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ORIGINAL ARTICLE

Genetic polymorphisms in *interleukin-6* and *interleukin-1-beta* were associated with dental caries and gingivitis

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ABSTRACT

Objective: Evaluate the association between single nucleotide polymorphisms (SNPs) in *Interleukin-6* (*IL-6*) gene (rs1800795) and in *Interleukin-1-beta* (*IL-1β*) gene (rs1143627 and rs1143629) with dental caries and gingivitis in Brazilian children.

Material and methods: Three hundred and fifty-three children aged 8–11 years were included. Visible biofilm and gingival bleeding were evaluated by Community Periodontal Index. The International System for Detection and Assessment of Carious Lesions (ICDAS) was used to investigate dental caries. Real-time PCR evaluated SNPs in the DNA. Chi-square test, haplotype analysis and logistic regression were applied (alpha of 5%).

Results: The GG genotype in rs1800795 (*IL-6*) decreases the risk of gingivitis in a co-dominant model ($p = .05$; OR = 0.64). The GG genotype in rs1143627 (*IL-1β*) reduces the risk of dental caries (Co-dominant model: $ICDAS_0$ versus $ICDAS_{1-6}$: $p = .05$; OR = 0.55. $ICDAS_{0-2}$ versus $ICDAS_{3-6}$: $p = .02$; OR = 0.49. Recessive model: $ICDAS_0$ versus $ICDAS_{1-6}$: $p = .005$; OR = 0.48. $ICDAS_{0-2}$ versus $ICDAS_{3-6}$: $p = .004$; OR = 0.45. Logistic regression: $ICDAS_{0-2}$ versus $ICDAS_{3-6}$: $p = .05$; OR = 0.24; CI 95%= 0.05–1.00). The GG genotype in rs1143629 was more frequent in $ICDAS_0$ ($p = .05$; OR: 0.60). In the haplotype analysis, *IL-1β* was associated with gingivitis.

Conclusion: The rs1800795 in *IL-6* gene was associated with gingivitis. The rs1143627 and rs1143629 in *IL-1β* were associated with dental caries and gingivitis.

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Introduction

Dental caries and gingivitis are multifactorial diseases that affect millions of people worldwide [1]. In the case of dental caries, the main causal agent appears to be the imbalance between remineralization and demineralization of the tooth tissues stimulate by bacterial metabolism, associated with low salivary flow, high sugar consumption and social conditions [2]. During the onset of the disease, there is an increase of local inflammatory mediators, such as cytokines, in response to bacteria and injuries from carious lesions. These proteins start the inflammatory process and activate the production of the antimicrobial peptides which protected the tooth and surrounding bone from polymicrobial infection [3–5]. Gingivitis, which is a mildest form of periodontal disease, is described as a reversible inflammation of the gingiva in an attempt to protect the periodontium against the invading bacteria. In gingivitis, cytokines are secreted in subgingival region due to the factors suffered by periodontal tissue and also by bacterial infection [6,7]. Cytokines are mediators secreted mainly for leukocytes and macrophages as to inflammatory and immune response to bacterial infections and successive trauma to the tissue. These mediators are divided in inflammatory cytokines: tumour necrosis factor alpha (TNF-α), interferon (IFN) and interleukins (IL) IL-2, IL-6, IL-8 and IL-1β (that intensify the inflammatory process); and anti-inflammatory cytokines: IL-4, IL-10 and IL-13 (that control de inflammatory process). IL-6 and IL-1β are one of the major proinflammatory interleukins secreted in dental caries and gingivitis [3–7].

Once cytokines are involved in host response to dental caries and gingivitis, and are potential biomarkers for the risk of both conditions [8–13], Single Nucleotide Polymorphisms (SNPs) in cytokine-encoding genes may be candidates to be studied in both conditions. In fact, the SNPs in *IL-6* and *IL-1β* genes has been associated with gingivitis susceptibility in Italian adults [14], Czech children [15], and England adults [16]. The rs1800795 in *IL-6* gene was associated as a protective factor for gingivitis in Italian adults [8]. Besides gingivitis, periodontal disease [9–12,17–19] and dental caries [12,13] were also associated with SNPs in *IL-6* and *IL-1β* genes, although the results were contradictory. Therefore, the aim of this study was to investigate the association between SNPs select from *IL-6* and *IL-1β* with dental caries and gingivitis susceptibility in a sample of children from southeastern Brazil.

