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Periodontal disease: a potential modifiable risk factor limiting conception

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BACKGROUND: Periodontal disease (PD) is a common chronic infectious and inflammatory disease of the gums and its supporting tissues, associated with several adverse health outcomes including significant obstetric consequences. PD is treatable with good oral hygiene and dental care, and consequently is a modifiable variable that may lead to improvements in adult health. To date, there are no published studies describing the influence of PD on a woman's time to conceive (TTC).

METHODS: This study formed part of the Smile study, which was a multi-centre randomized controlled trial of treatment for PD in mid-pregnancy. PD was defined as the presence of pockets ≥4-mm deep at ≥12 probing sites in fully erupted teeth. At the time of recruitment, women were asked about their TTC and whether they had required fertility treatment.

RESULTS: Of 3737 pregnant women recruited to the study, information was available from 3416 spontaneous conceptions, including 1014 cases with PD (29.7%). Planned pregnancies accounted for 1956 of the 3416 pregnancies available for study. For 146 women, the TTC was >12 months and PD was more prevalent in this group (34.9% versus 25.7%, $P = 0.015$). The mean TTC in women with PD was 7.1 months [confidence interval (CI): 5.7–8.6] compared with 5.0 months (CI: 4.4–5.5, $P = 0.019$) in those without PD. PD was present in 23.8% of Caucasian women and 41.4% of non-Caucasian women. Compared with Caucasian women without PD, non-Caucasian women with PD had an increased likelihood of TTC >12 months [13.9% versus 6.2%, odds ratio (OR): 2.88 (CI: 1.62–5.12), $P < 0.001$], but there was no difference for Caucasians with PD (8.6% versus 6.2%, OR: 1.15, CI: 0.74–1.79, $P = 0.534$). Other simultaneous predictors of TTC >1 year included age, BMI >25 and smoking.

CONCLUSIONS: In the non-Caucasian population, PD was associated with an increased TTC, but whether this is related to PD, or some other factor also present within this population, should be further investigated.

Key words: periodontal disease / fertility / inflammation / implantation / genetic polymorphism

Introduction

There is increasing evidence of an association between poor oral health and a number of clinically important medical conditions (Williams *et al.*, 2008). The purpose of this study was to assess time to conception in women with periodontal disease. Periodontal disease is a chronic infectious and inflammatory disease of the gums and supporting tissues and has been associated with cardiovascular disease, type 2 diabetes, respiratory disease, kidney disease and adverse pregnancy outcomes (Mattila *et al.*, 1989; Shultis *et al.*, 2007; Williams *et al.*, 2008; Newnham *et al.*, 2009; Shub *et al.*, 2009; Ford *et al.*, 2010). It is believed that up to 10% of the population have severe periodontal disease; however, the prevalence in indigenous populations may be substantially greater (Jamieson *et al.*, 2010). Periodontal disease is a descriptive term used to describe inflammation of the gingiva leading to damage of the supporting connective tissue and bone anchoring the teeth to the jaws. Specific anaerobic bacteria within the periodontal pocket are thought to be responsible for periodontal disease and as infection takes hold, a cascade of tissue-destructive pathways ensues, fuelled by inflammatory mediators (Williams *et al.*, 2008; Kebschull *et al.*, 2010). From a periodontal pocket, inflammatory mediators and pathogens may pass into the circulation and cause systemic consequences. For example, common periodontal pathogens such as *Porphyromonas gingivalis* and

Streptococcus sanguis have been found in arterial plaques from carotid endarterectomy samples (Williams *et al*., 2008).

Many of the published intervention studies to date have suggested that successful treatment of periodontal disease may alter or modify inflammatory markers, leading to a reduction in systemic levels of C-reactive protein and interleukins (IL; D'Aiuto *et al*., 2004a) and improvements in endothelial and vascular function after therapy (Tonetti *et al*., 2007). Despite the diverse consequences of periodontal infection, the oral condition itself is eminently treatable with good oral hygiene and good dental care and between one to four visits to the periodontist (Newnham *et al*., 2009; Teughels *et al*., 2009). Consequently, periodontal disease is a modifiable variable that may lead to improvements in adult health.

Initial intervention studies for periodontal disease in pregnancy suggested that treatment significantly reduced the incidence of preterm birth, but did not affect the rates of miscarriage and stillbirth (Polyzos *et al*., 2009). However, the most recent meta-analyses of 11 trials with 6558 women found that treatment had no significant effect on the overall rates of preterm birth, low birthweight infants, spontaneous abortions/stillbirths or overall adverse pregnancy outcomes (preterm births <37 weeks and spontaneous abortions/stillbirths; Polyzos *et al*., 2010). This suggests that either it is not periodontal disease that is causing the adverse pregnancy outcome, i.e. it is some other systemic associated factor that is present in women with periodontal disease, or that the intervention to treat periodontal disease was initiated too late in pregnancy, and should be initiated prior to conception to prevent the adverse pregnancy consequences.

