant spread of neurotoxins in tetanus, the spread of microorganisms in bacteraemia-associated heart valve damage and the cardiac problems following rheumatic fever (host responses causing distant damage).

However, over the past decade this concept has been revived, and periodontitis has been associated with cardiovascular disease and cerebrovascular events, diabetes and adverse pregnancy outcome. This culminated in the development of the term "periodontal medicine" to describe such possible relationships and the means by which they may be managed. As highlighted in the editorial to this journal volume, this is an inappropriate term and perhaps a better descriptor would be 'periodontitis and systemic disease'. The aim of this paper is to review the potential mechanisms by which periodontitis may influence pregnancy outcome and the role of other risk factors for both periodontitis and pregnancy outcome as they relate to the existence of such a relationship.

Periodontal Health During Pregnancy

There is a plethora of publications describing changes seen in the periodontium during pregnancy, which are reported to affect between 35 and 100% of mothers (Hasson, 1966; Lundgren et al, 1973; Gunay et al, 1991). The changes reported are believed to be plaque-dependant (Raber-Durlacher et al, 1993 and 1994), and may be summarised as:

- Increased gingival inflammation, bleeding and crevicular fluid flow, mediated by vasodilation and increased vessel permeability (Lindhe and Bränemark, 1967a, 1967b; Mohammed et al, 1974; Vittek et al, 1984).
- Development of gingival epulides, classically interproximally (Amar and Chung, 1994).

These variations have been ascribed to variations in the oral flora, the host response and interactions between these (Baines et al, 1977; Bulmer and Hancock, 1977; Clemens et al 1979; O'Neil 1979; Lopatin et al, 1980; Raber-Durlacher et al, 1993). It has been suggested that there are changes in the proportions of certain microorganisms, such as *Prevotella intermedia* within plaque during pregnancy, and that this is associated with increased amounts of selective metabolites present within crevicular fluid during pregnancy (Kornman and Loesche, 1980; Raber-Durlacher et al, 1994).

The chronology of these changes has been investigated by several workers. Early cross-sectional studies (Silness and Löe, 1964) implied that gingivitis steadily worsens through pregnancy and does not diminish until after delivery, but later longitudinal studies imply that there is a peak in inflammation during the second trimester and then a decrease in the third term (Cohen et al, 1969; Hugoson, 1970; O'Neil, 1979; Kornman and Loesche, 1980; Miyazaki et al, 1991; Cerna et al, 1992). A small cross-sectional study of Turkish women (Yalcin et al, 2002) supported these findings and reported that they were reflected in an increase in mean probing pocket depth between the first and third trimesters. More recently, Moss et al (2005) have studied predictors of progression of periodontal disease in 891 subjects from 24 weeks gestation to parturition, recording the number of sites that progressed by 2+ mm in probing, and recorded a range of medical and behavioural factors that may be associated with changes during pregnancy. Just under 2% of sites showed probing depth increases, but 46% of women had at least one site showing some progression, and the vast majority of sites only exhibited increased probing by 2 mm or less. Such changes were associated with previous bleeding on probing and so would appear to be an indication of locally enhanced inflammatory change, especially at proximal sites. Unfortunately the authors do not report summary variables for periodontal status such as mean probing depth or sites bleeding, and so it is hard to be sure what the level of disease at baseline actually was. It is, however, likely that these subjects were from the same pool as that reported by Lieff et al (2004). Regression analysis suggested that subject-based factors associated with increased probing were baseline probing and bleeding scores and ethnicity. There were no data on changes postpartum, and the authors did not relate these changes to possible future pregnancy risk.

Pregnancy Complications: Incidence and Long-Term Outcome

Preterm delivery and low birthweight are an unresolved clinical problem across the world. Statistics vary, but in 1998 the incidence of pre-term delivery (36 weeks gestation or less) was 11.6% of all live births in the USA (Mattison et al, 2001), and low birth weight deliveries accounted for 7.6% of all US deliveries in 2000. Unfavourable pregnancy outcome in terms of foetal health and development may have longer-lasting effects beyond the initial perinatal period and early childhood. A series of epidemiological studies (Barker, 1992; Goldberg and Prentice, 1994) based in the UK have suggested that intrauterine and immediately antenatal environmental, nutritional or infective factors influencing (mostly impairing) development and growth may be associated with a greater prevalence of ischaemic heart disease, bronchitis, cerebrovascular disease, higher plasma concentrations of clotting proteins and an altered pattern of fat deposition. These changes appeared to be dependent upon the pattern and timing of environmental change in relation to development, which could result in changes manifested as variations in relative placental weight, head circum-

ference and weight/length ratio. The adult changes are apparent by the fourth decade, and some as early as 10 years of age (such as alterations in blood pressure). However, some changes such as reduced maternal weight gain or increased placental weight may be a marker of other factors (examples may be maternal infection or anaemia), which may, like periodontal disease, be shared with adverse pregnancy outcome. However, some of these, such as socioeconomic status, were investigated and found to be of no significance.

