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Association of *CD14*, *IL1B*, *IL6*, *IL10* and *TNFA* functional gene polymorphisms with symptomatic dental abscesses

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Abstract

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Aim To investigate in individuals with symptomatic dental abscesses the occurrence of functional polymorphisms within five genes involved with the immune response. The functional gene polymorphisms analysed were *CD14* (−260 C/T), *IL1B* (+3954 C/T), *IL6* (−174 G/C,), *IL10* (−1082 G/A) and *TNFA* (−308 G/A).

Methodology Genomic DNA obtained from oral swabs from individuals with symptomatic dental abscesses and asymptomatic inflammatory periapical lesions, without previous exacerbation, was submitted to restriction fragment length polymorphism (RFLP) analyses to determine each individual genotype. The chi-square and principal components analysis tests were used for statistical analysis.

Results A significant association was observed between the occurrence of the GG genotype or the G allele expression of the polymorphic *locus* −174 (G/C) of the *IL6* gene, and the presence of the symptomatic dental abscesses in women and in individuals ≤35 years old. The principal components analysis suggested predominance of the symptomatic dental abscesses in individuals displaying: high-producer *IL6* genotype; intermediate and high-producer *IL1B* genotypes and low-producer *TNFA* genotype.

Conclusions The present study suggests that genetic factors are associated with susceptibility to develop symptomatic dental abscesses.

Keywords: cytokines, molecular biology, odontogenic abscess, polymorphism, receptor CD14.

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Introduction

Persistent injuries to the dental pulp usually cause irreversible pulpitis and pulpal necrosis (Torabinejad 1994). Irritants may be mechanical or chemical, but are most often bacterial. The interaction between these irritants and host defensive cells results in the release of numerous mediators that, through the root canal system, are capable of initiating immunologic reactions in periapical tissues that result in the formation of inflammatory periapical lesions. These reactions include immune response mediated by cells, through the actions of T lymphocytes and cytokines, and humoral immune response mediated by antibodies, activated by B-lymphocyte products (Takahashi 1998).

Cytokines play a major role in the inflammatory and immune responses, several of them displaying inflammatory and others anti-inflammatory properties.

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Interleukin (IL)-1, IL-6 and tumor necrosis factor-alpha (TNF-α) are examples of inflammatory cytokines. IL-1 plays a pivotal role in several chronic diseases and is also known to be one of the most active stimulators of osteoclastic bone reabsorption (Lang *et al.* 2000). IL-6, another cytokine important in bone reabsorption, acts in both innate and adaptive immune responses and its main functions involve the stimulation of haematopoiesis and the production of acute-phase proteins by hepatocyte and B-lymphocyte growth (Emery & Salmon 1991, Kishimoto *et al.* 1992). TNF-α is a potent immunologic mediator of acute and chronic inflammatory responses (Birkedal-Hansen 1993) and has the capacity to increase bone resorption (Manolagas 1995). On the other hand, IL-10 is a pleiotropic cytokine with strong anti-inflammatory properties, regulating B-cell proliferation and differentiation and exhibiting immunoregulatory activities, such as expansion of IL-4-producing T cells (Volk *et al.* 2001). The balance between inflammatory and anti-inflammatory cytokine expression is crucial in the maintenance of health and the expression of these mediators is often associated with the activation of cell surface markers. The receptor CD14 is involved in lipopolysaccharide (LPS) signal transduction and therefore is essential both for interfacing the innate immune system with microbial pathogens and for directing adaptive immune responses along specific T helper differentiation pathways though induction of cytokine expression (Medzhitov & Janeway 2000).

It is now recognized that cytokine and cell receptor production varies widely among individuals. These inter-individual differences can be explained at least partially by the presence of genetic polymorphisms within many of those genes (Di Giovine *et al.* 1992, McDowell *et al.* 1995, Turner *et al.* 1997). Some forms of variation in the genetic code may result in either altered expression or in functional changes of the encoded molecules, therefore perhaps making individuals with aberrant genotypes more susceptible to a given disease, or resulting in an increase of disease severity (Craandijk *et al.* 2002).

