J Clin Immunol (2008) 28:237–243
DOI 10.1007/s10875-007-9162-3

Interleukin-6-174 Genotype, Periodontal Disease and Adverse Pregnancy Outcomes: A Pilot Study

M. Dashash · J. Nugent · P. Baker · D. Tansinda · F. Blinkhorn

Received: 6 October 2007 / Accepted: 7 December 2007 / Published online: 8 January 2008

Abstract This study was undertaken to investigate whether maternal periodontal disease and variant genotypes of IL-6 gene are associated with adverse pregnancy outcomes. A total of 145 pregnant women were recruited from St Mary's Hospital, Manchester, UK. Bleeding on probing (BOP) and pocket depth indices were recorded on all teeth. Amplification refractory mutation system-polymerase chain reaction was used for −174 IL-6 genotyping. Birth weight was assessed using the individualized birth ratio (IBR) with intrauterine growth restriction (IUGR) defined as an IBR below the fifth percentile. The G/G genotype results in more BOP % sites in Caucasian($P<0.001$) and Afro-Caribbean pregnant women ($P=0.035$). In addition, a marginal significant association between the −174 C/C genotype and IUGR was observed ($P=0.06$). The −174* C allele was more frequent in women with IUGR than in normal women (63 vs 37%, $P=0.05$). Moreover, the combination between the carriage of −174C allele and increased bleeding sites have increased the risk of IUGR ($P=0.006$). Future studies, with a larger sample size, are required to better clarify the relationship between the IL-6 gene polymorphism, periodontal disease, and IUGR.

M. Dashash · F. Blinkhorn
Greater Manchester School for Professions Complementary to Dentistry,
Salford Primary Care Trust, St James House,
Pendleton Way,
Salford, UK

J. Nugent · P. Baker (✉) · D. Tansinda
Maternal and Fetal Health Research Centre,
University of Manchester, St Mary's Hospital,
Hathersage Road,
Manchester M13 0JH, UK
e-mail: philip.baker@manchester.ac.uk

Keywords Periodontitis · IL-6 · IUGR · immunology · pathology

Introduction

Recent investigation has shown that, during pregnancy, Gram-negative pathogens associated with periodontitis produce a variety of bioactive molecules and pro-inflammatory cytokines, such as interleukin (IL)-1, tumor necrosis factor (TNF)-, IL-6, and IL-8, that can directly affect the fetus [1, 2]. IL-6 has been considered as one of the most sensitive markers of the acute phase response to infections and inflammation. Overproduction of this cytokine has been noticed in the amniotic fluid during intrauterine infection. An increased level of IL-6 has also been observed in maternal serum, cervix, and amniotic fluid in preterm labor [2]. Several investigators have suggested using this cytokine as a diagnostic marker to predict women at increased risk of having preterm delivery [3, 4]. Overproduction of IL-6, because of periodontal infection, has been shown to affect the fetal membranes and cause preterm uterine contractions [1, 5, 6].

More recent work has shown that pregnant women of elevated PGE2, IL-6, and IL-8 and with periodontitis are at high risk for premature birth [7]. Genetic regulation of IL-6 has been demonstrated. The gene polymorphism in the promoter region of IL-6 at position −174 influences cytokine production; the G/G genotype of IL-6 gene polymorphism is associated with increased production of IL-6, whereas the C/C genotype is associated with decreased production of the cytokine [8].

To date, no studies have been undertaken to investigate the association between gene polymorphisms of IL-6, adverse pregnancy outcomes, and periodontal disease. Therefore, this study aimed to firstly investigate the

association between IL-6 gene polymorphisms and periodontal disease; secondly, the association between IL-6 gene polymorphisms and adverse pregnancy outcomes; and finally, to compare the association between IL-6 gene polymorphisms and adverse pregnancy outcomes in women with different levels of periodontal disease.

Materials and Methods

This study was approved by Central Manchester Ethics Committee. A pilot prospective observational study was carried out in St. Mary's Hospital, Manchester, which serves an area of marked social deprivation, ranked seventh of the 354 most deprived local Authority District in England.