Pathogenesis/Mechanisms of Pregnancy Complications

Investigations since the 1980s have led to an increase in the understanding of the aetiology and pathogenesis of complications such as low birth weight and preterm delivery. Whilst current concepts may not be able to explain all cases, they do serve to provide a framework around which links with periodontal diseases may be considered.

Established risk factors for such pregnancy complications include extremes of age, ethnicity, local anatomy, genitourinary infection during pregnancy (including bacterial vaginosis), socio-economic status, a previous history of such problems, and environmental factors such as smoking and alcohol intake (Kramer, 1987). There is considerable evidence suggesting that such complications are a response to local infection and inflammation. The infections widely considered to have a role in pregnancy outcome may be local (in the genitourinary region) or distant. The latter may include cytomegalovirus, rubella virus, various toxoplasma species, streptococci and *Escherichia coli* (Ledger, 1986). These are fortunately rare, seen (with the exception of cytomegalovirus) in 0 to 4% of newly born babies and may be asymptomatic, or present as a febrile illness with associated rash (Ledger, 1986).

During reproductive adult life, the microflora of the vagina is normally dominated by facultative lactobacilli, with small numbers of other organisms. However, a significant proportion of women can undergo a shift in the flora to an anaerobically-dominated state described as bacterial vaginosis, which may be asymptomatic in around 50% of cases (Hill, 1998; Amsel et al, 1983). Research investigating and clarifying the important role of infections during pregnancy in the aetiology of pregnancy complications was thoroughly reviewed by Hill (1998) and Gibbs (2001). Many studies have shown that bacterial vaginosis is a significant and important risk factor for preterm labour or premature rupture of membranes, independent of other risk factors. In addition the condition is consistent with amniotic fluid infection and local inflammation of membranes (chorioamnionitis), which in turn has been shown by a number of workers to be associated with preterm delivery and therefore also lower birth weight (Hillier et al, 1988).

Black pigmented anaerobes are common in the cervix and vaginal fornices, and 80% have been reported as being *Porphyromonas gingivalis (Pg)*, and 15% *Prevotella intermedia (Pi)* and *P. nigrescens* (Sleigh and Timbury, 1990). In addition, there is some evidence suggesting the presence of other, non-defined taxa resembling the *Prevotella* group (Devine et al, 1994). Other organisms seen as common commensals in the female urogenital (UG) tract include lactobacilli, *Staphylococcus epidermidis, Escherichia coli* and *Streptococcus faecalis*. Urinary tract infection (UTI) is primarily assumed to be caused by certain serotypes of *E. coli, Staph. saprophyticus, Strep. faecalis* and *Klebsiella* species. These infections may be caused by a single organism or may be mixed (Sleigh and Timbury, 1990).

Symptomless urinary tract infections have been reported to occur in 5% of adult women (Sleigh and Timbury, 1990). Consequently, screening for UTI has been advocated for all pregnant women, although oestrogen-dependent changes in the pH of local secretions may decrease the likelihood of local infection (Varma, 1986). Although relationships between asymptomatic bacteruria, large numbers of neutrophils within a midterm vaginal swab (Ramsey et al, 2005) and prematurity have been established (Norden and Kass, 1968), the ability of antimicrobial treatment to alter the prevalence of prematurity is debated (Elder et al, 1971; Kass, 1978), and it may be that urinary tract infection per se does not play a major role in initiating prematurity (Ledger, 1986). Even so, if mothers with bacterial vaginosis and a previous history of prematurity were treated prophylactically with a course of systemic metronidazole, the incidence of preterm births has been shown to be reduced