Little is known about the effect of treatment of periodontal disease on reproductive function, although treatment of periodontal disease has been shown to lead to improvement in semen parameters in male patients treated for periodontal disease (Bieniek and Riedel, 1993). To date, there are no published studies describing the effect of periodontal disease on a woman's chance of conceiving. The purpose of this observational study was to determine the time to conception for women who were recruited to the Smile study, a pregnancy intervention study for women with periodontal disease (Newnham *et al*., 2009). Our hypothesis was that periodontal disease would increase the time it took for a woman to conceive.

Materials and Methods

The Smile study was a randomized controlled trial of treatment for periodontal disease in mid-pregnancy conducted at seven sites in one city: King Edward Memorial Hospital, the Oral Health Centre of Western Australia, Osborne Park Hospital, Swan Districts Hospital, Armadale Hospital, Rockingham Hospital and Joondalup Hospital. The latter five hospitals are secondary-level centres across metropolitan Perth, while King Edward Memorial Hospital is the tertiary-level perinatal centre for the state of Western Australia. The ethics committees responsible for each of the study sites approved the experimental protocol. Women were eligible for recruitment if they were >16 years of age; did not have maternal cardiac disease that would warrant the need for antibiotics for periodontal examination or treatment; had not already received periodontal treatment during the current pregnancy; had at least 20 natural teeth; had a singleton pregnancy of >12 and <20 weeks of gestational age; did not have any known fetal anomalies or other risk factors such as polyhydramnios that would place the pregnancy at imminent risk of complications and were able to attend regularly for periodontal treatment if required.

Informed written consent to conduct the periodontal screening study was obtained by a research midwife or dental hygienist. These screening studies were performed by one of five research hygienists under the supervision of two specialist periodontists. The examinations were a modified Community Periodontal Index of Treatment Needs study conducted in a well-equipped dental environment (Cutress *et al*., 1987). Each study took ~20 min to conduct. Periodontal disease was defined as the presence of periodontal pockets ≥4 mm in depth at 12 or more probing sites in fully erupted teeth (typically excluding wisdom teeth). Periodontal pocketing was used to define the presence of periodontal disease rather than loss of clinical attachment because it better represents the microbial challenge and is the most frequently employed criterion of current disease.

Pregnant women were invited to participate in the treatment study if findings from a screening examination met the criteria for periodontal disease. At the time of recruitment to the study, women were asked to complete a questionnaire to obtain data on demographic, dental and medical aspects of the woman's health. In line with our hypothesis that periodontal disease may be a factor limiting conception women were asked whether the pregnancy was planned; the time taken to conceive (TTC); whether the woman had required any fertility treatment to conceive; whether surgery was required to conceive and whether ovulation induction, intrauterine insemination, *in vitro* fertilization or oocyte or sperm donation were used to assist conception.

Statistical analysis

Means and standard deviations or medians and interquartile ranges (IQR) and ranges (R) were used to summarize continuous data. Categorical characteristics were summarized using frequency distributions. Univariate comparisons between groups were conducted using *t*-tests and Mann–Whitney tests where appropriate for continuous data and χ^2 tests were used for categorical data. Evaluation of the effects of periodontal disease on other maternal characteristics on TTC >1 year was performed using univariate and multivariable logistic regression analyses, and odds ratios (OR) and their 95% confidence intervals (CI) were reported. All risk factors significantly associated with TTC >1 in univariate logistic regression analysis were considered in the multivariable logistic regression as candidate predators, and the final multivariable regression model was based on the simultaneously significant risk factors. Probabilities of conception over time were estimated using Kaplan–Meier survival probabilities. Cox proportional hazards regression modelling was also used to evaluate the effects of risk factors on time until conception. The results of this analysis were analogous to the logistic regression analysis of factors associated with TTC >1 year and are not shown. All hypothesis tests were two-sided and *P*-values < 0.05 were considered statistically significant. SAS (SAS Institute Inc., Cary NC) and SPSS (SPSS Inc., Chicago, IL, USA) statistical software were used for data analysis.