The study was confined to nulliparous patients to limit the effect of confounding variables (previous poor pregnancy outcome and parity). Women with a multiple pregnancy or any maternal disease (e.g., chronic hypertension, diabetes, renal compromise) were excluded from the study. Patients using antibiotics or anti-inflammatory drugs during pregnancy and with diseases that compromise immune system were also excluded. Other exclusion criteria included multiple pregnancy and fetal anomalies. Non-English speaking subjects were offered a translation service. Subjects unable to consent were excluded. Smoking was recorded as yes or no only. All women seen on these clinics were classed as using alcohol within acceptable limits. Drug abusers and those with a serious alcohol problem would be seen on a different clinic, which was run in conjunction with social services.

One hundred forty-five pregnant women were recruited at their initial visit, between 12 and 16 weeks gestation, to the antenatal clinic, St. Mary's Hospital, Manchester.

Clinical Examination

A full-mouth periodontal examination was performed by one trained dental hygienist on all teeth using the World Health Organization probe. The diagnosis of periodontal disease was based on recording pocket depth and the presence or absence of bleeding on probing at all sites of each tooth present (midbuccal, mesiobuccal, distobuccal, midlingual, mesiolingual, and ditolingual). All the participants had mean values of pocket depth of less than 2.5 mm, and all pregnant women had bleeding on probing. Bleeding on probing BOP has become a standard diagnostic test for identifying sites at risk for disease progression [9–11]. It was noted that higher percentages of sites with BOP is associated with increased risk for disease progression [10]. Therefore, subjects were classified as women with gingival bleeding present in more than 50% of the sites assessed or into those who had bleeding gingiva in less than 50% of the sites examined.

Assessment of Pregnancy Outcome

Pregnancy outcome information was collected; this included gestational age at delivery, sex, and weight of the baby. Delivery data was obtained from the St Mary's Hospital database (CMiS) or the subjects' General Practitioner if they delivered at another unit. Participants' weight, height, and ethnicity were also collected to facilitate the calculation of IBR.

Pregnancy outcome was categorized into three groups—preterm labor <32 weeks, preterm labor <37 weeks, and intrauterine growth restriction (IUGR) defined as an individualized birth ratio (IBR) of less than fifth percentile. Preterm labor is historically defined as less than 37 weeks gestation but with improved neonatal care; those at <32 weeks gestation are most at risk of perinatal mortality and long-term morbidity.

−174 IL-6 Genotyping

Buccal cells were collected from the cheeks. Genomic DNA was extracted as previously described [12]. Amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) was used to genotype the G → C nucleotide substitution at position −174 from the transcription start site of the IL-6 gene [13]. DNA was amplified in a 10-μl PCR reaction. The PCR master mix (ABgene, UK) was prepared with 10 μM of human growth hormone primer-mix (HGH) primers: sense, 5′gccttcccaacccttccctta-3′, and anti-sense, 5′tcacggatttctgttgtgtttc-3′.

A volume of 5 μl of DNA plus master mix was aliquoted into 96-well PCR plate containing 5 μl of either one or the other specific primer mix (10 μM each). The specific primer sequences (PCR product size=258 bp) used in the PCR reactions were as follows: generic primer (5′-gcctcagagacatctccagtcc-3′); primer G (sense) (5′-cccctagttgtgtcttgcg-3′); and primer C (sense) (5′-cccctagttgtgtcttgcc-3′).

Primer design used sequence data for IL-6 alleles from GenBank (accession number Y00081).The PCR reaction was carried out using a DNA thermal cycler Programmable (PTC-100, MJ Research Inc. Technical sales, Edmonton, Canada). Cycles of ARMS-PCR were as follows: 1 min at 95°C followed by 10 cycles of 15 s at 95°C, 50 s at 65°C, and 40 s at 72°C followed by 20 cycles of 20 s at 95°C, 50 s at 59°C, and 50 s at 72°C.

The amplified products were visualized on a 2% agarose gel against a 200-bp DNA ladder and stained with 0.5 μg/ml of ethidium bromide. Two readings of the gel were taken on different occasions by one investigator (MD), who was blinded to the clinical classification.

Statistical Tests

The frequency of gingivitis, alleles, and genotypes was calculated using SPSS 11.5 (SPSS Inc, Chicago, IL) and

EPI-ENFO 2002 (Centers for Disease Control and Prevention, Atlanta, GA). The IBR was calculated using Gestation Related Optimal Weight software package produced by the Perinatal Research Monitoring Unit (Nottingham University). The χ^2 tests and Fisher's exact test were also utilized to detect any association between BOP sits, IUGR, and IL-6 alleles at position −174. Student's *t* test were also used to determine the difference between healthy subjects and those with IUGR in terms of the mean values of pocket depth, bleeding sites, number of teeth examined, and birth weight. A corrected *P* value less than 0.05 was considered statistically significant.