(Morales et al, 1994). Although genitourinary tract infections are considered capable of causing low birth weight, preterm delivery (Holst et al, 1994) and spontaneous abortions in humans, there is limited evidence to implicate direct infection of the foetal-placental complex (Gibbs et al, 1992). Certain GU infections and histological chorioamnionitis have however been described as consistent findings associated with prematurity, but it is uncertain whether the 30% of patients with premature delivery and concurrent amniotic infection (Gibbs et al, 1992) actually are infected prior to labour beginning (Lopez-Bernal et al, 1993). A further complication is that not all women who have demonstrably infected amniotic fluid have ruptured membranes when they go into preterm labour, and the organisms found do not correlate well with those found vaginally. Whilst organisms can penetrate membranes, Hill (1998) has reported how *Fusobacteria* (including F. *nucleatum*) are a frequent finding within amniotic fluid, and that these are rarely found within the vaginal flora even during vaginosis. Interestingly, Holst et al (1994) reported that *F. nucleatum* was only recovered from women with bacterial vaginosis who had experienced preterm delivery. This would support a possible role for haematogenous spread of maternal oral organisms in pregnancy complications. Alternatively, the organisms could be inoculated locally (and hence may not be related to maternal periodontitis, as proposed by Dixon et al in 1994) and only survive within the cervix thereafter, where conditions are most favourable.

Pregnancy complications may be related to the production of maternal cytokines either at the site of original infection, or within the placenta (Lopez-Bernal et al, 1993; Collins et al, 1994a and b): these may in turn cause the release of cytokines from foetal tissue, or the foetal tissues may themselves respond to bacterial chemicals (Dudley et al, 1993). These mediators, including tumour necrosis factor alpha (TNF-α) and prostaglandin-E_2 (PGE_2) have been shown to induce rupture of membranes, cervical dilation, uterine contraction and abortion when administered to pregnant animals (Chaouat et al, 1990), and human studies (Hillier et al, 1993; Foulon et al, 1995) have indicated that delivery at or before 34 weeks gestation was associated with elevated amniotic fluid levels of interleukin-6 (IL-6), IL-8, IL-1α and IL-1β, TNF-α and PGE_2 in women who were afebrile. However, levels of TNF-α and PGE_2 both increase within amniotic fluid during pregnancy, and these are considered regulators of normal delivery (Drife and Calder, 1992; Hillier et al, 1993; Collins et al, 1994; Romero et al, 1994). Prostaglandins E_2 or F may be used to induce labour or as a means of inducing abortion either alone or with surgical intervention (Varma, 1986). Romero et al (1998) have shown how maternal and foetal elevations in circulating IL-6 are associated with a positive amniotic fluid culture. In this study only 3 of 16 cases with positive cultures were found to have oral organisms present locally. Such elevations in IL-6 have been shown to be associated with preterm birth and associated complications (Goepfert et al 2004a). Thus it would seem likely that local infection may cause preterm delivery or low birth weight, acting via a mechanism involving induction of cytokine production (Hillier et al, 1993; Foulon et al, 1995; Gomez et al, 1998). This in turn could be superimposed on other factors, such as recognised variation in pregnancy duration by ethnicity (Patel et al, 2003), increasing the risk for certain groups to have further complications.

It is considered that various possible pathways of initiation converge upon one, in which prostaglandin production affects the responsiveness of local uterine and cervical muscle (Grillin, 1994). In addition, prostaglandins, when applied topically, cause a rapid and profound increase in collagenase, elastase and other protease activity in cervical tissue: this is a major element of cervical ripening prior to delivery and thus may be involved in inducing delivery (Granstrom et al, 1992, Rath et al, 1993), as well as having a role during labour itself (Osmers et al, 1994).

There is evidence, based on both in vitro and animal studies supporting a possible role for certain elements of the periodontal microflora in inducing obstetric problems. Lindemann and Economou (1988) have demonstrated that *Pg* and *Actinobacillus actinomycetemcomitans (Aa)* are both capable of causing human monocytes to release IL-1 and TNF-α, whereas others (McFarlane et al, 1990) have shown that such cells taken from patients with periodontitis release these cytokines spontaneously in culture. Interleukin-1 may then promote the release of other cytokines such as IL-6 from gingival fibroblasts, amongst other cells (Yamazaki et al, 1992).