A polymorphism of the *IL1B* gene at +3954 (C/T) was found to result in an increased production of this cytokine. Homozygous individuals for the T allele produce a four-fold higher amount of IL-1β compared with individuals displaying the CC genotype (Pociot *et al.* 1992). A single nucleotide polymorphism in the promoter of the *IL6* gene −174 (G/C) has recently been described, showing that the allele C entails reduced transcription compared with the −174G allele (Fishman *et al.* 1998). In the *IL10* gene, the low production of IL-10 *ex vivo* was significantly associated with the presence of allele A at position −1082 (Turner *et al.* 1997). Furthermore, in the *TNFA* gene, a polymorphism at position −308 (G/A) was associated with altered TNF-α production; the allele A having six to seven-fold more transcriptional activity in a gene report assay than the G variant (Wilson *et al.* 1997). The polymorphic allele T of the polymorphism −260 (C/T), in the promoter of the *CD14* LPS receptor gene, is reported to show increased expression of CD14 (Ito *et al.* 2000).

Considering that many studies have shown an association between polymorphisms in cytokine and cell receptor genes and the development of several inflammatory diseases and that gene polymorphisms are important determinants of disease risk or severity for a number of conditions in which the immune system plays a pivotal role, the aim of our study was to investigate the possible occurrence of the functional polymorphisms within five genes involved with the immune response, in a sample of Brazilian individuals with symptomatic periapical abscess. The functional gene polymorphisms analysed were *CD14* (−260 C/T), *IL1B* (+3954 C/T), *IL6* (−174 G/C), *IL10* (−1082 G/A) and *TNFA* (−308 G/A).

Material and methods

Subjects and sample collection

The study employed a cross-sectional design involving individuals from the State of Minas Gerais in the southeastern region of Brazil. A total of 98 patients were recruited from the Endodontics and Surgery Clinics at the Universidade Federal de Minas Gerais and included in the study. A questionnaire was given to all individuals enrolled in this study, in order to obtain information regarding general health concerns, history of diabetes, hepatitis or HIV infection, immunosupressive chemotherapy, bleeding disorders, severely compromised immune function, that were considered exclusion criteria.

On the basis of their clinical presentations, the patients were stratified into two groups: 45 patients with symptomatic dental abscesses (case group) and 53 patients with asymptomatic inflammatory periapical lesions, without previous exacerbation, age and sex matched (control group). Patients in the case group exhibited symptomatic dental abscesses, as the result of infection of endodontic origin, with swelling of the tissues, with or without systemic manifestations, such

as fever, malaise, headache and lymphadenopathy. Patients in the control group exhibited pulpal necrosis, and apical radiolucencies, suggesting chronic periapical pathosis. They were asymptomatic at the time of sample collection and had no history of a previous exacerbation. Presence of pus or a sinus tract in intra oral or extra oral sites was regarded as exclusion criteria. The patients of both case and control groups were from the same geographic area and had identical socioeconomic status. Ethnicity was not established, as the hazards of judging Brazilians by colour, race and geographical origin was recently demonstrated (Parra *et al.* 2003). This study was approved by the University's Ethics Committee (n°235/04) and a signed informed consent was obtained from all participants.

Oral mucosal swabs were taken once from the subjects. The swabs were performed with sterile plastic tips, placed immediately in Eppendorf microtubes containing 500 μL of Krebs buffer, and the pellet obtained after 5 min of centrifugation at 13 000 *g* was stored at −20 °C until processing.

DNA isolation

The extraction of DNA was carried out as described by Boom *et al.* (1990) and modified as below. A 450 μL volume of lyses buffer (6.0 mol L^{-1} GuSCN, 65 mmol L^{-1} Tris HCl pH 6.4, 25 mmol L^{-1} EDTA, 1.5% TritonX-100) and 20 μL silica (SiO_2, Sigma S-5631, St. Louis, MO, USA) was added to a microcentrifuge tube containing the oral mucosa swab pellet. The tube was vortexed and incubated for 10 min at 56 °C, centrifuged at 3000 *g* for 1 min and the supernatant discharged. The pellet obtained (DNA adsorbed to the SiO_2) was washed twice with 450 μL washing buffer (6.0 mol L^{-1} GuSCN, 65 mmol L^{-1} Tris HCl), twice with 70% ethanol, once with 450 μL acetone and dried at 56 °C for 10 min. Finally, 100 μL of TE buffer (10 mmol L^{-1} Tris-HCl pH 8.0, 1 mmol L^{-1} EDTA) was added and incubated at 56 °C for 10 min to elute the DNA. After incubation the solution was homogenized and centrifuged at 5000 *g* for 2 min and the supernatant containing DNA transferred to a new tube.

