IL-10 Gene Polymorphisms, Periodontal Disease and Intrauterine Growth Restriction: A Pilot Study

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Abstract

IL-10 has several immuno-protective functions in the placenta The expression of IL-10 is genetically determined. The aim of this study was to investigate whether maternal periodontal disease and IL-10 gene polymorphisms, are associated with intra uterine growth restriction (IUGR). A total of 160 pregnant women were recruited from the Antenatal Clinic, St Mary's Hospital, Manchester, UK and enrolled into a prospective pilot study. The study included 92 Caucasians, 41 Asians and 27 Afro Caribbeans. The periodontal examination involved recording bleeding on probing and pocket depth indices on all teeth. ARMS-PCR was used for genotyping IL-10 gene at position -1082, -819 and -592. Birth weight was assessed using the individualised birth weight ratio (IBR), with intrauterine growth restriction IUGR defined as an IBR below the 5th percentile. χ^2 test was performed for statistical analysis. The –1082 A/A genotype, is associated with more increased BOP% sites (23% vs14% , P = 0.069), and significantly with more IUGR in Afro-Caribbean women (75% vs 9%, P = 0.002). Moreover, the combination between the –1082 A genotype and increased percentages of bleeding sites have increased the risk of IUGR in Afro Caribbean women. However, this genotype had only a marginal influence on IUGR (P = 0.07) and no influence on periodontal disease in Caucasian women (P = 0.55). There was no significant association between IUGR, periodontal disease and IL-10 gene polymorphisms at position –819 and –592. Also, there was no significant difference between IL-10 haplotypes, periodontal disease and IUGR in any of the ethnic groups examined. Future studies, with a larger sample size, are required to ascertain the genetic association between IL-10 gene polymorphisms maternal periodontal disease and IUGR in different ethnic groups.

Introduction

Intrauterine growth restriction (IUGR) is an important pregnancy complication associated with significant increases in perinatal mortality and morbidity. IUGR results from inadequate transfer of nutrients and oxygen to the developing fetus. A baby is growth restricted if its individualised birth weight ratio IBR is less than the 5th percentile [Wilcox, *et al.*, 1993, Sanderson, *et al.*, 1994].

Maternal infections by gram-negative anaerobic pathogens, which are implicated in periodontitis, have been shown to cause preeclampsia, preterm birth, fetal growth restriction, and fetal loss [Hillier, *et al.*, 1995]. Offenbacher and co-workers (1998) were the first research team to report that women having low birth weight (LBW) infants had greater attachment loss than women having normal birth weight (NBW) infants [Offenbacher, *et al.*, 1998]. During pregnancy, it has been found that the ratio of anaerobic gram-negative bacterial species to aerobic species in dental plaque increases in the second trimester [Kornman and Loesche, 1980]. The gram-negative pathogens associated with periodontitis such as *Actinobacillus actinmycetmcomitans Aa* and *Porphyromonas gingivalis Pg* have been found to produce a variety of bioactive molecules and pro-inflammatory cytokines such as IL-1β, TNF-α, IL-6, and PGE2 that can directly affect the foetus [Li, *et al.*, 2000]. Over production of IL-1β, TNF-α, because of periodontal infection, has been shown to affect the placental membrane and cause preterm uterine contractions [Offenbacher, *et al.*, 1998, Wang, *et al.*, 2001, Romero, *et al.*, 2003].

In normal conditions, an anti-inflammatory cytokine such as IL-10, which has immunoprotective functions in the placenta [Denison, *et al.*, 1998, Rivera, *et al.*, 1998, Mitchell, *et al.*, 2004], interferes with the inflammatory process, suppresses pro-inflammatory cytokines and PGE2 production. It also inhibits metaloprotease expression in the fetal membrane. However, the expression of IL-10 is genetically determined, and the gene of IL-10 is polymorphic. This means that any polymorphism in the promoter region of IL-10 gene can affect its production [Turner, *et al.*, 1997]. If the production of this cytokine is insufficient, then oral pathogens may