The exact mechanism by which these cytokines induce complications is uncertain. TNF-α may act to stimulate intrauterine tissues to produce prostaglandins and so induce parturition, it may impair uptake of lipids by the foetus or it may act to induce ischaemic necrosis in the foetal-placenta unit (Collins et al, 1994b). Prostaglandins in turn may lead to a direct increase in collagenase activity, or an indirect effect mediated by increased local blood flow and allowing increased concentrations of neutrophils and neutrophil collagenase. Ylikorkala and Viinikka (1992) have suggested that silent infection of the type described above may trigger early onset of labour by increases in prostaglandin production initiated by cytokines released as a response to infection. This was supported by Ohno et al (1994), who demonstrated that amniotic fluid levels of interleukin 2 (IL-2) were elevated in pregnant women with intrauterine infection, and that this could interact with an IL-2 receptor in foetal tissues. These tissues were shown to respond to increased concentrations of IL-1 or IL-2 by greater production of PGE_2 and reduced release of progesterone. Murine studies in which IL-1 was injected subcutaneously into pregnant preterm mice indicated that such an administration caused delivery within 12 hours (Romero et al, 1991b). IL-1α and IL-1β are both elevated in most women with premature rupture of membranes (defined as 21 to 36 weeks) and local infection (Romero et al, 1992; Fidel et al, 1994).

It has been shown that foetal neutrophil precursors in liver and bone marrow both respond readily to stimulation by IL-1α, as indicated by elevated granulocyte colony stimulating factor production (G-CSF, Ohls et al, 1995), and similar elevations in maternal G-CSF have been identified and related to raised neutrophil counts preceding labour (Umesaki et al, 1995). Raised IL-1 associated with infection may promote labour via G-CSF production, elevated neutrophil numbers and activity and elevated prostaglandin production (Russell et al 1994). This may be modulated or enhanced by increases in maternal expression of neutrophil and monocyte integrin expression (Thilaganathan et al, 1995). Furthermore, IL-1 has been shown to stimulate amnion cells to produce PGE_2 within 2 hours of exposure (Mitchell et al, 1993), as well as in gingival fibroblasts, together with the production of other cytokines such as IL-6 and IL-8 (Richards and Rutherford, 1988; Bartold and Haynes, 1991; Uchiyama et al, 1992; Dudley et al, 1993; Fidel et al, 1994), which may be a good predictor for preterm delivery (Foulon et al, 1995). PGE_2 may additionally modulate the degree by which IL-1β further elevates cytokine activity (Czuszak et al, 1996). In addition, Taniguchi et al (1993) and Foulon et al (1995) have demonstrated that bacterial LPS present in chorioamnionitis is capable of promoting the release of IL-8, TNF and IL-6 from foetal mononuclear cells into amniotic fluid (Romero et al, 1991a) and Saito et al (1993) have indicated that amniotic levels of IL-6, IL-8 and G-CSF are all elevated in the presence of intrauterine infection, especially in cases of premature delivery.

Periodontitis: Possible Systemic Effects

The mechanisms described above would seem to be well suited to interactions with maternal periodontitis. This may either be by the effects of local microorganisms, effects of a local inflammatory response, or due to the systemic effects of cytokines released in response to distant infection.

The mechanism by which *Porphyromonas gingivalis (Pg)* causes complications was investigated by Odle et al (1989), who administered endotoxins from *Pg* or *Escherichia coli (Ec)* as an intravenous bolus to pregnant hamsters, in doses ranging from 1 ng to 1 mg per 100 g body weight (equivalent to 7 mg to 7 g for an adult human). The dose dependent pathogenicity of the toxins was different between bacterial species. A dose of 30 to 300 µg per 100 g body weight of *Ec* toxin always resulted in foetal death, and 25% of foetuses were found to be dead if 3 µg per 100 g body weight of *Ec* toxin was administered. In contrast, *Pg* toxin gave a maximum lethality of only 25%, at 0.3 µg of toxin per 100 g body weight, with no fatalities at higher doses but an increased risk of developmental abnormalities. Both preparations were found to reduce foetal weight, except when doses over 100 µg per 100 g body weight of toxin were administered. However, this report did not discuss the effects of such doses on the mothers and whether these were representative of the dosage likely to be experienced by an adult human with periodontal disease. Similar results were reported by Silver et al (1995) using 10 µg lipopolysaccharide (LPS) injected into pregnant mice. This study indicated that much prostaglandin

output may be derived from the foetus itself, with production commencing within 30 minutes of exposure to the LPS.