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Methods

The guidelines STREGA (Strengthening the Reporting of Genetic Association) was followed for this study [20].

Ethics statement

The Human Ethics Committee of Federal University of Alfenas, Minas Gerais, Brazil, approved this study (protocol #: 78568217.7.0000.5142). Children were included after informed written consent from the parents and assent from the children, in an age-appropriate document were signed.

Study population

Children included in the sample were enrolled in public schools in the city of Alfenas, Minas Gerais, Brazil. The city is located in the southeast of the country and has 79,996 inhabitants of European and African ancestry, mostly The Human Development Index of Alfenas is 0.761 and public water supply has controlled fluoride adjustment [21]

Sample size calculation was performed with a power of 0.80% and alpha of 0.05. Based on the genotypes' differences of 15% between groups according to the finds in a previous study [13], the calculation predicted a minimum of 276 children (http://clincalc.com). The total number of children selected was 353, which was the sum of all children enrolled in four schools. This sample was previously described in Barbosa et al. [21].

Anamnesis and clinical examination

A single trained and calibrated dentist (kappa intra-examiner= 0.87) evaluated dental caries, biofilm and bleeding on probe. The dentist performed the recruitment of children, both genders, aged ranged between 8 and 11 years-old and biologically unrelated, in the first semester of 2018 during the school year in schools from the distinct regions of the city. The exclusion criteria were as follows: Children with any syndrome and with any systemic or chronic disease or infectious diseases. Clinical examination was carried out at the school's yard, under natural light and used standard mouth mirrors and ball point probe (World Health Investigation), cotton rollers and gauze.

Visible biofilm average of the scores of six sextants was assessed using the index described by Silness and Löe [22]. The Community Periodontal Index was also performed [23] using a ball point probe to evaluate the presence or absence of gingival bleeding. ICDAS (International System for Detection and Assessment of Carious Lesions) was performed for the dental caries diagnosis [24].

The information about health habits were obtained through the anamnesis answered by parents or caregivers.

Saliva collection and DNA extraction and genotyping

Saliva samples was the source for obtaining of buccal epithelial cells genomic DNA. Children were asked to rinse the mouth with 5 ml saline solution and expectorating in a propylene tube. Genomic DNA was extracted and measured from saliva following a published protocol [25].

Three SNPs in *IL-6* (rs1800795) and *IL-1β* (rs1143629 and rs1143627) gene were studied. Characteristics of each SNP are shown in Table 1. The SNPs were chosen based on previous studies that demonstrate the potential role of these SNPs [8–13]. The genotyping of SNPs was performed by real-time polymerase chain reaction (StepOnePlus® Real-time PCR System, Applied Biosystems, Foster City, CA) using TaqMan® technology (Applied Biosystems, Foster City, CA) according to a stablished protocol.

Statistical analysis

The presence of gingivitis was defined as categorized variable as Non-Gingivitis and Gingivitis. Caries experience was assessed as a continuous variable (mean and standard deviation -SD) and, also, as a categorized variable: healthy and non-cavitated lesion ($ICDAS_{0-2}$) versus cavitated dental caries lesion ($ICDAS_{3-6}$); or healthy ($ICDAS_0$) versus dental caries ($ICDAS_{1-6}$) [21].

For normality analysis, Shapiro–Wilk test was used. For comparisons of means and standard deviations (SD), one-way ANOVA with Tukey's post-test was performed. Pearson's Chi-square was applied for overdispersion analysis. The allele and genotype (in the co-dominant, recessive and dominant models) distributions among the groups were compared by Chi-square test. Chi-square test was also used to evaluate Hardy–Weinberg equilibrium. Multivariate analysis was performed using biofilm as co-variant once this was identified as a potential cofounder factor, and was also used to evaluate the SNP-SNP interaction. Haplotype analysis was performed for IL-1β. Alpha of 5% was considered statistically significant. The GraphPad Prism 8.3 (Graph-Pad, San Diego, CA), SPSS (IBM Corp. Released 2015 IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.) and Plink software were used.