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be able to cross intact membranes into the amniotic cavity, where they can also stimulate the production of extra-inflammatory cytokines by macrophages and other host cells inducing preterm uterine contractions [Romero, *et al.*, 2003]. A Previous study has shown an association between under-production of IL-10 and the –1082 *A allele, and also between high production of IL-10 and the –1082* G allele [Turner et al 1997]. In addition, a closed linkage between alleles –819 *C and –592* C and between alleles –819 *T and –592 * A has been identified [Dashash, *et al.*, 2006]. It is still not clear whether over-production of pro-inflammatory cytokines or under-production of anti-inflammatory cytokines in periodontitis is implicated in preterm uterine contractions. All previous studies have investigated the possible association of gene polymorphisms of pro-inflammatory cytokines with preterm delivery and periodontal disease [Offenbacher, *et al.*, 1996, Jeffcoat, *et al.*, 2003, Louro, *et al.*, 2001, Lopez, *et al.*, 2002, Hasegawa, *et al.*, 2003, Dasanayake, *et al.*, 2003, Moore, *et al.*, 2004].

To date no studies have been undertaken to investigate the association between gene polymorphisms of anti-inflammatory cytokines including IL-10, IUGR and periodontal disease. Therefore, this study aimed to firstly investigate the association between IL-10 gene polymorphisms and IUGR. Secondarily, the association between IL-10 gene polymorphisms and periodontal disease and finally to compare the association between IL-10 gene polymorphisms and IUGR in women with different levels of periodontal disease.

Materials and Methods:

This study was approved by Central Manchester Ethics Committee and was a pilot prospective observational study undertaken at St. Mary's Hospital, Manchester, which serves an area of marked social deprivation, ranked 7th/354 of the most deprived local Authority Districts in England. This area has a higher rate of unemployment, a lower life expectancy, and an increased rate of stillbirth, perinatal and childhood mortality when compared the rest of the country. Smoking was recorded as yes or no only. All women seen on these clinics were classed as using alcohol within acceptable limits. No specific information was collected by the medical team, just a self assessment by the women on their alcohol consumption. The women in our study were classed as normal and not high risk. Drug abusers and those with a serious alcohol problem would be seen on a different clinic which was run in conjunction with social services. 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. Subjects unable to consent were excluded. Subjects of mixed ethnicity were excluded. Non-English speaking subjects were offered a translation service.

One hundred and sixty pregnant women, between 12 and 16 weeks gestation, were recruited at the antenatal-clinic on their initial visit.

Clinical Examination:

All periodontal examinations were carried out by one trained dental hygienist in the maternity ward in the antenatal clinic, St Mary's Hospital, Manchester. Women were supine on a hospital bed. A full mouth periodontal examination was undertaken using a WHO probe with a mean number of teeth examined of 27.59 (SD 2.08). The diagnosis of periodontal disease was based on recording pocket depth and the presence or absence of bleeding on probing at six sites of each tooth present (midbuccal, mesiobuccal, distobuccal, midlingual, mesiolingual and ditolingual). Bleeding on probing BOP has become a standard diagnostic test for identifying sites at risk for disease progression [Josh, *et al.*, 1994, Lang, *et al.*, 2000]. It was noted that higher percentages of sites with BOP are associated with an increased risk for disease progression [Lang, *et al.*, 2000]. All the participants had mean values of pocket depths for all teeth examined of less than 2.5mm and had sites with BOP. Therefore, subjects were classified, according to the percentages of bleeding sites they had, into women with BOP present in more than 50% of the sites examined and those with less than 50% of sites assessed.

Assessment of IUGR:

Pregnancy outcome information included: gestational age at delivery, plus 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. Weight, height, and ethnicity were also collected to facilitate the calculation of the individualized birth weight ratio (IBR).