Results

Out of 3737 pregnant women recruited into the study, pregnancy planning and pregnancy outcomes were available in 3576 women. After further exclusion of 160 women who conceived after fertility treatment, the outcomes in 3416 women (91.4%) were analysed. Of 3416 pregnant patients assessed for evidence of periodontal disease, 1014 (29.6%) were confirmed to have evidence of periodontal disease. A flow chart is presented in Fig. 1. Of the 3416 women available for study, 1956 reported that their pregnancies were planned and reported their TTC (57.3%). Only women who

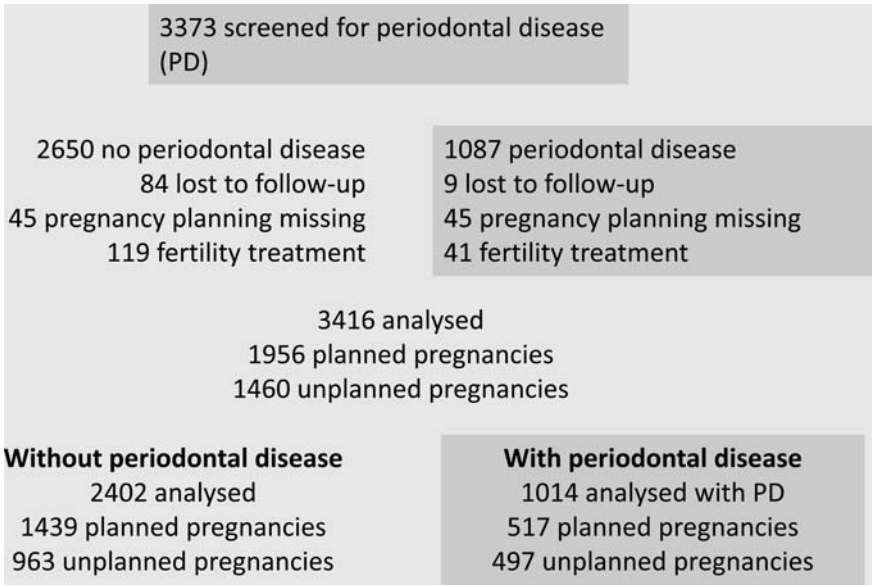

Figure 1 Flow chart of patients at time of recruitment to the Smile study.

had planned pregnancies were analysed further. A woman who has an unplanned pregnancy is more likely to be younger, be a smoker, have a BMI >25, have received <10 years education and be from a lower socio-economic group than if she had planned the pregnancy. There were no statistical differences in the obstetric and antenatal complications recorded between the women who had a planned pregnancy and those who did not plan their pregnancy with regard to threatened miscarriage, threatened pre-term labour, prevalence of pre-eclampsia, ante-partum haemorrhage, gestational diabetes, fetal distress in labour, gestational age at delivery, pre-term birth, Apgar scores at birth, requirement for resuscitation at delivery and admission to the special care nursery (data not shown). However, a pregnancy that was unplanned was significantly more likely to result in a small-for-gestational age baby (prevalence of 8.9% compared with 6.1% if planned; $P = 0.002$), to be lighter at birth (mean: 3.425 kg compared with 3.480 kg; $P = 0.010$), to result in a spontaneous onset of labour ($P = 0.020$) and to have a vaginal delivery ($P = 0.040$).

Demographic data of women within the cohort analysed (those with planned pregnancies) are presented in Table I. Of particular relevance is the fact that a woman who has periodontal disease is more likely to be older, be a smoker, have a BMI >25, have received <10 years education and be non-Caucasian, compared with women without periodontal disease.

For 146 of the 1956 women with planned pregnancies, the TTC was >12 months (Table II). These women were significantly more likely to be older, be non-Caucasian, have a BMI exceeding 25 and to smoke compared with those with TTC <12 months. The prevalence of periodontal disease was also significantly greater in the TTC >12 months group (34.9% versus 25.7%, $P = 0.015$). The mean TTC in women with periodontal disease was 7.1 months [95% confidence intervals (CI): 5.7–8.6 months] and was 5.0 months in women without disease (95% CI: 4.4–5.5 months, $P = 0.019$).

Periodontal disease was present in 23.8% of Caucasian women and in 41.4% of non-Caucasian women, and there were significant differences between the risk of TTC >12 months for combinations of ethnicity and presence of periodontal disease (Table III). Of the 295 non-Caucasian women, 198 (67.1%) were Asian, 44 were African (14.9%), 25 were Aboriginal (8.5%) and 28 were of other ethnicities (9.5%). The multivariable logistic regression analysis including the other simultaneously statistically significant risk factors (maternal age, BMI and smoking) found that compared with Caucasian women without periodontal disease, non-Caucasian women with periodontal disease were at an increased likelihood of TTC >12 months (13.9% versus 6.2%; adjusted OR = 2.88 95% CI: 1.62–5.12, $P < 0.001$), while no increased risk was seen in either Caucasian women with periodontal disease (8.6% versus 6.2%; adjusted OR: 1.15; CI: 0.74–1.79, $P = 0.534$) or non-Caucasian women without periodontal disease (9.2% versus 6.2%; adjusted OR: 1.69; CI: 0.92–3.11, $P = 0.091$; Table III). Alternative parametrization of periodontal disease and ethnicity for this multivariable model showed that relative to non-Caucasian women with periodontal disease, Caucasian women with periodontal disease were at significantly decreased risk of TTC >12 months (8.6% versus 13.9%; adjusted OR: 0.40 CI: 0.21–0.77, $P = 0.006$), while the risk was not significantly different in non-Caucasian women without periodontal disease (9.2% versus 13.9%; adjusted OR: 0.59 CI: 0.27–1.26, $P = 0.171$).