These theories have been further tested in small animal models (Collins et al, 1994a, 1994 b), and data from these studies would support the proposed hypotheses. Pregnant hamsters, challenged with either living or dead *Pg*, or a combination of the two in two stages via a subcutaneous tissue chamber, showed progressively greater rates and degrees of pregnancy complications as the severity of the bacterial challenge increased. As expected, pregnant animals tended to have higher levels of inflammatory mediators than a comparator group of non-pregnant controls, both pre- and post-inoculation. There was an association between increasing levels of both PGE_2 and TNF-α within the chamber and foetal growth retardation and embryolethality, supporting an inflammatory mechanism. The mean foetal weight was reduced by 24% with respect to controls in those animals challenged by killed and then living *Pg*, and the percentage embryo death increased to 26.5% of animals. There was a dose-dependent effect of the amount of LPS administered on foetal weight. These changes were statistically significant ($p<0.05$). There was no evidence of systemic infection or sepsis within the animals, and maternal weight was unaffected. Similar results were reported by the same group for intravenous challenges with LPS preparations from *Ec* and *Pg*. In addition, the authors indicated that previous exposure to the inoculated microorganism resulted in a greater risk of adverse pregnancy outcome and a greater elevation of cytokine levels locally. They suggested that this was indicative of a cell-mediated response, and this would imply that if this were so, chronic exposure to potentially pathogenic organisms over the long term, as in marginal periodontitis, may cause an amplification of the host response by immunological as well as inflammatory means, such that a smaller infecting dose may cause obstetric problems. Schlafer et al (1994) carried out a similar study on pregnant ewes, and found that intravenous administration of 1µg LPS per kg body weight caused foetal hypoxaemia within hours, followed by preterm labour and intra-uterine foetal death.

Pg has been show to spread to the placenta in pregnant female mice from a subcutaneous chamber, indicative of haematogeous spread, and to result in significant elevations in maternal TNF-α and IL-6, accompanied by foetal growth retardation (Lin et al, 2003a, 2003b).

A similar effect, both in terms of cytokine responses and pregnancy outcome, has also been demonstrated in pregnant mice following exposure to lipoteichoic acid, a cell wall component from Gram positive organisms (Kajikawa et al, 1998). In contrast studies using ligature-induced periodontitis in rats before and during pregnancy (Galvao et al, 2003) have failed to show an association with birth weight.

In humans, Madianos et al (2001) reported levels of certain organisms in maternal plaque and antibodies in maternal serum and foetal cord blood after delivery: it is assumed but not stated that the plaque was collected at the same time as the other samples. There were no significant differences in prevalence of established periodontal pathogens (several of which may be present during bacterial vaginosis) between cases and controls. Whilst no difference was seen in maternal IgG levels to pathogens, there were higher levels of foetal IgM to all the periodontal pathogens tested in the case group. No data on oral hygiene, periodontal disease, vaginosis, chorioamnionitis or smoking levels are presented for these subjects. Hence it is uncertain whether these organisms are associated with ongoing periodontal disease or may be part of a more local bacterial vaginosis flora, which is known to involve such organisms and which is strongly associated with pregnancy complications. When maternal and foetal responses are assessed together, the former has a protective effect, whilst the latter appears to increase the risk of complications. However, the possibility that the maternal and foetal responses are to local events in the birth canal, as opposed to distant changes in the mouth, was not considered, and data were not published to address this. More recently Dörtbudak et al (2005) found that amniotic fluid from 6 preterm cases had higher levels of IL-6 and PGE_2 whereas the levels of IL-8 were higher in the normal birth control group. Again, none of the amniotic fluid samples gave a positive bacterial culture.

Even so, it may be possible for inflammation intra-orally to lead to changes elsewhere in the body, mediated by changes in levels of proinflammatory mediators, such as interleukins. Other changes in acute phase reactants may be an indicator of sys-

temic inflammatory changes and have been associated with periodontitis in several studies (Ebersole et al, 1997; Noack et al, 2001; Montebugnoli et al, 2005). Periodontal instrumentation can result in rapid elevations of circulating TNF-α and IL-6 (Ide et al 2004), possibly related to systemic dissemination of bacterial endotoxins as well as bacteraemia, and Geerts et al (2002) have suggested that even chewing on teeth with periodontitis results in measurable elevations in circulating endotoxin concentrations which may in turn result in raised levels of proinflammatory mediators. One would expect that periodontal treatment would therefore reverse such changes, but data published on the effects of treatment is contradictory, although this may be related to the severity and effectiveness of the treatment provided (Ebersole et al, 1997; Ide et al, 2003; D'Autio et al, 2005; Montebugnoli et al, 2005; Rahman et al, 2005).