Genotyping

Polymorphisms were assessed by polymerase chain reaction (PCR) amplification and digestion. The sequences of PCR primers with expected PCR product size are shown in Table 1. The PCR was carried out in a total volume of 50 μL, containing 10 μL of solution DNA (approximately 400 ng), primers (20 pmol per reaction), 25 μL premix buffer (50 mmol L^{-1} KCl, 10 mmol L^{-1} Tris-HCl pH 8.4, 0.1% Triton X-100, 1.5 mmol L^{-1} $MgCl_2$, deoxynucleoside triphosphates and 1.25 units of Taq DNA polymerase) (Phoneutria Biotecnologia, Belo Horizonte, Brazil). The amplification conditions consisted of 94 °C for 3 min followed by 40 cycles of 94 °C for 30 s, 54 °C for 35 s and 72 °C for 30 s. The run was terminated by final elongation at 72 °C for 5 min. In all steps the lid temperature was 103°. The products were digested with restriction enzyme according to manufacturer protocols (see Table 1). The visualization of the products was

Table 1 Primers sequences, restriction enzymes and genotypes for each polymorphism

Genes	Primers	Digestion (conditions)	Genotypes
CD14 −260 (C/T)	5′-CTAAGGCACTGAGGATCATCC3-′ 5′-CATGGTCGATAAGTCTTCCG-3′ (Ito *et al.* 2000)	HaeIII (37 °C/3 h)	TT = 418 bp CT = 418 + 263 + 155 bp CC = 263 + 155 bp
IL1B +3954(C/T)	5′-CTCAGGTGTCCTCGAAGAAATCAAA-3′ 5′-GCTTTTTTGCTGTGAGTCCCG-3′ (Kornman *et al.* 1997)	TaqI[a] (65 °C/4 h)	TT = 182 + 12 bp CT = 182 + 97 + 85 + 12 bp CC = 97 + 85 + 12 bp
IL6 −174(G/C)	5′-CAGAAGAACTCAGATGACCTG-3′ 5′-GTGGGGCTGATTGGAAACC-3′ (Klein *et al.* 2001)	HSP92II[a] (37 °C/8 h)	CC = 229 + 122 + 51 + 29 bp GC = 229 + 173 + 122 + 51 + 29 bp GG = 229 + 173 + 29 bp
IL10 −1082(G/A)	5′-CCAAGACAACACTACTAAGGCTCCTTT-3′ 5′-GCTTCTTATATGCTAGTCAGGTA-3′ (Koch *et al.* 2001)	XagI[b] (37 °C/4 h)	AA = 280 + 97 bp GA = 280 + 253 + 97 + 27 bp GG = 253 + 97 + 27 bp
TNFA −308(G/A)	5′-AGGCAATAGGTTTTGAGGGCCAT-3′ 5′-TCCTCCCTGCTCCGATTCCG-3′ (Wilson *et al.* 1993)	NcoI[a] (37 °C/12 h)	AA = 107 bp GA = 107 + 87 + 20 bp GG = 87 + 20 bp

[a]Promega, Madison, WI, USA.
[b]MBI Fermentas, Bath, UK.