In addition to PD and ethnicity, other significant predictors of TTC >12 months were older age, BMI >25 and smoking (Table III). Smoking in pregnancy was more common in Caucasian women ($P < 0.001$) with 21.8% of Caucasian women, with periodontal disease smoking (86 out of 395 women) and 10.2% of women without periodontal disease smoking (129 out of 1262 women) during pregnancy. Smoking among the non-Caucasian women was reported in 4.9% (6 out of 122) and 6.4% (11 out of 173) of women with and without periodontal disease, respectively.

Table I Frequencies of factors and time-to-event variables in planned pregnancies (n = 1956).

Factors	Periodontal disease		*P*-value
	No (n = 1439)	Yes (n = 517)	
Maternal age[#a] (years)	30.4 (26.7, 33.9), [19.2, 43.5]	31.0 (28.1, 34.3), [20.1, 44.2]	0.005
Maternal age			
<20 years	40 (2.8%)	4 (0.8%)	0.013
20–35 years	1130 (78.5%)	401 (77.6%)	
>35 years	268 (18.6%)	112 (23.6%)	
Caucasian	1266 (88.0%)	35 (76.4%)	<0.001
Nulliparous	661 (45.9%)	213 (44.2%)	0.063
Education			
≤10 years	91 (6.3%)	63 (12.5%)	<0.001
11–12 years	209 (14.5%)	67 (13.0%)	
>12 years	612 (42.5%)	225 (43.5%)	
University	521 (36.2%)	155 (30.0%)	
BMI[a] (kg/m^2)	23.0 (20.8, 26.0), [17.0, 56.0]	23.7 (21.1, 27.1), [16.7, 52.5]	0.004
BMI category (kg/m^2)			
<18.5	70 (4.9%)	34 (6.6%)	0.003
18.5–25	898 (62.4%)	262 (50.7%)	
25–30	280 (19.5%)	130 (25.1%)	
≥30	152 (10.6%)	77 (14.9%)	
BMI ≥25 kg/m^2	432 (30.0%)	207 (40.0%)	<0.001
SEIFA			
1–3	860 (59.8%)	320 (61.9%)	0.193
4–5	530 (36.8%)	171 (33.1%)	
Smoking in current pregnancy	140 (9.7%)	92 (17.8%)	<0.001
Illicit drugs in 2 last years	257 (17.9%)	99 (19.1%)	0.028
Tonsils			
No problem	1139 (79.2%)	402 (77.8%)	0.003
Recurrent tonsillitis	68 (4.7%)	33 (6.4%)	0.341
Removed	228 (15.8%)	80 (15.5%)	
Sinusitis			
No problem	1188 (82.6%)	423 (81.8%)	0.688
Mild	153 (10.6%)	53 (10.3%)	
Moderate/severe	98 (6.8%)	41 (7.9%)	

Women undergoing fertility treatment are excluded (n = 31). Percentages may not add to 100 due to rounding or missing data.
SEIFA (socio-economic index for areas) based on Australian SEIFA index of relative disadvantage and advantage quintiles, from the lowest to the highest quintile, 1–5.
[a]Data shown as median, interquartile range (IQR), range [R].

Discussion

This observational study of the prevalence of periodontal disease in pregnant women in the second trimester suggests that periodontal disease may be a factor affecting a woman's TTC, as a woman with periodontal disease, on average, takes an extra 2 months to conceive, which is a negative influence on conception of the same order of magnitude as obesity. The negative influence upon conception was only significant in the non-Caucasian population, in line with the known differing prevalence, influence and severity of periodontal disease among different ethnic groups (Horton *et al.*, 2008; Kinane and Bouchard, 2008). Furthermore, this study confirms the previous negative influences upon time to conception of an age >35 years, being overweight or obese, and being a smoker. We believe that this is the first report suggesting that periodontal disease presents a further modifiable risk factor that may affect a non-Caucasian woman's chance of conceiving. Consequently, as periodontal disease can be treated by up to four dental visits, in the future, pending supportive prospective intervention studies, all patients could be advised to have a dental check-up prior to attempting to conceive, as well as receiving information about smoking discontinuation, weight loss and folate supplementation.