Results

A total of 145 pregnant women aged between 17 and 43 years old with a mean age of 26.64 years (SD±5.3) were enrolled in this study. The study population included 84 Caucasians, 37 Asians, 24 Afro-Caribbeans. Clinical characteristics are presented in Table I. A total of 76(52%) women had bleeding in less than 50% of the sites examined, whereas 69(48%) had bleeding in more than 50% of the sites. Three women (2%) delivered preterm at less than 32 weeks of gestation and a total of 10 (7%) of women before 37 weeks of gestation. Sixteen women (11%) had IUGR defined as an IBR less than fifth percentile.

There was no significant difference between different ethnic groups (P=0.81) or between smokers and non-smokers (P=0.58) in the distribution of IUGR In addition, no significant association was found between IUGR and maternal age at ≤20 years (P=0.15) or at ≥35 years (p= 0.38). There was no significant association (P=0.97) between increased bleeding sites and IBR(P=0.81).There was also no significant association between increased bleeding sites and preterm delivery at <37 weeks (P= 0.63), or <32 weeks (P=0.46) of gestation.

−174 IL-6 Gene Polymorphism and Periodontal Disease

Table II summarizes the association between −174 IL-6 genotypes, alleles, and the percentages of bleeding sites in pregnant women with gingivitis. The G/G genotype results in more BOP % sites (38 vs 9% in C/C genotype positives P< 0.001). In addition, a subgroup analysis according to ethnicity has shown that the association between IL-6 gene polymorphism and periodontal disease remained significant in Caucasian (P=0.001) and Afro-Caribbean women (P=0.035).

−174 IL-6 Gene Polymorphism and Adverse Pregnancy Outcomes

Table III details the pregnancy outcomes stratified by −174 IL-6 alleles and genotypes. A marginal significant association between the −174 C/C genotype and IUGR was observed (P= 0.06). The −174 C allele positive subjects exhibited higher occurrence of IUGR than allele C negatives. In addition, the −174* C allele was more frequent in women with IUGR

Table I Summary of Clinical Characteristics of Pregnant Women

All subjects=145	IUGR IBR<5th percentile (n=16)	Non- IUGR IBR>5th percentile (n=129)	P value
Maternal age			
Age(year±SD)	27.06±4.02	26.94±5.31	0.64[a]
≤20 years	0(0%)	15 (11.6%)	0.15[b]
>20 years	16(100%)	114(88.4%)	
≥35 years	16 (100%)	121(94%)	0.38[b]
<35 years	0 (%)	8(6%)	
Ethnicity			
Caucasians	9(%)	75 (58%)	P=0.81[b]
Asians	5(%)	32(25%)	
Blacks	2 (%)	22(17%)	
Smoking status			
Smokers	2 (13%)	19(15%)	P=0. 58[b]
Non smokers	14 (87%)	110(85%)	
Characteristics of gestation			
Birth weight (g)	Q	3355.446±567.81	P=0.000[a]
Pre-term (<37 week)	2 (13%)	8(6%)	P=0. 3[b]
Pre-term (<32 week)	1 (6%)	2(2%)	P=0.29[b]
Periodontal examination			
BOP >50%	8 (50%)	61 (47%)	P=0.52[b]
Bleeding sites (%)	47.63±0.19	49.03±0.223	P=0.81[a]
Pocket depth (mm)	0.71±0.33	0.735±0.39	P=0.87[a]
Number of teeth examined	27.44±2.03	27.56±1.944	P=0.81[a]

[a] Student's *t* test
[b] χ^2 test

Table II Percentages of IL-6 Alleles and Genotypes at −174 in Pregnant Women with Periodontal Disease

	Total=145			Caucasian=84			Asians=37			Afro-Caribbeans=24		
	BOP <50%	BOP >50%	*P* value	BOP <50%	BOP >50%	*P* value	BOP <50%	BOP >50%	*P* value	BOP <50%	BOP >50%	*P* value
−174 IL-6 G/C												
GG	5	26	0.00[a]	1	9	0.001[b]	3	11	0.38[b]	1	6	0.035[b]
	7%	38%		2%	26%		23%	46%		8%	54%	
GC	56	37		37	22		8	11		11	4	
	74%	54%		74%	65%		62%	46%		84%	37%	
CC	15	6		12	3		2	2		1	1	
	20%	9%		24%	9%		15%	8%		8%	9%	
Total	76	79		50	34		13	24		13	11	
G	66	89	0.00	39	40	0.012[a]	14	33	0.20[a]	13	16	0.10[a]
	43%	64%		39%	59%		54%	69%		50%	73%	
C	86	49		61	28		12	15		13	6	
	57%	36%		61%	41%		46%	31%		50%	27%	
Total	152	138		100	68		26	48		26	22	