IL-10 Genotyping:

Genomic DNA was extracted from buccal cells of the cheeks as previously described [Dashash, *et al.*, 2005]. Briefly, Automatic DNA extraction was performed with a KingFisher™ Purification Kit (KingFisher™ Purification Kit (N.630 0041) Thermo Labsystems), and a KingFisher mL machine (Thermo Labsystems OY. Finland), in accordance with the manufacturer's instructions, and selecting the Genomic_DNA_1 program.

Amplification Refractory Mutation System (ARMS) –PCR was used to genotype IL-10 gene at position –1082, –819 and –592[13]. The specific primer sequences used for each polymorphism,

together with those for human growth hormone primer (GenBank accession number: X78437), are presented in Table 1.

DNA was amplified and the PCR reaction was carried out using a DNA thermal cycler (Programmable Thermal Cycler, PTC-100, MJ Research Inc. Technical sales. Edmonton, Alberta, Canada).

Cycles of ARMS-PCR were as follows: 1 minute at 95°C followed by 10 cycles of 15 seconds at 95°C, 50 seconds at 65°C, and 40 seconds at 72°C followed by 20 cycles of 20 seconds at 95°C, 50 seconds at 59°C and 50 seconds at 72°C.

The amplified products were visualized on a 2% agarose gel against a 200 bp DNA ladder and stained with 0.5mg/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 Analysis:

The frequency of gingivitis, alleles and genotypes was calculated using SPSS 13.00. The IBR was calculated using Gestation Related Optimal Weight software package (Perinatal Research Monitoring Unit, Nottingham University).

Allele and haplotype frequencies were calculated on patients and control groups by direct counting. The χ^2 tests and Fisher's exact test were also utilized to detect any association between BOP sits, IUGR and IL-10 gene polymorphisms at position –1082, –819 and –592. Student's *T*-test were also used to determine the difference between healthy subjects and those with IUGR in term 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 160 pregnant women aged between 17 to 43 years old with a mean age of 26.76(SD 5.3) were enrolled in this study. Table 2 presents the clinical characteristics of pregnant women with and without IUGR. The study included 92 Caucasians, 41 Asians and 27 Afro Caribbeans. Based on IBR, 22 (14%) had IUGR and 138 (86%) were healthy. There was no significant difference between different ethnic groups (P = 0.95) or between smokers and non smokers (P = 0.86) in the distribution of IUGR. In addition, no significant association was found between IUGR and maternal age at ≤ 20 years (P = 0.5) or at ≥ 35 years (p = 0.61).

Periodontal Disease and IUGR:

A total of 90 (56 %) women had BOP present in less than 50% of the sites examined, whilst 70 (44%) had BOP involving more than 50% of the sites. There was no significant difference between periodontal disease and IUGR (P = 0.86). There was no significant association between periodontal disease and preterm delivery at < 37 weeks (P = 0.16), or < 32 weeks (P = 0.32) of gestation (Table 2).

IL-10 Gene Polymorphism and Periodontal Disease:

Table (III) presents the association between IL-10 genotypes, alleles and the percentages of bleeding sites in pregnant women with gingivitis. The –1082 A/A genotype was observed more frequently among Afro-Caribbean women with increased percentages of BOP sites, however, this difference was not statistically significant (P = 0.069). In addition, there was no significant association between –1082 IL-10 gene polymorphism and periodontal disease in Caucasians or Asians. There was no significant association between –819, –592 IL-10 gene polymorphisms and periodontal disease (Table 3). Also, no association was found between IL-10 haplotypes and periodontal disease in all ethnic groups (Table III).

IL-10 Gene Polymorphism and IUGR:

The relation between IUGR and IL-10 gene polymorphism at position –1082, –819, –592 is shown in Table (IV). A significant association was observed between the IL-10 genotypes at position –1082 and IUGR in Afro Caribbean women (P=0.002). Allele A homozygotes (A/A) exhibited a significantly higher occurrence of IUGR than allele A hetrozygotes (G/A) (75%vs 25%). Similarly, a trend to a higher frequency of the –1082 A allele in Afro Caribbean women with IUGR was observed (88% vs 54% with normal values) although this did not reach statistical significance

TABLE 1 Primers used for the analysis of IL-10 gene at positions –1082 –819 and –592.