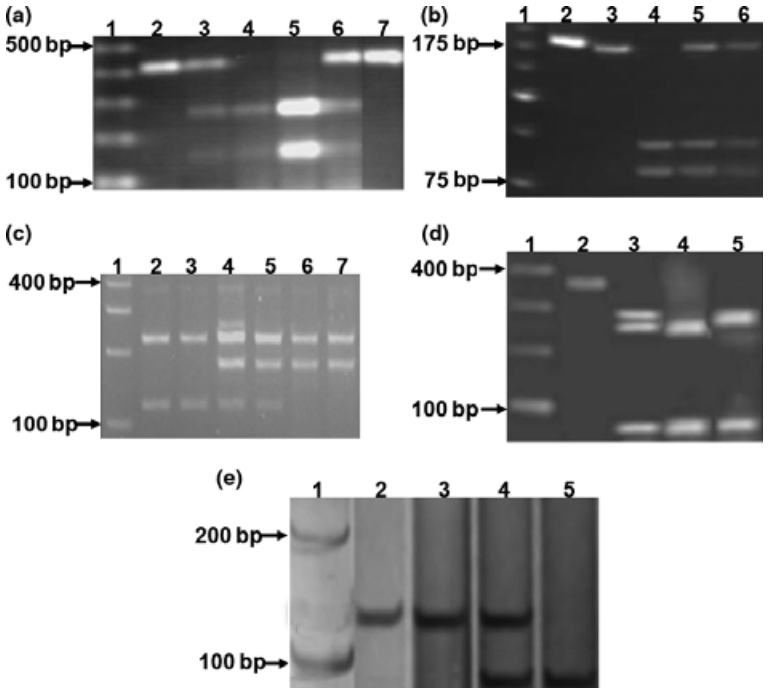

Figure 1 (a) 2% agarose gel electrophoresis for detection of CD14 (−260 C/T) polymorphism. Base pair ladder (100 bp) (lane 1); PCR product without digestion = 418 bp (lane 2); genotype CT = 418 + 263 + 155 bp (lanes 3,6); genotype CC = 263 + 155 bp (lanes 4,5); genotype TT = 418 bp (lane 7). (b) 10% polyacrylamide gel electrophoresis for detection of IL1B polymorphism (+3954 C/T). Base pair ladder (25 bp) (lane 1); PCR product without digestion = 194 bp (lane 2); genotype TT = 182 bp (lane 3); genotype CC = 97 + 85 bp (lane 4); genotype CT = 182 + 97 + 85 bp (lanes 5,6). (c) 2% agarose gel electrophoresis for detection of IL6 (−174 G/C) polymorphism. Base pair ladder (100 bp) (lane 1); genotype CC = 229 + 122 bp (lanes 2,3); genotype GC = 229 + 173 + 122 bp (lanes 4,5); genotype GG = 229 + 173 bp (lanes 6,7). (d) 2% agarose gel electrophoresis for detection of IL10 (−1082 G/A) polymorphism. Base pair ladder (100 bp) (lane 1); PCR product without digestion = 377 bp (lane 2); genotype GA = 280 + 253 + 97 bp (lane 3); genotype GG = 253 + 97 bp (lane 4); genotype AA = 280 + 97 bp (lane 5). (e) 10% polyacrylamide gel electrophoresis for detection of TNFA (−308 G/A) polymorphism. Base pair ladder (100 bp) (lane 1); PCR product without digestion = 107 bp (lane 2); genotype AA = 107 bp (lane 3); genotype G/A = 107 + 87 bp (lane 4); genotype GG = 87 bp (lane 5).

performed in a 2% agarose gel eletrophoresis (for analysis of the *CD14*, *IL6* and *IL10*), 18 × 16 cm 10% polyacrylamide gel eletrophoresis (for analysis of *IL1B*), both of them stained with ethidium bromide (0.5 μg mL^{-1}) and 10 × 7 cm 10% polyacrylamide gel eletrophoresis (for the analysis of *TNFA*) with silver staining. Individuals with known genotypes and exclusion of the restriction enzyme were used as controls (Fig. 1).

Statistical analysis

Statistical significance of differences between case and control group distributions for alleles and genotypes were determined using chi-square test. A significant level of $P \geq 0.05$ was used. The observed genotype frequencies were compared with those calculated from Hardy–Weinberg equilibrium theory. These statistical analyses were performed using BioStat 3.0 software (Optical Digital Optical Technology, Belém, Brazil). As the medians in both groups were close to 35 years, this value was considered to stratify the groups according to age (> or ≤35 years old).

The principal components analysis was used in this study on the multivariate analysis. This test is a descriptive analysis of variables displayed in a graphic in three axes (F1, F2 and F3). Each group has a specific score according to the clinic pattern and each genotype has a specific score according to the rate of transcription. For example, score 1 was used for case group and score 0 for control group. Low producer genotype was scored 1, moderate 2 and high producer 3. The

INFOSTAT software (Infostat Group, Cordoba, Argentina) was used for the analysis.

Results

The distribution of genotype and allele frequencies of all polymorphisms in patients with symptomatic dental abscesses (case group) and asymptomatic inflammatory periapical lesions, without previous exacerbation (control group) are shown in Tables 2 and 3. Significant statistical differences were not observed in any of the evaluated polymorphisms between the individuals from both groups. However, a significant association was observed between GG genotype and G allele of the polymorphism in the *locus* −174 (G/C) of the *IL6* gene, in women and in individuals ≤35 years old with presence of the symptomatic odontogenic abscess.