[a] χ^2 test, [b] Fisher's exact test.

than in normal women (63 vs 45%, *P*=0.05). However, no significant difference was found between IL-6 gene polymorphism and preterm delivery at <32 weeks (*P*=0.42), or <37 weeks of gestation (*P*=0.18).

−174 IL-6 Gene Polymorphism, Periodontal Disease, and IUGR

The findings of −174 IL-6 gene polymorphisms in women with and without IUGR stratified by percentages of bleeding sites are presented in Table IV. An increased risk of having IUGR was observed in Caucasian women who were Allele C positives (GC/CC) and had increased number of bleeding sites(*P*=0.018). In addition, the combination between the carriage of the −174 C allele and having increased bleeding sites (>50%) seemed to be risk factor for IUGR in Caucasian women(70 vs 30% in G positives *P*= 0.045), whereas the carriage of −174 G allele seemed to be protective against IUGR women who had increased percentages of bleeding sites. An increased risk of having IUGR was also observed in Caucasian smoker women who were Allele C homozygotes (CC) and had increased

Table III Percentages of IL-16 Alleles and Genotypes at Positions −174 in Pregnant Women with IUGR

	Total=145			Caucasian=84			Asians=37			Afro-Caribbeans=24		
	IBR<5	IBR >5	*P* value [c]	IBR<5	IBR >5	*P* value	IBR<5	IBR >5	*P* value	IBR<5	IBR >5	*P* value
−174 IL-6 G/C												
GG	1	30	0.066[b]	0	10	0.27 [b]	1	13	0.07 [b]	0	7	0.52 [b]
	6%	23%		0%	13%		20%	41%		0%	29%	
GC	10	83		6	53		2	17		2	13	
	62%	64%		67%	71%		40%	53%		100%	59%	
CC	5	16		3	12		2	2		0	2	
	31%	12%		33%	16%		40%	6%		0%	9%	
Total	16	129		9	75		5	32		2	22	
										100%	100%	
G	12	143	0.05[a]	6	73	0.21[a]	4	43	0.09[a]	2	27	0.65[b]
	37%	55%		33%	49%		40%	67%		50%	61%	
C	20	115		12	77		6	21		2	17	
	63%	45%		67%	51%		60%	33%		50%	39%	
Total	32	258		18	150		10	64		4	44	

[a] χ^2 test
[b] Fisher's exact test.
[c] No significant difference was found between IL-6 gene polymorphism and preterm delivery at <32 weeks (*P*=0.42), or <37 weeks of gestation (*P*=0.18).

Table IV −174 IL-6 Gene Polymorphisms in Women with and Without IUGR Stratified by Bleeding on Probing Sites

		IBR <5 Total	IBR >5	*P* value[a]	IBR <5 Caucasians[c]	IBR >5	*P* value[a]	IBR <5 Asians	IBR >5	*P* value[a]	IBR <5 Afro-Caribbeans	IBR >5	*P* value[a]
Bleeding sites < 50%	GG	0	5	0.7	0	1	0.95	0	3	0.29	0	1	0.80
		0%	7%		0%	2%		0%	27%		0%	9%	
	GC	6	50		3	34		1	7		2	9	
		75%	74%		75%	74%		50%	64%		100%	82%	
	CC	2	13		1	11		1	1		0	1	
		25%	19%		25%	24%		50%	9%		100%	9%	
	Total	8	68		4	46		2	11		2	11	
		100%	100%					100%	100%		100%	100	
	Allele												
	G	6	60	0.61	3	36	0.92	1	13		2	11	0.20
		37%	44%		37%	39%		25%	60%		50%	50%	
	C	10	76		5	56		3	9		2	11	
		63%	56%		63%	61%		75%	40%		50%	50%	
	Total	16	136		8	92		4	22		4	22	
Bleeding sites >50%	GG	1	25	0.006	0	9	0.018	1	10	0.24	0	6	NA[b]
		13%	41%		0%	31%		33%	48%		0%	55%	
	GC	4	33		3	19		1	10		0	4	
		50%	54%		60%	66%		33%	48%		0%	36%	
	CC	3	3		2	1		1	1		0	1	
		37%	5%		40%	3%		33%	5%		0%	9%	
	Total	8	61		5	29		3	21		0	11	
		100%	100 %		100%	100%		100%	100		0%	100%	
	Allele												
	G	6	83	0.016	3	37	0.045	3	30	0.28	0	16	NA[b]
		37%	68%		30%	64%		50%	71%		0%		
	C	10	39		7	21		3	12		0	6	
		63%	32%		70%	36%		50%	29%		0%		
	Total	16	122		10	58		6	42		0	22	