–1082 G→A (PCR product size= 258bp)	Generic primer (antisense) Primer G (sense) Primer A (sense)	5´-cagtgccaactgagaatttgg-3´ 5´-ctactaa ggcttctttgggag-3´ 5´-actactaaggcttctttgggaa-3´
–592 (C→A), –819(C→T): (PCR product size = 233bp)	Generic primer (antisense) Primer C (sense) Primer T (sense)	5´-aggatgtgttccaggctcct-3´ 5´-cccttgtacaggtgatgtaac-3´ 5´-acccttgtacaggtgatgtaat-3´
Human growth hormone (HGH)	Anti-sense Sense	5´-tcaggatttctgttgtgtttc-3´ 5´-gccttcccaaccattcccctta-3´

TABLE 2 Summary of clinical characteristics of pregnant women

All subjects=160	IUGR IBR < 5th percentile (n = 22)	Non- IUGR IBR > 5th percentile (n = 138)	P value
Maternal age			
Age(yr± SD)	26.09 ± 4.79	26.86 ± 5.43	0.09[a]
≤ 20 years	3 (14%)	16 (12%)	
> 20 years	19 (86%)	122 (88%)	0.5[b]
≥ 35 years	11 (50%)	61 (44%)	
< 35 years	11 (50%)	77 (56%)	0.61[b]
Ethnicity			
Caucasians	12 (54%)	80 (58%)	
Asians	6 (27%)	35 (25%)	P = 0.95[b]
Blacks	4 (19%)	23 (17%)	
Smoking			
Smokers	3 (14%)	17 (12%)	P = 0.86[b]
Non smokers	19 (86%)	121 (88%)	
Characteristics of gestation			
Birth weight (g)	2488.27 ± 596.10	3329.73 ± 556.84	P = 0.48[a]
Pre-term (< 37 week)	4 (18%)	12 (9%)	P = 0.16[b]
Pre-term (< 32 week)	1 (5%)	2 (3%)	P = 0.32[b]
Periodontal Examination			
BOP > 50%	10 (45%)	60 (43%)	P = 0.86[b]
Bleeding sites (%)	40.9 ± 23.69	45.94 ± 29.35	P = 0.24[a]
Pocket Depth (mm)	0.728 ± 0.39	0.73 ± 0.43	P = 0.51[a]
Number of teeth examined	27.59 ± 2.08	27.59 ± 2.28	P = 0.87[a]

[a] Student's T-test
[b] χ^2 test

(P = 0.07). There was no significant association between IUGR and IL-10 gene polymorphisms at position –819 and –592 (Table 4). Also, there was no significant difference between IL-10 haplotypes and IUGR in any of the ethnic groups examined (Table IV).

IL-10 Gene Polymorphism, periodontal disease and IUGR:

The findings of –1082 gene polymorphisms in women with and without IUGR stratified by percentages of bleeding sites are presented in Table V. An increased risk of having IUGR was observed in Afro Caribbean women who were Allele A homozygotes (A/A) and had increased number of bleeding sites (P = 0.038) whilst the carriage of –1082 G allele (GA) seemed to be protective against IUGR in Caucasian (P = 0.024) and Afro-Caribbean women (P = 0.038) who had increased percentages of bleeding sites. Non smokers, Afro Caribbean women with increased percentages of bleeding sites and were A allele homozygotes, were also significantly (P = 0.04) more susceptible to have IUGR (Table 5).

Discussion

IL-10 is an anti-inflammatory cytokine, which is highly expressed in the uterus and placenta to control inflammation – induced adverse pregnancy outcomes [Lin, *et al.*, 2003]. Previous studies have shown that *Porphyromonas gingivalis* infection during pregnancy increases maternal tumour necrosis factor α, suppresses maternal IL-10 and enhances fetal growth restriction in animal models [Bassani, *et al.*, 2007].