Results

The mean age in years of the included children was 8.88 (SD = 0.88). Three hundred and eight (87.2%) children were in the mixed dentition. One hundred and eighty-three children

Table 1. Studied SNP and their characteristics.

Gene	Initials	rs#	Base change in the context	Function	Average heterozygosity	Assay ID
Interleukin 6	IL-6	rs1800795	GTCTTGC[C/G]ATGCTAA	Intron Variant	0.242 ± 0.250	C___1839697_20
Interleukin 1 beta	IL-1β	rs1143627	TTTTAT[G/A]GCTTTCA	5 Prime UTR Variant	0.498 ± 0.028	C___1839944_10
		rs1143629	GGGCTGA[A/G]TCTTTTC	Intron Variant	0.500 ± 0.013	C___1839945_1_

Obtained from databases: http://www.thermofisher.com and http://www.ncbi.nlm.nih.gov.

(51.8%) were girls, while 170 were boys (48.2%). More details about characteristics of the children and the distribution among the groups are shown in Table 2.

All SNPs were in equilibrium according Hardy–Weinberg analysis ($p > .50$). The estimates of overdispersion were less than 1. The genotypes and alleles distributions of the SNPs among dental caries groups are shown in Table 3. The dominant homozygote (GG) in rs1800795 decreases the risk of gingivitis in a co-dominant model ($p = .05$; OR = 0.64; CI 95%= 0.40–1.00). There was no association between rs1800795 in *IL-6* and dental caries.

The analysis performed in rs1143627 (*IL-1β*) in the co-dominant model showed that the genotype GG reduces the risk of dental caries in both analysis ($ICDAS_0$ versus $ICDAS_{1-6}$: $p = .05$; OR = 0.55; CI 95%=0.30–1.00) and ($ICDAS_{0-2}$ versus $ICDAS_{3-6}$: $p = .02$; OR = 0.49; CI 95%=0.26–0.93). In the recessive model, the same genotype was also associated with low risk of caries ($ICDAS_0$ versus $ICDAS_{1-6}$: $p = .005$; OR = 0.48; CI95%=0.28–0.80) and ($ICDAS_{0-2}$ versus $ICDAS_{3-6}$: $p = .004$; OR = 0.45; CI 95%=0.27–0.78). In the allelic distribution, the G allele was more frequent in the non-affected groups in both analysis ($ICDAS_0$ versus $ICDAS_{1-6}$: $p = .05$; OR = 0.60; CI

Table 2. Characteristics of the children and the distribution among the groups.

	Healthy versus gingivitis			Healthy versus dental caries (ICDAS0 × ICDAS1-6)			Healthy and non-cavitated caries lesion versus cavitated caries lesion (ICDAS1-2 × ICDAS3-6)		
Characteristics	Health ($n = 206$)	Gingivitis ($n = 147$)	*p*-value	$ICDAS_0$ ($n = 187$)	$ICDAS_{1-6}$ ($n = 166$)	*p*-value	$ICDAS_{0-2}$ ($n = 203$)	$ICDAS_{3-6}$ ($n = 150$)	*p* Value
Age (mean, SD)	8.91 (0.88)	8.84 (0.90)	0.89	8.95 (0.92)	8.79 (0.84)	0.87	8.92 (0.81)	8.83 (0.95)	.78
Sex (n %)									
Male	96 (46.6)	74 (50.3)	0.48	92 (48.7)	78 (47.6)	0.83	98 (48.3)	72 (48.0)	.81
Female	110 (53.4)	73 (49.7)		97 (51.3)	86 (52.4)		105 (51.7)	78 (52.0)	
Visible biofilm index (mean, SD)	1.73 (0.31)	1.86 (0.23)	**0.0001**	1.75 (0.30)	1.83 (0.26)	**0.007**	1.74 (0.30)	1.85 (0.24)	**.002**
Self-report habit of use of dental floss (n, %)									
No	65 (32.3)	63 (43.4)	**0.03**	61 (33.0)	67 (41.6)	0.09	65 (32.7)	63 (42.8)	.05
Yes	136 (67.7)	82 (56.6)		124 (67.0)	94 (58.4)		134 (67.3)	84 (57.2)	
Self-report habit of brush teeth before sleep (n, %)									
No	44 (22.3)	33 (24.1)	0.86	43 (24.2)	34 (21.8)	0.30	45 (23.4)	32 (22.5)	.30
Yes	153 (77.7)	104 (75.9)		135 (75.8)	122 (78.2)		147 (76.6)	110 (77.5)	
How often brush teeth per day (mean, SD)	2.37 (0.69)	2.32 (0.69)	0.50	2.38 (0.69)	2.31 (0.69)	0.34	2.38 (0.69)	2.30 (0.69)	.31