The data obtained from the principal components analysis are outlined in Fig. 2. The euclidean distance (distance between two points) and the position on the quadrants on the graphic depict the relation between variables. Variables situated in the same quadrant and close to each other are strongly associated; while variables situated in the opposite quadrants are inversely associated. Variables distant from each other situated on the same axis show a weak association. The principal components analysis showed a strong positive correlation between *IL6* high producer genotype and patients with symptomatic odontogenic abscess (close to each other). Although a positive correlation between *IL1B* high producer and intermediate producer genotype and patients with symptomatic odontogenic abscess was observed, it was not as strong as the former (situated in the same quadrant but distant from each other). A strong inverse correlation was detected between *TNFA* and patients with symptomatic odontogenic abscess (situated in the opposite quadrants).

Discussion

Although polymorphisms associated with immune responses are widely investigated in inflammatory conditions, there is no reports in the literature about the possible influence of these genetic variations in the formation of symptomatic dental abscesses.

Considering the univariate analysis, the results demonstrated association between the GG genotype and the occurrence of the G allele of the *IL6* −174 (G/C) polymorphism and the manifestation of symptomatic dental abscesses. The GC and CC genotypes and the C allele were also related to a reduced risk of acute exacerbation in women and in patients under 35 years of age. There is no epidemiologic evidence demonstrating these findings. Although the samples were collected at the same institution, geographic location and socio-economic status, the possibility of some unidentified bias should not be ruled out. Further studies with different communities should be performed in order to confirm or refute such evidence.

The multivariate analysis showed a strong positive correlation between variables *IL6* and the case group, which suggests the predominance of patients with symptomatic dental abscesses expressing the genotype related to the high rate of *IL6* transcription. Although speculation about the functional dynamics of the inflammatory process in question is not possible, there may be an association between the GG genotype and susceptibility to the manifestation of symptomatic dental abscess. It could also be speculated that there is a possible association of other important genes related to the inflammatory response, which can be in linkage disequilibrium with *IL6*, and susceptibility to the manifestation of symptomatic dental abscesses.

It is worth remembering that the G allele is associated with high levels of IL-6 production, when compared with the C allele (Fishman *et al.* 1998). Considering that IL-6 is an important cytokine of both innate and adaptive immune response (Feyen *et al.* 1989, Löwik *et al.* 1989, Ishimi *et al.* 1990, Kurihara *et al.* 1990), the increase of its production may cause major osteoclastic bone destruction and stimulation of the synthesis of acute phase proteins by hepatocytes, which contribute to the systemic effects of the inflammation (Crowl *et al.* 1991). These aspects are important characteristics in the evolution of the symptomatic dental abscesses.

Although dental abscesses are a multifactorial inflammatory condition, it was expected that patients with symptomatic dental abscesses, could have high frequencies of both the polymorphic variants associated with the high production of CD14, IL-1β e TNF-α (pro-inflammatory mediators), and the polymorphic variant associated with the low production of IL-10 (immunoregulated cytokine). However, no relation between the functional polymorphisms in the genes *CD14* (−260 C/T), *IL1B* (+3954 C/T), *IL10* (−1082 G/A) and *TNFA* (−308 G/A) and the development of dental abscesses was found in the univariate analysis, even after stratification of the individuals by sex and age.

In the multivariate analysis of the Principal Components regarding the *IL1B* +3954 (C/T) polymorphisms, a positive correlation was found between the variables

Table 2 Distribution of the genotypes in patients with symptomatic dental abscesses (case group) and with asymptomatic inflammatory periapical lesions, without previous exacerbation (control group), using chi-squared test