We aimed, in this study, to investigate whether maternal periodontal disease and IL-10 gene polymorphisms, are associated with IUGR among Caucasians, Asians and Afro-Caribbeans.

In the present study, having more than 50% of sites with bleeding on probing was considered to be a marker of periodontal disease in pregnant women. Using this marker, no association between periodontal disease, preterm birth (< 32 or 37 week of gestation) and IUGR (IBR < 5th percentile) was found in any of the ethnic groups.

Considering the clinical attachment loss of more than 3 mm in 3 sites and the presence of bleeding on probing of more than 50% of the sites as periodontal markers of chronic periodontitis and after adjusting for maternal age, previous pregnancies, pre-natal care, smoking previous low birth or premature birth and medical conditions, similar findings to ours were reported on Brazilian pregnant women. No association between periodontal disease, preterm (< 37 week of gestation) low birth weight (< 2500g) and IUGR (> 10th percentile of ponderal index which is birth weight x 100/ crown heel length) was observed [Bassani, *et al.*, 2007].

In contrast, our findings differ from those reported by Santos-Pereira, *et al.*, (2007) which suggested that chronic periodontitis in pregnant women could increase the risk of having a preterm birth at less than 37 week by a factor of 4.9, 95% CI: 1.9-12.8. The authors concluded that the

TABLE 3 Percentages of IL-10 alleles, genotypes and haplotypes at position –1082, –819 and –592 in pregnant women with periodontal disease

	Total = 160			Caucasian = 92			Asians = 41			Afro-Caribbeans = 27		
	BOP < 50%	BOP > 50%	P value	BOP < 50%	BOP > 50%	P value	BOP < 50%	BOP > 50%	P value	BOP < 50%	BOP > 50%	P value
Position –1082												
GG	9 10%	2 3%	0.15[b]	9 16%	1 3%	0.55[b]	0 0%	1 4.5%	0.55[b]	0	0	0.069[a]
GA	64 71%	57 81%		39 69%	31 89%		13 69%	16 73%		12 86%	10 77%	
AA	17 19%	11 16%		9 16%	3 9%		6 32%	5 23%		2 14%	3 23%	
Total	**90**	**70**		**57**	**35**		**19**	**22**		**14**	**13**	
G	82 45%	61 44%	0.72[a]	57 50%	33 47%	0.7[a]	13 34%	18 41%	0.53[a]	12 43%	10 38%	0.74[a]
A	98 55%	79 56%		57 50%	37 53%		25 66%	26 59%		16 57%	16 62%	
Total	**180**	**140**		**114**	**70**		**38**	**44**		**28**	**26**	
Positions –819, –592												
CC/CC	24 27%	16 23%	0.64[b]	19 33%	8 23%	0.2[b]	2 10.5%	2 9%	0.8[b]	3 21%	6 46%	0.17[b]
CT/CA	65 72%	52 74%		38 67%	27 77%		16 84%	18 82%		11 79%	7 54%	
TT/AA	1 1%	2 3%		0 0%	0 0%		1 5%	2 9%		0 0%	0 0%	
Total	**90**	**70**		**57**	**35**		**19**	**22**		**14**	**13**	
–819*C/ –592*C	113 63%	84 60%	0.61[a]	76 66%	43 61%	0.77[a]	20 53%	22 50%	0.81[a]	17 61%	19 73%	0.31[a]
–819*T/ –592*A	67 37%	56 40%		38 34%	27 39%		18 47%	22 50%		11 39%	7 27%	
Total	**180**	**140**		**114**	**70**		**38**	**44**		**28**	**26**	
IL-10 haplotype												
GCC	82 46%	61 44%	0.87[a]	57 50%	33 47%	0.75[a]	13 34%	18 41%	0.45[a]	15 54%	15 58%	0.47[b]
ACC	31 17%	23 16%		19 17%	10 14%		7 18%	4 9%		2 7%	4 15%	
ATA	67 37%	56 40%		38 33%	27 38%		18 47%	22 50%		11 39%	7 27%	
Total	**180**	**140**		**114**	**70**		**38**	**44**		**28**	**26**	