Other organisms have been considered: Dasanayake et al (2005) studied salivary levels of microorganisms associated with dental caries in around 300 pregnant women in Birmingham, Alabama. The levels of *Lactobacillus casei* and *Actinomyces naeslundii* were associated with pregnancy outcome. However, they formed a part of a model which explained less than 10% of variation in birth weight and gestation, and no other classical confounders for pregnancy outcome were analysed.

Experimental work led to initial cross sectional human studies. That by Offenbacher et al (1996) led to a great increase in interest regarding periodontal-pregnancy interactions, and will be discussed further in the subsequent paper in this journal. This work was extended by a further case control study (Offenbacher et al, 1998) on 48 women in which levels of PGE_2 and IL-1β in gingival crevicular fluid were compared together with the levels of certain organisms associated with periodontitis. Unfortunately no other data on smoking, ethnicity, age and other risk factors were reported. This latter study suggested both increased levels of the proteolytic organisms of interest and of crevicular fluid mediators in the case women. The authors report that this implies that periodontitis may be causing elevated local inflammation which may have a systemic effect: the other possibility is that crevicular fluid levels of these molecules are merely raised as a reflection of systemic changes, induced by vaginal, cervical or amniotic infection, and that the increased crevicular fluid flow seen during this change also helps to favour the growth of proteolytic organisms.

SHARED RISK FACTORS

Pregnancy outcome and periodontitis susceptibility may be influenced by a number of shared risk factors, including age, ethnicity, socio-economic status, smoking, psychosocial factors and possibly genetic factors (Oliver et al, 1998; Haste et al, 1991; Larroque et al, 1992 and 1993; Scholl and Hediger, 1994; Gardosi et al, 1995; Peacock et al, 1995; David and Collins, 1997; Machuca et al, 1999; Yalcin et al, 2002; Ahern et al, 2003; Bracken et al, 2003; Patel et al, 2003; Dole et al, 2004).

Smoking

Smoking is a widely recognised risk factor for pregnancy complications and for the development and progression of periodontitis (Wang et al, 2002; Bergström, 1989; Haber et al, 1993; Papapanou, 1996). This means that smoking can act as a major confounder when trying to investigate links between periodontitis and other conditions in which smoking plays a role. When this fact is combined with difficulties in recording lifetime exposure to smoking, it becomes apparent that study design and data handling must be carefully managed to try to deal with this problem (Hujoel, 2002; Spiekerman et al, 2003). Even so it is difficult to easily exclude the effects of smoking (which is also linked to other behavioural and clinical variables) merely using statistical modelling, and it would seem that the only reliable way to truly account for this is to design studies involving only "never smoker" subjects.

Are these Conditions Linked by Common Genetic Factors?

The work described above illustrates current concepts of the pathophysiology of adverse pregnancy outcomes. The processes involved are largely related to proinflammatory molecules, many of which are also involved in the pathology of periodontitis. Hence it may be possible that a factor

which could globally raise levels of these molecules within the host would increase the risk of both diseases and give the appearance of a relationship between the two, when in fact they would be different manifestations of the same underlying phenomenon. One such factor would be the concept of a genetically determined hyper-inflammatory trait, as suggested by Beck et al (1998) and Kornman et al (2001) for links between cardiovascular disease and periodontitis. Ongoing work in the USA should answer this question further, but there is currently contradictory literature for such genetic factors and both periodontitis and preterm birth (Ehmke et al, 1999; Roberts et al, 1999; Amory et al, 2004; Moore et al, 2004b).

If periodontitis in pregnancy and adverse pregnancy outcome were two entities of a generalised hyper-inflammatory trait (Beck et al, 2000), an exaggerated response may take place in the chronic presence of bacteria, thereby making the individual more susceptible to periodontal disease and infection-associated preterm birth. This trait may be associated with polymorphic variants of genes that code for inflammatory mediators such as interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α). These cytokines were chosen in a study by Moore et al (2004b) as there is evidence that the IL-1β +3953 variant is associated with periodontitis (Kornman et al, 1997; Michalowicz et al, 2000) and likewise the TNF-α –308 variant was shown to be more prevalent in preterm birth (Roberts et al, 1999), although this was only seen in African-American subjects. Blood samples from 48 case subjects (those with adverse pregnancy outcome) and 82 control subjects (normal pregnancy outcome), defined by a larger case-control study (Moore et al, 2005), were taken and these underwent restriction fragment length polymorphism analysis which facilitated the identification of study subjects as being either homozygous or heterozygous for the two allelic variants or homozygous for the non-variant (common) genes.