	Total			Males			Females			Age ≤35 years			Age > 35 years		
Genotypes	Case ($n = 45$)	Control ($n = 53$)	*P*-value	Case ($n = 21$)	Control ($n = 27$)	*P*-value	Case ($n = 24$)	Control ($n = 26$)	*P*-value	Case ($n = 26$)	Control ($n = 28$)	*P*-value	Case ($n = 19$)	Control ($n = 25$)	*P*-value
CD14 −260 (C/T)															
CC *n* (%)	9 (20)	10 (18.9)	0.92	5 (23.8)	4 (14.8)	0.64	4 (16.7)	6 (23.1)	0.52	5 (19.2)	4 (14.3)	0.88	4 (21.1)	6 (24)	0.82
CT *n* (%)	22 (48.9)	28 (52.8)		11 (52.4)	14 (51.8)		11 (45.8)	14 (53.8)		14 (53.9)	16 (57.1)		8 (42.1)	12 (48)	
TT *n* (%)	14 (31.1)	15 (28.3)		5 (23.8)	9 (33.4)		9 (37.5)	6 (23.1)		7 (26.9)	8 (28.6)		7 (36.8)	7 (28)	
IL1B +3954 (C/T)															
CC *n* (%)	25 (55.6)	33 (62.2)	0.47	10 (47.6)	15 (55.6)	0.53	15 (62.5)	18 (69.2)	0.83	15 (57.7)	16 (57.1)	0.61	10 (52.6)	17 (68)	0.46
CT *n* (%)	20 (44.4)	19 (35.9)		11 (52.4)	11 (40.7)		9 (37.5)	8 (30.8)		11 (42.3)	11 (39.3)		9 (47.4)	8 (32)	
TT *n* (%)	0	1 (1.9)		0	1 (3.7)		0	0		0	1 (3.6)		0	0	
IL6 −174 (G/C)															
CC *n* (%)	3 (6.7)	4 (7.6)	0.15	3 (14.3)	2 (7.4)	0.71	0	2 (7.7)	0.005*	1 (3.8)	2 (7.1)	0.04*	2 (10.5)	2 (8)	0.94
GC *n* (%)	13 (28.9)	25 (47.2)		8 (38.1)	10 (37)		5 (20.8)	15 (57.7)		6 (23.1)	15 (53.6)		7 (36.9)	10 (40)	
GG *n* (%)	29 (64.4)	24 (45.3)		10 (47.6)	15 (55.6)		19 (79.2)	9 (34.6)		19 (73.1)	11 (39.3)		10 (52.6)	13 (52)	
IL10 −1082 (G/A)															
AA *n* (%)	26 (57.8)	28 (52.8)	0.78	12 (57.2)	15 (55.6)	0.98	14 (58.3)	13 (50)	0.59	16 (61.5)	16 (57.2)	0.91	10 (52.6)	12 (48)	0.87
GA *n* (%)	14 (31.1)	20 (37.8)		7 (33.3)	9 (33.3)		7 (29.2)	11 (42.3)		7 (26.9)	9 (32.1)		7 (36.9)	11 (44)	
GG *n* (%)	5 (11.1)	5 (9.4)		2 (9.5)	3 (11.1)		3 (12.5)	2 (7.7)		3 (11.6)	3 (10.7)		2 (10.5)	2 (8)	
TNFA −308 (G/A)															
GG *n* (%)	29 (64.4)	40 (75.5)	0.26	12 (57.1)	21 (77.8)	0.14	17 (70.8)	19 (73.1)	0.88	17 (65.4)	24 (85.7)	0.15	12 (63.2)	16 (64)	0.65
GA *n* (%)	16 (35.6)	12 (22.6)		9 (42.9)	5 (18.5)		7 (29.2)	7 (26.9)		9 (34.6)	4 (14.3)		7 (36.8)	8 (32)	
AA *n* (%)	0	1 (1.9)		0	1 (3.7)		0	0		0	0		0	1 (4)	

*$P \leq 0.05$ was used.

Table 3 Distribution of the alleles in patients with symptomatic dental abscesses (case group) and with asymptomatic inflammatory periapical lesions, without previous exacerbation (control group), using chi-squared test

	Total			Males			Females			Age ≤35 years			Age > 35 years		
Alleles	Case (%)	Control ($n = 106$)	*P*-value	Case ($n = 42$)	Control ($n = 54$)	*P*-value	Case ($n = 48$)	Control ($n = 52$)	*P*-value	Case ($n = 52$)	Control ($n = 56$)	*P*-value	Case ($n = 38$)	Control ($n = 50$)	*P*-value
CD14 −260(C/T)															
C *n*(%)	44.4	45.3	0.97	50	40.7	0.48	39.6	50	0.39	46.1	42.8	0.88	42.1	48	0.73
T *n*(%)	55.6	54.7		50	59.3		60.4	50		53.9	57.2		57.9	52	
IL1B +3954(C/T)															
C *n*(%)	77.8	80.2	0.81	73.8	75.9	1	81.3	84.6	0.85	78.8	76.8	0.97	76.3	84	0.52
T *n*(%)	22.2	19.8		26.2	24.1		18.7	15.4		21.2	23.2		23.7	16	
IL6 −174(G/C)															
C *n*(%)	21.1	31.1	0.15	33.3	25.9	0.57	10.4	36.5	0.004*	15.4	33.9	0.04*	28.9	28	0.88
G *n*(%)	78.9	68.9		66.7	74.1		89.6	63.5		84.6	66.1		71.1	72	
IL10 −1082(G/A)															
A *n*(%)	73.3	71.7	0.92	73.8	72.2	0.95	72.9	71.2	0.97	75	73.2	0.99	71.1	70	0.89
G *n*(%)	26.7	28.3		26.2	27.8		27.1	28.8		25	26.8		28.9	30	
TNFA −308(G/A)															
G *n*(%)	82.2	86.8	0.49	78.6	87	0.4	85.4	86.5	0.89	82.7	92.9	0.18	81.6	80	0.93
A *n*(%)	17.8	13.2		21.4	13		14.6	13.5		17.3	7.1		18.4	20	