[a] Fisher's exact test

[b] χ^2 test not applicable

[c] No significant difference between IL-6 gene polymorphism, periodontal disease, and IUGR was observed in Caucasian smoker women (n=14, P=0.082).

number of bleeding sites (Table IV). However, this did not reach the significance (n=14, P=0.082).

Discussion

IL-6 is an anti-inflammatory cytokine that maintains normal pregnancy, and it is essential for angiogenesis, development of ovarian follicles, and formation of the decidua after embryonic implantation [14]. Previous work has shown that the production of this cytokine is genetically determined. Fishman et al [15] have shown that the G/G genotype of IL-6 gene polymorphism at position −174 is associated with increased production of IL-6, whereas the C/C genotype is associated with decreased production of the cytokine.

The aim of this study was to investigate the association between −174 IL-6 gene polymorphism, periodontal disease, and adverse pregnancy outcomes including preterm delivery at less than 32 and less than 37 weeks gestation and IUGR.

In the present study, the implication of the −174 IL-6 G/G genotype in increasing sites with BOP has been demonstrated in Caucasian pregnant women(n=84). These findings are similar to those reported previously in adult Caucasian Brazilians with periodontitis [16].

Our results are also in agreement with other studies, which have reported higher levels of IL-6 in sites and tissues with gingivitis and periodontitis when compared to healthy controls [17, 18, 19].

IL-6, which has been implicated in periodontitis, was previously regarded a risk factor for several adverse pregnancy outcomes. El-Bastawissi et al. [20] have found the strongest association with preterm labor as a result of chorioamnionitis. An elevated concentration of this cytokine has also been observed in spontaneous preterm delivery and some fetal systemic responses such as periventricular leukomalacia, bronchopulmonary dysplasia, and composite neonatal morbidity [21]. An increased level of IL-6 has also been observed in maternal serum, cervix, and amniotic fluid in preterm labor [3].

In our hypothesis, we tested whether the G/G genotype of IL-6, which results in increased production of IL-6, is associated with adverse pregnancy outcomes. There was no significant association between IL-6 gene polymorphism and preterm delivery at less than 32 weeks or at less than 37 weeks of gestation. However, the G/G genotype, which has been identified as risk factor for periodontal disease in Caucasian and Afro-Caribbean women, has been shown in this study to be a protective marker against IUGR. A marginal significant association was observed between IUGR and the C/C genotype rather than the G/G genotype. The C/C genotype, which is associated with decreased production of IL-6, was more frequent in pregnancies complicated with IUGR than in normal pregnancies (P=0.06). Moreover, the combination between the −174C/C genotype and increased bleeding sites have increased the risk of having IUGR in Caucasian women (P=0.018).

In fact, previous work has shown that IL-6 insufficiency in the cord serum could affect trophoblast function and can cause several adverse pregnancy outcomes such as pre-eclampsia and preterm low birth [14]. This might well explain our findings. In contrast, our results are in contrary to previous work, which showed that the carriage of −174 *C allele is protective against preterm delivery [3] and also with that which showed a significant association between IL-6 G/G genotype (increased production of IL-6) and preterm delivery at <37 weeks of gestation [22].

Although IL-6 has anti-inflammatory properties, it is a pro-inflammatory cytokine. Overexpression of this cytokine has been implicated in the pathophysiology of abnormal pregnancies [8]. IL-6 has been considered as a major mediator of the host response to infection and tissue damage, and it can stimulate the production of C-reactive protein by liver cells, the acute phase plasma protein response, and activation of T and natural killer cells [6]. Therefore, it was suggested that women who produce less IL-6 in response to infection or inflammation are less likely to have dramatic feed-forward inflammatory cascade of events such as preterm labor and delivery [3]. This may well explain the differences between our study and other studies, which were undertaken on infants with spontaneous preterm birth [3, 23].