If the association between periodontal disease and time to conception is not explained by associated factors such as ethnicity, age, BMI and

Table II Maternal characteristics of women who planned their pregnancies stratified by duration until pregnancy (<12 months versus ≥12 months).

	All (n = 1956)	<12 months (n = 1810)	≥12 months (n = 146)	P-value
Periodontal disease	517 (26.4%)	466 (25.7%)	51 (34.9%)	0.015
Caucasian	1661 (84.9%)	1548 (85.5%)	113 (77.4%)	0.008
Maternal age[a] (years)	30.5, [17.9, 44.2]	30.5, (27.1, 33.9)	31.6, (27.0, 36.0)	0.007
Maternal age				
<20 years	44 (2.2%)	41 (2.3%)	3 (2.1%)	0.006
20–35 years	1531 (78.3%)	1431 (79.1%)	100 (68.5%)	
>35 years	380 (19.4%)	337 (18.6%)	43 (29.5%)	
Nulliparous	874 (44.7%)	815 (45.0%)	874 (44.7%)	0.280
Education				
≤10 years	154 (7.9%)	141 (7.8%)	13 (8.9%)	0.690
11–12 years	276 (14.1%)	251 (13.9%)	25 (17.1%)	
>12 years	837 (42.8%)	778 (43.0%)	59 (40.4%)	
University	676 (34.6%)	627 (34.6%)	49 (33.6%)	
BMI[a] (kg/m^2)	23.1, [13.0, 56.0]	23.1, (20.9, 26.3)	24.4, (21.5, 27.6)	0.012
BMI category (kg/m^2)				
<18.5	104 (5.3%)	98 (5.4%)	6 (4.1%)	0.004
18.5–25	1160 (59.3%)	1093 (60.4%)	67 (45.9%)	
25–30	410 (21.0%)	370 (20.4%)	40 (27.4%)	
≥30	229 (11.7%)	203 (11.2%)	26 (17.8%)	
BMI ≥5 kg/m^2	639 (32.7%)	573 (31.7%)	66 (45.2%)	<0.001
SEIFA				
1–3	1180 (60.3%)	1084 (59.9%)	96 (65.8%)	0.172
4–5	701 (35.8%)	656 (36.2%)	45 (30.8%)	
Smoking in current pregnancy	232 (11.9%)	201 (11.1%)	232 (21.2%)	<0.001
Illicit drugs in 2 last years	372 (19.0%)	347 (19.2%)	25 (17.1%)	0.555
Tonsils				
No problem	1541 (78.8%)	1432 (79.1%)	109 (74.7%)	0.571
Recurrent tonsillitis	334 (16.2%)	93 (5.1%)	8 (5.5%)	
Removed	308 (15.7%)	281 (15.5%)	27 (18.5%)	
Sinusitis				
No problem	1611 (82.4%)	1498 (82.8%)	113 (77.4%)	0.260
Mild	206 (10.5%)	126 (7.0%)	20 (13.7%)	
Moderate/severe	139 (7.1%)	99 (6.8%)	13 (8.9%)	

SEIFA (socio-economic index for areas) based on Australian SEIFA index of relative disadvantage and advantage quintiles, from the lowest to the highest quintile, 1–5.
[a]Data shown as median, interquartile range (IQR) or range [R].

smoking, there are two potential mechanisms whereby periodontal disease may directly influence TTC. One potential mechanism responsible for the negative influence upon conception of periodontal disease may be the low-grade systemic inflammation associated with periodontal disease that may have a local effect within the endometrium (Hypothesis 1). The other potential mechanism purports that periodontal disease is a marker of systemic inflammation (Hypothesis 2; Fig. 2).

Hypothesis 1

Many inflammatory conditions have a negative impact on conception such as the presence of hydrosalpinges (Johnson *et al.*, 2010), endometriosis (Barnhart *et al.*, 2002) and polycystic ovarian syndrome (Hart, 2007). In these medical conditions it is believed that the negative influence on fertility relates in part to an endometrial effect (Barnhart *et al.*, 2002; Hart and Norman, 2006; Weiss *et al.*, 2009; Johnson *et al.*, 2010). Patients treated by non-surgical periodontal therapy have been shown to display a significant increase in plasma TNF-alpha, CRP and IL-6 levels immediately after intervention, suggestive of a substantial bacterial inoculation in conjunction with the mechanical instrumentation of the periodontal tissues (Kebschull *et al.*, 2010). With this in mind, it may be wise for a couple undergoing fertility treatment to wait for a few weeks after the periodontal treatment before embarking on their fertility intervention (Gibbs, 2001; Kebschull *et al.*, 2010).

Table III Effects of risk factors on TTC >1 year.