SD means standard deviation. Bold forms means statistical significance difference ($p < .05$).

Table 3. Genotype and allele distributions among gingivitis group.

				Healthy versus gingivitis			
Gene	SNP	Model	Genotypes	Control (%)	Gingivitis (%)	*p* Value	OR (CI 95%)
IL-6	*Rs1800795*	Co-dominant	GG	114 (56.7)	65 (46.8)	Reference	
			CG	73 (36.3)	65 (46.8)	**0.05**	**0.64 (0.40–1.00)**
			CC	14 (7.0)	09 (6.4)	0.79	0.88 (0.35–2.11)
		Recessive	GG + GC	187 (93.0)	130 (93.6)	Reference	
			CC	14 (7.0)	09 (6.4)	0.85	1.08 (0.46–2.71)
		Dominant	GG	114 (56.7)	65 (46.8)	Reference	
			GC + CC	87 (43.3)	74 (53.2)	0.07	0.67 (0.43–1.04)
		Allele	G	301 (74.9)	195 (70.1)	Reference	
			C	101 (25.1)	83 (29.9)	0.17	1.26 (0.90–1.79)
IL-1β	*Rs1143627*	Co-dominant	AA	57 (29.5)	40 (29.4)	Reference	
			GA	86 (44.6)	64 (47.1)	0.82	0.94 (0.55–1.58)
			GG	50 (25.9)	32 (23.5)	0.76	1.09 (0.59–2.03)
		Recessive	AA + GA	143 (74.1)	104 (76.5)	Reference	
			GG	50 (25.9)	32 (23.5)	0.62	1.13 (0.69–1.86)
		Dominant	AA	57 (29.5)	40 (29.4)	Reference	
			GA + GG	136 (70.5)	96 (70.6)	0.98	0.99 (0.60–1.60)
		Allele	A	200 (51.8)	144 (52.9)	Reference	
			G	186 (48.2)	128 (47.1)	0.77	0.95 (0.69–1.30)
	Rs1143629	Co-dominant	AA	61 (31.1}	46 (33.3)	Reference	
			AG	87 (44.4)	65 (47.1)	0.97	1.00 (0.61–1.64)
			GG	48 (24.5)	27 (19.6)	0.32	1.32 (0.74–2.36)
		Recessive	AA + AG	148 (75.5)	111 (80.4)	Reference	
			GG	48 (24.5)	27 (19.6)	0.28	1.33 (0.78–2.30)
		Dominant	AA	61 (31.1)	46 (33.3)	Reference	
			AG + GG	135 (68.9)	92 (66.7)	0.66	1.10 (0.69–1.78)
		Allele	A	209 (53.3)	157 (56.9)	Reference	
			G	183 (46.7)	119 (43.1)	0.36	0.86 (0.63–1.18)

Bold forms means statistical significance difference ($p < .05$).

Table 4. Genotype and allele distributions among caries groups in the two cut-offs.