[a] χ^2 test
[b] Fisher's exact test

presence of bleeding on probing (54.61 ± 30.76), deep pockets (1.33 ± 0.29) and clinical attachment loss (1.00 ± 0.94) are important risk factors for adverse pregnancy outcomes, as the deep pocket can facilitate the entrance of periodontal pathogens exaggerating the immunoinflammatory responses that can compromise pregnancy [Santos-Pereira 2007]. The decreased mean value of pocket depth of 0.7262 (SD 0.423) in our population when compared to that reported by Santos-Pereira, *et al.*, may well explain differences with our results.

Radnai, *et al.*, (2006) also found an increased risk of having low birth weight and preterm birth in Caucasian pregnant women who had bleeding on probing of more than 50% of the teeth examined and had pocket depth of more than 4mm [28]. However, it should be emphasised that our study have used a different method for the assessment of low birth weight babies. The IBR < 5th percentile rather than a birth weight of less than 2.5Kg has been shown to be more accurate for assessing low birth weight babies. Nugent and Baker have pointed out that using birth weight for gestational age, < 10th centile or < 2.5kg at term, does not reliably detect babies who are growth restricted. In the present study, the individualised birth weight ratio (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

TABLE 4 Percentages of IL-10 alleles, genotypes and haplotypes at positions –1082, –819 and –592 in pregnant women with IUGR

	Total			Caucasian			Asians			Afro-Caribbeans		
	IBR < 5	IBR > 5	P value	IBR < 5	IBR > 5	P value	IBR < 5	IBR > 5	P value	IBR < 5	IBR > 5	P value
Position –1082												
GG	3 14%	8 6%	0.04[a]	3 25%	7 9%	0.07[b]	0 0%	1 3%	0.74[a]	0 0%	0 0%	0.002[b]
GA	12 55%	109 79%		6 50%	64 80%		5 83%	24 69%		1 25%	21 91%	
AA	7 32%	21 15%		3 25%	9 11%		1 17%	10 29%		3 75%	2 9%	
Total	**22**	**138**		**12**	**80**		**6**	**35**		**4**	**23**	
G	18 41%	125 45%	0.58[a]	12 50%	78 49%	0.9[a]	5 42%	26 37%	0.76[a]	1 12%	21 46%	0.07[b]
A	26 59%	151 55%		12 50%	82 51%		7 58%	44 63%		7 88%	25 54%	
Total	**44**	**276**		**24**	**160**		**12**	**70**		**8**	**46**	
Positions –819, –592												
CC/CC	4 18%	36 26%	0.54[b]	4 33%	23 29%	0.49[b]	0 0%	4 11%	0.48[b]	0 0%	9 39%	0.12[b]
CT/CA	18 82%	99 72%		8 67%	57 71%		6 18%	28 82%		4 22%	14 78%	
TT/AA	0 2%	3 2%		0 0%	0 0%		0 0%	3 9%		0 0%	0 0%	
Total	**22**	**138**		**12**	**80**		**6**	**35**		**4**	**23**	
–819*C/ –592*C	26 59%	171 62%	0.71[a]	16 66%	103 64%	0.82[a]	6 50%	36 51%	0.92[a]	4 50%	32 70%	0.27[b]
–819*T/ –592*A	18 41%	105 38%		8 34%	57 36%		6 50%	34 49%		4 50%	14 30%	
Total	**44**	**276**		**24**	**160**		**12**	**70**		**8**	**46**	
IL-10 haplotype												
GCC	18 41%	125 45%	0.86[a]	12 50%	78 49%	0.97[a]	5 42%	26 31%	0.84[b]	1 12%	21 46%	0.21[b]
ACC	8 18%	46 17%		4 17%	25 16%		1 6%	10 14%		3 38%	11 24%	
ATA	18 41%	105 38%		8 33%	57 36%		6 50%	34 49%		4 50%	14 30%	
T	**44**	**276**		**24**	**160**		**12**	**70**		**8**	**46**	