There was no difference in periodontal health between the case and control subjects. Carriage of the IL-1β +3953 variant was not associated with either periodontal disease or preterm birth. Likewise the TNF-α –308 variant was not associated with periodontal disease, but there was a higher prevalence of TNF-α –308 variant carriage in the preterm birth (case) subjects (odds ratio 2.22, p = 0.026). This odds ratio was higher when only the non-smoking subjects were analysed (3.90, p = 0.004). The lack of an association between the IL-1β +3953 variant and periodontal disease in this study population may have been due to the low level of periodontal disease seen. This study does support the theory that adverse pregnancy outcome may be associated with polymorphisms in certain cytokine genes, however the cascade of cytokine involvement in inflammation is very complex and therefore the genetic control of a hyper-inflammatory response trait is probably more complex than we have described. In addition, the presence of a polymorphic gene does not infer functional changes and so further work in this field is required.

A further factor considered relevant is nutritional status. A prime example of this is the possible role of zinc deficiency in altering pregnancy outcome (Swanson and King, 1987). In addition, zinc has been reported as a possible factor influencing the host response in periodontitis (Woessner, 1991). Zinc has been identified as being a vital catalytic component in over 200 enzymes and a structural component of many proteins and also has powerful antibacterial properties. Plasma zinc is related to dietary intake but may also be modified by factors such as pregnancy, infection, stress, exercise and aging (Simmer and Thompson, 1985; King, 1990), although intracellular zinc levels may not be so readily affected (Simmer and Thompson, 1985). A double blind placebo controlled study by Simmer et al (1991) indicated that daily maternal zinc supplementation significantly reduced intrauterine growth retardation and enhanced foetal health. Goldenberg et al (1995) have indicated that daily provision of oral zinc supplementation to a population of pregnant women exhibiting low plasma zinc at baseline (19 weeks gestation) resulted in a significantly higher infant birth weight and head circumference with respect to a control group without zinc supplementation. Differences in gestational age and percentage of deliveries below 2.5 kg were seen to be approaching significance (p=0.06), such that controls tended to deliver on average three days earlier and were at at least at 50% greater risk of delivering below 2.5 kg. This work supported the earlier finding (Simmer and Thompson, 1985; Simmer et al, 1985; Haste et al, 1991) that leucocyte zinc levels were reduced in mothers giving birth to low birth weight babies regardless of

smoking, that smoking also reduced intracellular zinc concentrations, and that smoking and low leucocyte zinc levels could collectively be seen in 85% of study subjects delivering small babies. Such zinc depletion was found to be associated with an alteration in the pattern of maternal prostaglandin production, with reduced levels of prostaglandin $F_2\alpha$ ($PGF_2\alpha$) and elevated PGE_2: the authors suggest that this change in PG production may be partly responsible for premature delivery or adverse pregnancy outcome. Jameson (1993) has additionally identified foetal malformation, perinatal death and prematurity, maternal morbidity and bleeding problems to be associated with zinc deficiency during pregnancy.

SUMMARY

Relationship Between Periodontitis and Pregnancy Outcome

There is an increasing amount of published work investigating potential links between pregnancy outcome and maternal periodontitis. A number of plausible mechanisms exist to support such associations, and these are supported by work in vitro and in animal models. However human epidemiological data are more equivocal. These data and the associated publications will be discussed in the following paper.

Acknowledgements

This report was based on work originally funded by the DHSC – London, Research and Development, Responsive Funding Programme. The views expressed in the publication are those of the authors and not necessarily those of the NHS or the Department of Health.

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Reprint requests
Dr Mark Ide
Senior Lecturer / Honorary Consultant,
Periodontology and Preventive Dentistry
Guy's, King's and St. Thomas' Dental Institute,
King's College London,
London Bridge, London SE1 9RT
United Kingdom
Tel +44 (0) 207 188 5391
Fax +44 (0) 207 407 6736
E-mail mark.ide@kcl.ac.uk