Another explanation for the differences seen in our results compared to those from other studies may be related to the methods employed for the assessment of adverse pregnancy outcomes. Our study is unique because it used the more accurate assessment of growth restriction, an IBR less than fifth percentile rather than a birth weight of less than 2.5 kg.

Nugent and Baker have pointed out that using birth weight for gestational age, <10th centile or <2.5 kg at term, does not reliably detect babies who are growth restricted. In the present study, the IBR calculated a baby's predicted birth weight using birth weight at delivery, gestation at delivery, baby's sex and maternal height, weight, ethnicity, and parity. The baby's actual birth weight is divided by the predicted weight and expressed as a percentage. A baby is growth-restricted if its IBR is less than the fifth percentile [24, 25].

Our study is also the first to investigate the association between IL-6 gene polymorphism, periodontal disease, and pregnancy outcomes. Previous studies have investigated gene polymorphisms of other pro-inflammatory cytokines such as IL-1 and TNF [26, 27]. Moore et al. have shown that the combination between the variant genotype of IL-1ß +3953 and TNF-308 and severe periodontal disease did not increase the risk of having preterm birth. In the present study, the combination between −174 CC genotype and increased bleeding sites (>50%) have increased the risk of IUGR (P=0.006).

Further studies including several pro/anti-inflammatory cytokines are still needed to further clarify the association between cytokine gene polymorphisms, periodontal disease, and poor pregnancy outcomes.

Although this pilot study is limited by the small size, we could show that the G/G genotype, which is associated with increased production of IL-6 and is implicated in periodontitis, is protective against IUGR in Caucasians. This is in contrast to previous work by Offenbacher et al. [1] in 1998, which found that overproduction of IL-6, because of periodontal infection, can affect the fetal membranes causing preterm uterine contractions. A further study, with a larger sample size, is required to confirm our results in different ethnic groups.

In conclusion, this pilot study has shown that the −174 IL-6 G/G genotype could be a risk factor for periodontitis, but also may be protective against IUGR. Future research with a larger sample size is needed to better clarify the exact role of IL-6 gene polymorphism in periodontitis and adverse pregnancy outcomes in different ethnic groups.

Acknowledgment We would like to thank Prof. Anthony Blinkhorn for his invaluable scientific advice and Prof. Ian Hutchinson for assistance. We are also grateful to all pregnant women who agreed to take part in this study and to Mrs Rosemary Hollowfield (Dental hygienist), Mrs Janet Weatherby (Dental Nurse), and Ms Jenny Robinson for their contribution. This study was funded by the Department of Health for England.