[a] χ^2 test
[b] Fisher's exact test

a percentage. A baby is growth restricted if its IBR is less than the 5th percentile [Sanderson, *et al.*, 1994]. Given this definition of IUGR, the differences between our findings and others could strongly attributed to the differences in method employed for IUGR assessment. Clearly, it is important that researchers take note of the advice offered by Nugent and Baker on defining IUGR when planning future studies [Nugent and Baker 2006]. It should also be emphasised that our study recruited pregnant women from an area of marked social deprivation with a higher rate of unemployment, a lower life expectancy, an increased rate of stillbirth, perinatal and childhood mortality when compared the rest of the country. Moreover, to limit the effects of extra variables, women with multiple pregnancy or with any maternal disease including urinary infection were excluded from the study. This may explain the disagreement between our findings when compared to those reported on Brazilian population by Santos-Pereira, *et al.*, [2007].

Interestingly, we found that the –1082 A/A genotype, which was observed more frequently in Afro-Caribbean women with increased percentages of BOP sites when compared with those with less bleeding sites (23% vs 14%, P = 0.069), was also significantly more frequent in women with IUGR (75% vs 9%, P = 0.002).

Previous work has shown that Placental AA genotype in the promoter region results in significantly less placental IL-10[30]. In addition, another investigation has shown that increased production of IL-10 in gestation could protect against preterm labour [Robertson, *et al.*, 2006]. Our

TABLE 5 Findings of IL-10 gene polymorphism at position –1082 in women with and without IUGR stratified by bleeding on probing sites

		IBR < 5	IBR > 5	P Value[a]	IBR < 5	IBR > 5	P Value[a]	IBR < 5	IBR > 5	P Value[a]	IBR < 5	IBR > 5	P Value[a]
		Total			**Caucasians**			**Asians**			**Afro Caribbeans**		
Bleeding sites <50%	GG	2 16.7%	7 8.8%	0.2	2 28.6%	7 14%	0.30	0 0%	0 0%	1.0	0 0%	0 0%	0.27
	GA	6 50%	60 75%		3 42.9%	36 72%		2 66.7%	11 68.8%		1 50%	11 91.7%	
	AA	4 33.3%	13 16.3%		2 28.6%	7 14%		1 33.3%	5 31.3%		1 50%	1 50%	
	Total	**12 100%**	**80 100%**		**7 100%**	**50 100%**		**3 100%**	**16 100%**		**2 100%**	**12 100%**	
Bleeding sites >50%	GG	1 10%	1 1.7%	P = 0.13	1 20%	0 0%	0.024	0 0%	1 5.3%	0.52	0 0%	0 0%	0.038[b]
	GA	6 60%	49 84.5%		3 60%	28 93.3%		3 100%	13 68.4%		0 0%	10 100%	
	AA	3 30%	8 13.8%		1 20%	2 6.7%		0 0%	5 26.3%		2 100%	1 9.1%	
	Total	**10 100%**	**58 100%**		**5 100%**	**30 100%**		**3 100%**	**19 100%**		**2 100%**	**11 100%**	

[a] Fisher's Exact Test.
[b] For non smoker AfroCaribbean women (p = 0.04).

findings are in agreement with other investigators who have reported that A/A which is associated with decreased production of IL-10 is a risk factor for many human diseases including gingivitis in Caucasian children [Dashash, *et al.*, 2005], Epstein Barr virus infection [Helminen, *et al.*, 1996], inflammatory bowel disease [Tagore, *et al.*, 1999], sporadic Alzheimer's disease [Lio, *et al.*, 2002] and chronic hepatitis [Abbas, *et al.*, 2003], and also with the work which reported that infants homozygous for the IL-10 high producer –1082 G allele might be at reduced risk for prematurity-associated disorders [Dordelmann *et al.*, 2006].