	%[a]	OR	Univariable (95% CI)	*P*-value	OR	Multivariable (95% CI)	*P*-value
Maternal age (years)							
20–35	6.5	1.00			1.00		
<20	6.8	1.05	(0.32–3.44)	0.940	1.01	(0.30–3.37)	0.992
>35	11.3	1.83	(1.25–2.66)	0.002	2.02	(1.34–3.01)	0.001
Caucasian, no PD	6.2	1.00			1.00		
Non-Caucasian, no PD	9.2	1.42	(0.78–2.57)	0.251	1.69	(0.92–3.11)	0.091
Caucasian, PD	8.6	1.45	(0.94–2.22)	0.090	1.15	(0.74–1.79)	0.534
Non-Caucasian, PD	13.9	2.56	(1.46–4.51)	0.001	2.88	(1.62–5.12)	<0.001
Multiparous	8.0	1.00					
Nulliparous	6.8	0.83	(0.59–1.17)	0.281			
Education							
University	7.2	1.00					
≤10 years	8.4	1.18	(0.62–2.23)	0.258			
11–12 years	9.1	1.27	(0.77–2.10)	0.345			
>12 years	7.0	0.97	(0.6–1.43)	0.881			
BMI (kg/m^2)							
18.5–25	5.8	1.00			1.00		
<18.5	5.8	1.00	(0.42–2.36)	0.998	0.87	(0.37–2.09)	0.762
25–30	9.8	1.76	(1.17–2.66)	0.007	1.71	(1.13–2.61)	0.011
≥30	11.4	2.09	(1.30–3.37)	0.002	2.11	(1.29–3.43)	<0.001
No smoking in pregnancy	6.7	1.00					
Smoking in pregnancy	13.4	2.15	(1.41–3.29)	<0.001	2.45	(1.56–3.86)	<0.001
SEIFA							
1–3	8.1	1.00					
4–5	6.4	0.78	(0.54–1.12)	0.173			
No illicit drugs in last 2 years	7.6	1.00					
Illicit drugs in 2 last years	6.7	0.87	(0.56–1.37)	0.555			
Tonsils							
No problem	7.1	1.00					
Recurrent tonsillitis	7.9	1.26	(0.81–1.96)	0.300			
Removed	8.8	1.13	(0.54–2.39)	0.749			
Sinusitis							
No problem	7.0	1.00					
Mild	9.7	1.43	(0.87–2.35)	0.164			
Moderate/severe	9.4	1.37	(0.75–2.50)	0.308			

OR and their 95% CIs are shown. All simultaneously significant predictors of TTC >1 are presented in the multivariable analysis.
Education = duration or level of education attended.
PD, periodontal disease.
[a]Percentage of cases with TTC >12 months in categories of each maternal characteristic.

In this study, the association between periodontal disease and increased TTC was limited to non-Caucasian women with no association seen in Caucasian women. One possible explanation for this observation is that the non-Caucasian women with periodontal disease in this study are immunologically different from Caucasian women with periodontal disease and are therefore more susceptible to periodontal disease and its systemic consequences (Fig. 2).

Over the last 15 years, there have been >100 publications describing associations between specific single nucleotide polymorphisms (SNPs) and periodontal disease. These studies have been performed in multiple populations, including American, Chilean, Brazilian, British, Dutch, German, Greek, Czech, Japanese, Thai and Chinese (Hart and Kornman, 1997; Kornman *et al.*, 1997; Loos *et al.*, 2009; Nibali *et al.*, 2009; Trombone *et al.*, 2009; Anovazzi *et al.*, 2010; Cao *et al.*, 2010; Chai *et al.*, 2010a,b; Laine *et al.*, 2010; Schaefer *et al.*, 2010a,b; Shete *et al.*, 2010; Folwaczny *et al.*, 2011; Scarel-Caminaga *et al.*, 2011; Schafer *et al.*, 2011). These studies have primarily focused on SNPs in the IL-1 cluster (Kornman *et al.*,