References

1. Offenbacher S, Jared HL, O' Reilly PG, Wells SR, Salvi GE, Lawrence HP, Socransky SS, Beck JD. Potential pathogenic mechanisms of periodontitis-associated pregnancy complications. Ann Periodontol. 1998;3:233–50.
2. Li X, Kolltveit KM, Tronstad L, Olsen I. Systemic diseases caused by oral infection. Clin Microbiol Rev. 2000;13:547–58.
3. Simhan HN, Krohn MA, Roberts JM, Zeevi A, Caritis SN. Interleukin-6-promoter- 174 polymorphism and spontaneous preterm birth. Am J Obstet Gynecol. 2003;189:915–8.
4. Lange M, Chen FK, Wessel J, Buscher U, Dudenhausen JW. Elevation of interleukin-6 levels in cervical secretion as a predictor of preterm delivery. Acta Obstet Gynecol Scand. 2003;82:326–9.
5. Wang X, Zuckerman B, Kaufman G. Molecular epidemiology of preterm delivery: methodology and challenges. Paed Perinat Epidemiol. 2001;15(suppl 2):63–77.
6. Romero R, Tinnakorn C, Espinoza J. Micronutrients and intrauterine infection, preterm birth and the fetal inflammatory response syndrome. J Nutr. 2003;133:1668S–73S.
7. Dörtbudak O, Eberhardt R, Ulm M, Persson GR. Periodontitis, a marker of risk in pregnancy for preterm birth. J Clin Periodontol 2005;32:45–52.
8. Unfried G, Bocskor S, Endler G, Nagele F, Huber JC, Tempfer CB. A polymorphism of the interleukin-6 gene promoter and idiopathic recurrent miscarriage. Hum Reprod. 2003;18:267–70.
9. Joss A, Adler R, Lang NP. Bleeding on probing. A parameter for monitoring periodontal conditions in clinical practice. J Clin Periodontol. 1994;21:402–8.
10. Lang NP, Tonetti MS, Suter J, Sorrell J, Duff GW, Kornman KS. Effect of interleukin-1 gene polymorphisms on gingival inflammation assessed by bleeding on probing in a periodontal maintenance population. J Periodontal Res. 2000;35:102–7.
11. Bassani DG, Olinto MT, Kreiger N. Periodontal disease and perinatal outcomes: a case-control study. J Clin Periodontol. 2007;34:31–9.
12. Dashash M, Blinkhorn AS, Hutchinson IV, Pravica V, Drucker DB. The relationship between interleukin-10 gene polymorphism at position −1082 and susceptibility to gingivitis in children. J Periodontol. 2005;76:1455–62.
13. Perrey C, Pravica V, Sinnott PJ, Hutchinson IV. Genotyping for polymorphisms in interferon-gamma, interleukin-10, transforming growth factor-beta 1 and tumour necrosis factor-alpha genes: a technical report. Transpl Immunol. 1998;6:193–7.
14. von Linsingen R, Bompeixe EP, Bicalho MdaG. A case-control study in IL6 and TGFB1 gene polymorphisms and recurrent spontaneous abortion in southern Brazilian patients. Am J Reprod Immunol. 2005;53:94–9.
15. Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, Woo P. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest. 1998;102:1369–76.
16. Trevilatto PC, Scarel-Caminaga RM, Brito RB Jr. Polymorphism at position −174 of IL-6 gene is associated with susceptibility to chronic periodontitis. J Clin Periodontol. 2003;30:438–42.
17. Gemmell E, Seymour GJ. Interleukin 1, interleukin 6 and transforming growth factor-beta production by human gingival mononuclear cells following stimulation with Porphyromonas gingivalis and Fusobacterium nucleatum. J Periodontal Res. 1993;28:122–9.
18. Wu Y, Zhao C, Zhang J. Interleukin-6 levels in the gingival crevicular fluid before and after periodontal treatment (Abstract). Hua Xi Kou Qiang Yi Xue Za Zhi. 2001;19:99–101.
19. Atilla G, Kutukculer N. Crevicular fluid interleukin-1beta, tumor necrosis factor-alpha, and interleukin-6 levels in renal transplant patients receiving cyclosporine. J Periodontol. 1998;69:784–90.
20. El-Bastawissi AY, Williams MA, Riley DE, Hitti J, Krieger JN. Amniotic fluid interleukin-6 and preterm delivery: a review. Obstet Gynecol. 2001;95:1056–64.
21. Jamie WE, Edwards RK, Ferguson RJ, Duff P. The interleukin-6-174 single nucleotide polymorphism: cervical protein production and the risk of preterm delivery. Am J Obstet Gynecol. 2005;4:1023–7.
22. Hartel Ch, Finas D, Ahrens P. Polymorphisms of genes involved in innate immunity: association with preterm delivery. Mol Hum Reprod. 2004;10:911–5.
23. Ahrens P, Kattner E, Kohler B, Härtel C, Seidenberg J, Segerer H, Möller J, Göpel W, Genetic Factors in Neonatology Study Group. Mutations of genes involved in the innate immune system as predictors of sepsis in very low birth weight infants. Pediatr Res 2004;55:652–6.
24. Sanderson DA, Wilcox MA, Johnson IR. The individualised birth weight ratio: a new method of identifying intrauterine growth retardation. Br J Obstet Gynaecol. 1994;101:310–4.
25. Nugent JL, Baker PN. Periodontal disease and adverse pregnancy outcomes: a systematic review. BJOG. 2006;113:848.
26. Moore S, Ide M, Randhawa M, Walker JJ, Reid JG, Simpson NA. An investigation into the association among preterm birth, cytokine gene polymorphisms and periodontal disease. Br J Obstet Gynaecol. 2004;111:125–32.
27. Hasegawa K, Furuichi Y, Shimotsu A. Associations between systemic status, periodontal status, serum cytokine levels, and delivery outcomes in pregnant women with a diagnosis of threatened premature labor. J Periodontol. 2003;74:1764–70.