From our findings and others, it can be speculated that IL-10, which is an anti-inflammatory cytokine, is protective against IUGR and periodontal disease in Afro-Caribbean women. The A/A genotype which is associated with decreased production of IL-10, was more frequent in Afro Caribbean women, with IUGR and periodontal disease. In fact, differences in the genetic predisposition to intrauterine growth restriction, among different ethnic groups, have been reported [Nguyen, *et al.*, 2004]. A significant difference was observed between Caucasians and African Americans in the cytokine responses of the placental membrane [Menon, *et al.*, 2006]. It has been noted that lipopolysaccharide stimulation resulted in increased presence of IL-10 in Caucasians but not in Afro Caribbeans. In the same investigation, it was also observed that Caucasians, during pregnancy, show more balance in inflammatory responses when compared with Afro-American [Menon, *et al.*, 2006]. However, it should be emphasised that the sample size of Afro Caribbean women in our study was small and the results should be interpreted with caution.

To confirm the gene-environment interaction between IL-10 gene polymorphism and periodontal disease, we investigated whether the combination between the –1082 A genotype and increased percentages of bleeding sites could increase the risk of IUGR in this group of population. we have found that gene-environment interaction could play a role in Afro Caribbean women with IUGR as the carriage of the –1082 A genotype with increased percentages of bleeding sites have increased the risk of IUGR. Moreover, non smokers, A allele homozygotes Afro Caribbean women with increased percentages of bleeding sites were also significantly more susceptible to have IUGR (P = 0.04). However, it should be noted that the number of this group is small (n = 12) and larger samples size is necessary to confirm these interesting findings.

Although this pilot study is limited by the small size, our findings show that the IL-10 gene polymorphisms could have an influence on IUGR in Caucasian pregnant women and on periodontal disease and IUGR in Afro Caribbean women, and explain the variant prevalence of IUGR among racial groups. Our research group is the first to investigate the association between periodontal disease, IUGR and gene polymorphisms of anti-inflammatory cytokine. We have previously found that the combination between –174 CC genotype, which is associated with decreased production of IL-6 and increased bleeding sites (>50%) have increased the risk of IUGR (P = 0.006). We also found that increased production of IL-6, which is an anti-inflammatory cytokine, could be risk factor for periodontitis but

is a protective marker against IUGR [Dashash, *et al.*, 2009]. Previous studies have investigated the possible association of gene polymorphisms of pro-inflammatory cytokines with preterm delivery and periodontal disease [Offenbacher, *et al.*, 1996, Jeffcoat, *et al.*, 2003, Louro, *et al.*, 2001, Lopez, *et al.*, 2002, Hasegawa, *et al.*, 2003, Dasanayake, *et al.*, 2003, Moore, *et al.*, 2004]. For instance, Moore, *et al.*, (2004) have investigated the role of gene polymorphisms of pro-inflammatory cytokines including IL-1β +3953 and TNF-308 in periodontal disease and preterm birth. An association between preterm birth and the carriage of variant TNF-308 allele was found in non smoker women [Moore, *et al.*, 2004]. However, the authors concluded, in contrast to our findings on –1082 IL-10, that the combination between the variant genotype and severe periodontal disease did not increase the risk of having preterm birth.

To date, IL-10 has been used for preventing cytokine-induced preterm uterine contractions in cases with under-production of IL-10 [Mitchell, *et al.*, 2004]. Similarly, the administration of IL-10 together with dexamethasone has been found to reduce the prostaglandin production and to reduce TNFα and IL-1β induced uterine contractility [Sadowsky, *et al.*, 2003]. If the role of IL-10 against periodontal disease and IUGR is well established, then a therapeutic application of IL-10 could be considered for the management of IUGR in Afro Caribbean women.

A prospective study, which assesses whether improved periodontal health is associated with improved pregnancy outcomes should be undertaken.

In Conclusion, this pilot study has shown that the IL-10 gene polymorphisms could have an influence on IUGR and periodontal disease in Afro Caribbean women. However, studies with a larger sample size, are required to ascertain the genetic association between IL-10 gene polymorphisms, maternal periodontal disease and IUGR in different ethnic groups.

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