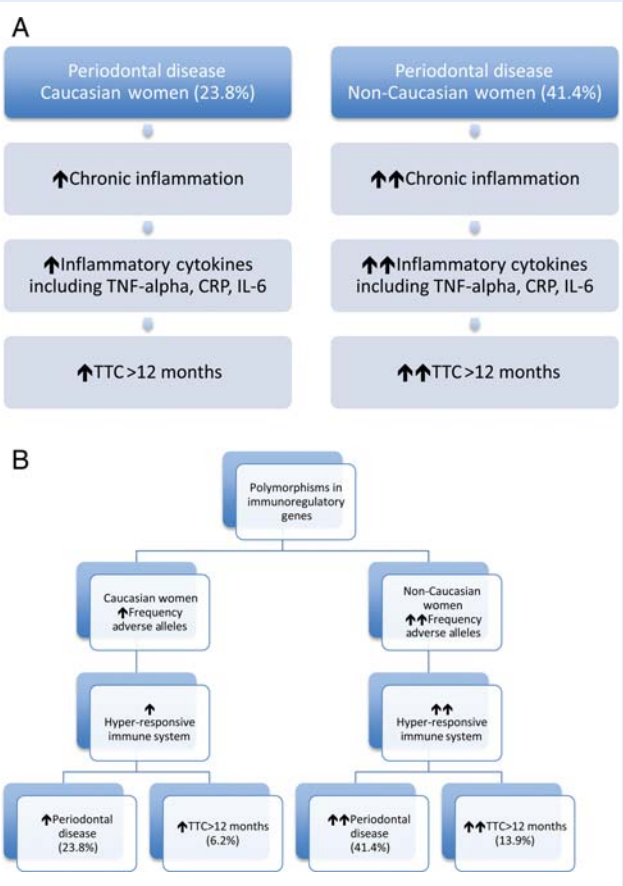

Figure 2 Potential mechanisms responsible for the increased rates of periodontal disease and increased TTC in non-Caucasian compared with Caucasian women. (**A**) One possibility is through a causal pathway mediated by an increased rate of inflammatory cytokines in women with periodontal disease. (**B**) Alternatively or synergistically, an increased frequency of polymorphisms in inflammatory cytokines could result in an increased proportion of women with hyper-responsive immune responses and subsequently an increased frequency of inflammatory conditions such as periodontal disease and increased risk of TTC >12 months.

1997; Laine *et al.*, 2001; Meisel *et al.*, 2002a; D'Aiuto *et al.*, 2004b; Moore *et al.*, 2004; Quappe *et al.*, 2004); however, specific SNPs in TNF-alpha (Craandijk *et al.*, 2002; Fassmann *et al.*, 2003; Soga *et al.*, 2003; D'Aiuto *et al.*, 2004b; Folwaczny *et al.*, 2004d; Moore *et al.*, 2004), TNF receptor type 2 (TNFR2; Shimada *et al.*, 2004), Toll receptor 2 and 4 (D'Aiuto *et al.*, 2004b; Folwaczny *et al.*, 2004b), IL-2 (Scarel-Caminaga *et al.*, 2002), IL-4 (Scarel-Caminaga *et al.*, 2003; Pontes *et al.*, 2004), IL-6 (D'Aiuto *et al.*, 2004b), IL-10 (McDevitt *et al.*, 2000), metalloproteinases (MMP)-1 (Itagaki *et al.*, 2004), MMP-3 (Itagaki *et al.*, 2004), chemokine receptor 5 (Folwaczny *et al.*, 2003), lymphotoxin alpha (Fassmann *et al.*, 2003), NOD2/CARD15 (Folwaczny *et al.*, 2004c), beta-defensin-1 (Boniotto *et al.*, 2004), CD14 (Folwaczny *et al.*, 2004a), IgG Fc receptors IIIa and IIIb (Sugita *et al.*, 2001), myeloperoxidase (MPO; Meisel *et al.*, 2002a), fibrinogen gene (Sechi *et al.*, 2000) and plasminogen-activator inhibitor-1 (Izakovicova Holla *et al.*, 2002) have also been evaluated. Most studies have reported that the presence of specific SNPs are associated with increased risk of periodontal disease (OR range from 2 to 4); and some studies have reported odds ratios for PD as high as 6.4 [e.g. H2H2-455G/A fibrinogen gene (Sechi *et al.*, 2000)] after controlling for smoking status. Several studies have also shown that different SNPs in the same gene [e.g. IL-1B, lymphotoxin alpha (Fassmann *et al.*, 2003), MPO (Meisel *et al.*, 2002a) and IgG Fc IIIb (Sugita *et al.*, 2001)] can be associated with either an increased or decreased risk of PD. Further, specific SNPs have also been shown to be associated with a failure to respond to individualized periodontal therapy (Persson *et al.*, 2003) and there is considerable evidence of gene–environment interactions with smoking (Laine *et al.*, 2001; Izakovicova Holla *et al.*, 2002; Meisel *et al.*, 2002b; Meisel *et al.*, 2004).

Many of the SNPs associated with periodontal disease are within immunoregulatory genes. Further, there are well-established ethnic variations in SNP frequency in many of the genes associated with periodontal disease (Nguyen *et al.*, 2004). Many of the cytokines involved with the pathogenesis of periodontal disease (including IL-1, IL-6, TNF-alpha and MMPs) are also involved in the regulation of pregnancy implantation. We speculate that the observed ethnic disparity in allelic frequencies may potentially contribute to differences in TTC in different ethnic populations with periodontal disease (Fig. 2). This hypothesis requires more detailed evaluation in future prospective studies.