*$P \leq 0.05$ was used.

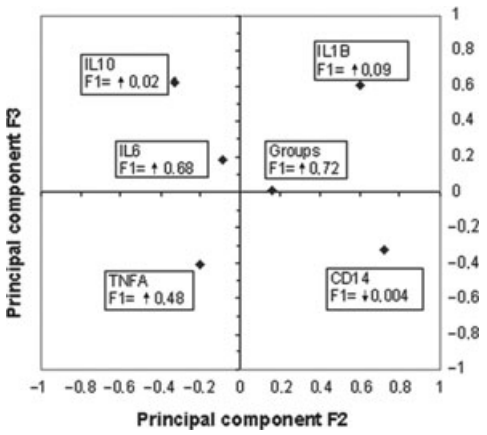

Figure 2 Position of the variables according to axis 2 and 3 and their respective co-ordinates in axis 1. Arrows: ↑ if positive and ↓ if negative in F1 axis. Note a positive correlation between *IL6* high producer genotype and patients with symptomatic odontogenic abscess (the IL-6 variable is the closest point in the graphic to the group variable).

IL1B and case groups, although to a lesser extent as the one observed with *IL6*. Such a result suggests the predominance of individuals with symptomatic dental abscesses expressing both the CT and the TT *IL1B* genotypes, which would relate the susceptibility to the manifestation of symptomatic dental abscesses. The polymorphic variant T presents as functional alteration with an augmentation in the production of IL-1β, where homozygous individuals for the T allele produce four-fold more IL-1β and heterozygous individuals produce approximately two-fold more IL-1β than homozygous individuals for C allele (Pociot *et al.* 1992).

An inverse correlation between *TNFA* genotype and patients with symptomatic dental abscesses was observed by the principal components analysis. These data suggest a predominance of patients with symptomatic dental abscesses, expressing genotype GG associated with low rate of transcription of the *TNFA*. Also, it indicates a predominance of patients with asymptomatic inflammatory periapical lesions, without previous exacerbation, expressing genotype AA associated with the high rate of transcription of the same gene. Based on these results, it could be speculated that patients who carry the AA genotype may express a protector phenotype against the disease. Such data also suggest that, at the same time that the TNF-α may not be relevant to symptomatic dental abscesses pathogenesis, it may also play an important role during chronic tissue inflammation, or still be in linkage disequilibrium with some gene involved in the immunomodulatory mechanisms which could explain the limited evolution of the inflammatory process existent in asymptomatic inflammatory periapical lesions.

Previous studies have evaluated the functional gene polymorphisms for *IL1B* (+3954 C/T), *IL6* (−174 G/C), *IL10* (−1082 G/A) and *TNFA* (−308 G/A) in the Brazilian population and the results observed in those studies were similar to the ones obtained by evaluating the present group of patients (Moraes *et al.* 2003, Albuquerque *et al.* 2004, Scarel-Caminaga *et al.* 2004, Moreira *et al.* 2005, 2006, Guimarães *et al.* 2006a,b). The present study demonstrates, for the first time, the evaluation of the functional polymorphism in *CD14* (−260 C/T) gene in the Brazilian population. These data are similar to the data obtained from the analysis of this gene in Czech, German and Japanese populations (Shimada *et al.* 2000, Holla *et al.* 2002, Koenig *et al.* 2002). Although polymorphisms associated with cytokine production are important to understand the development of inflammatory conditions, there is no information in the literature addressing the occurrence of cytokine gene polymorphisms in patients with periapical lesions of endodontic origin.

Conclusions

In conclusion, the present study indicates that genetic factors can influence the development of symptomatic dental abscesses.

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