Hypothesis 2

Multiple studies have indicated a strong genetic basis for inter-individual differences in infectious disease manifestations (Segal and Hill, 2003). Upon recognition of the presence of invading micro-organisms, the innate immune system mounts a response aimed at controlling infection. Activation of macrophages, dendritic cells and neutrophils is key to this process (Iwasaki, 2003; Zhao *et al.*, 2003), while their deactivation is central to preserve homeostasis (Bellingan, 1999).

A balanced and appropriate response is beneficial to clear infectious agents; however, individuals differ in their ability to mount an inflammatory response (Romero *et al.*, 2004). Some individuals are hyper-responders and have an excessive local or systemic inflammatory response, which can lead to tissue damage. Others are hypo-responders, and their ability to generate an adequate inflammatory response predisposes them to overwhelming infection. The effect of a dysregulated inflammatory response can be generalized (e.g. systemic inflammatory response syndrome, which often progresses to multi-organ failure and septic shock) or local, such as in periodontal disease.

A number of studies have provided evidence that individuals with a hyper-responsive immune response are more likely to have periodontal disease (Kornman *et al.*, 1997; Galbraith *et al.*, 1998; Kornman and di Giovine, 1998). Similarly, women with hyper-responsive immune responses may have an impaired ability to conceive as immunological mechanisms have been thought to play a role in reproductive problems such as recurrent miscarriage, infertility and implantation failure. A successful pregnancy involves a maternal adaptation of the immune response to the semi-allogenic developing embryo (Tang *et al.*, 2011). A number of recent reports have suggested that modulating the immune system in women with hyper-responsive immune responses may improve IVF success rates

(Winger *et al*., 2009; Winger *et al*., 2011). This may in part explain some of the racial disparity of the success of fertility treatment reported, where non-Caucasian women appear to have a reduced chance of conception, although it is acknowledged that there are differences in the prevalence of various fertility-related variables such as fibroids, obesity, PCOS and tubal disease, across different ethnic groups (Feinberg *et al*., 2006; Purcell *et al*., 2007; Seifer *et al*., 2008; Baker *et al*., 2010; Fujimoto *et al*., 2010).

Implications

In several countries there are incentives for couples to have a child, indeed in Australia there is a financial bonus paid to a couple for each child born (Office, 2009), although in many countries there is limited health coverage provided for dental care. As periodontal disease appears to be associated with a longer TTC, couples attempting to conceive should be advised to have a dental health check prior to attempting to conceive. The fact that semen parameters have been reported to improve in men treated for periodontal disease suggests that the male partner should also attend (Bieniek and Riedel, 1993), particularly as many medical conditions presenting later in life (Williams *et al*., 2008) are also associated with periodontal disease.

A potential bias in our study is that early miscarriages were excluded for our study, as only patients who were at least 12 weeks pregnant were recruited. It is unknown whether periodontal disease has an influence on the miscarriage rate, although it is believed that treatment of periodontal disease has a limited effect upon the chance of a miscarriage (Polyzos *et al*., 2009). However, it is recognized that treatment to prevent periodontal disease would have to commence prior to conception to have a significant influence on the early miscarriage rate.

A further limitation of our study is that some women may have been smoking prior to conception but discontinued when they conceived. This effect would tend to reduce the influence of smoking on TTC in our results, and may exaggerate the influence of periodontal disease as it is more prevalent in smokers. In addition we did not have information on TTC for >40% of our patients, whose pregnancies were unplanned, potentially introducing a bias to the study. In addition as no preconception assessments of the couples were performed, we cannot rule out that there may be a medical factor, such as Chlamydia exposure, tubal disease, the presence of fibroids, anovulation or even semen impairment, which may be more prevalent within the non-Caucasian PD group. Furthermore, we cannot rule out that there may be social or behavioural differences between women with and without periodontal disease, and as such they may have reduced coital frequency, potentially reducing the chance of conceiving due to a social or behavioural factor rather than to an inflammatory cause as proposed.

In conclusion our study suggests that periodontal disease is potentially a further modifiable risk factor influencing the time to conception in non-Caucasian women. As this condition is easily treated, couples trying to conceive may consider attending for a dental health check prior to attempting to conceive. It is important to note that this study demonstrates an association between PD and an increased TTC rather than it being a cause of infertility; a prospective study is required to determine whether dental intervention does indeed reduce the TTC and to further evaluate the racial differences identified in this study.

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Authors' roles

R.H. developed the hypothesis that periodontal disease may limit conception, devised the questionnaire and was primarily responsible for writing the paper. D.D. was responsible for analysing the data. Craig Pennell assisted with data collection and writing the paper. I.N. assisted with data collection, periodontal assessment and writing the paper. J.N. conceived the Smile study and assisted with writing the paper.

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Conflict of interest

None declared.

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