				Healthy versus dental caries (ICDAS0 × ICDAS1-6) (%)				Healthy and non-cavitated caries lesion versus cavitated caries lesion (ICDAS0-2 × ICDAS3-6) (%)			
Gene	SNP	Model	Genotypes	$ICDAS_0$	$ICDAS_{1-6}$	*p* Value	OR (CI95%)	$ICDAS_{0-2}$	$ICDAS_{3-6}$	*p* Value	OR (CI95%)
IL-6	*rs1800795*	Co-dominant	GG	92 (50.8)	87 (54.7)	Reference		101 (51.5)	78 (54.2)	Reference	
			CG	73 (40.4)	65 (40.9)	.79	0.94 (0.60–1.45)	79 (40.3)	59 (41.0)	.88	0.96 (0.61–1.50)
			CC	16 (8.8)	7 (4.4)	.10	0.46 (0.18–1.16)	16 (8.2)	7 (4.8)	.22	0.56 (0.22–1.43)
		Recessive	GG + CG	165 (91.2)	152 (95.6)	Reference		180 (91.8)	137 (95.1)	Reference	
			CC	16 (8.8)	7 (4.4)	.10	2.10 (0.87– 5.52)	16 (8.2)	7 (4.9)	.23	0.57 (0.21–1.39
		Dominant	GG	92 (50.8)	87 (54.7)	Reference		101 (51.5)	78 (54.2)	Reference	
			CG + CC	89 (49.2)	72 (45.3)	.47	1.16 (0.76–1.79)	95 (48.5)	66 (55.8)	.63	0.89 (0.58–1.38)
		Allele	G	257 (71.0)	239 (75.2)	Reference		281 (71.7)	215 (74.7)	Reference	
			C	105 (29.0)	79 (24.8)	.22	0.80 (0.57–1.14)	111 (28.3)	73 (25.3)	.38	0.85 (0.60–1.22)
IL-1β	*rs1143627*	Co-dominant	AA	50 (28.9)	47 (30.1)	Reference		53 (28.2)	44 (31.2)	Reference	
			GA	69 (39.9)	81 (51.9)	.39	1.24 (0.74–2.09)	77 (41.0)	73 (51.8)	.61	1.14 (0.68–1.91)
			GG	54 (31.2)	28 (18.0)	**.05**	**0.55 (0.30–1.00)**	58 (30.8)	24 (17)	**.02**	**0.49 (0.26–0.93)**
		Recessive	AA + GA	119 (68.8)	128 (82.0)	Reference		130 (69.1)	117 (83.0)	Reference	
			GG	54 (31.2)	28 (18.0)	**.005**	**0.48 (0.28– 0.80)**	58 (30.9)	24 (17.0)	**.004**	**0.45 (0.27–0.78)**
		Dominant	AA	50 (28.9)	47 (32.2)	Reference		53 (28.2)	44 (31.2)	Reference	
			GA + GG	123 (71.1)	99 (67.8)	.52	1.16 (0.72–1.88)	135 (71.8)	97 (68.8)	.55	0.86 (0.53–1.40)
		Allele	A	169 (48.8)	175 (58.9)	Reference		183 (48.7)	161 (57.1)	Reference	
			G	177 (51.2)	122 (41.1)	**.01**	**0.66 (0.48–0.91)**	193 (51.3)	121 (42.9)	**.03**	**0.71 (0.52–0.97)**
	rs1143629	Co-dominant	AA	58 (32.6)	49 (31.0)	Reference		61 (31.8)	46 (32.0)	Reference	
			AG	73 (41.0)	81 (51.3)	.27	1.31 (0.79–2.12)	83 (43.2)	71 (49.3)	.61	1.13 (0.68–1.84)
			GG	47 (26.4)	28 (17.7)	.25	0.70 (0.39–1.29)	48 (25.0)	27 (18.7)	.34	0.74 (0.41–1.37)
		Recessive	AA + AG	131 (73.6)	130 (82.3)	Reference		144 (75.0)	117 (81.2)	Reference	
			GG	47 (26.4)	28 (17.7)	**.05**	**0.60 (0.35–1.00)**	48 (25.0)	27 (18.8)	.17	0.69 (0.40–1.17)
		Dominant	AA	58 (32.6)	49 (31.0)	Reference		61 (31.8)	46 (32.0)	Reference	
			AG + GG	120 (67.4)	109 (69.0)	.75	0.93 (0.59–1.48)	131 (68.2)	98 (68.0)	.97	0.99 (0.61–1.56)
		Allele	A	189 (53.1)	179 (56.6)	Reference		205 (53.4)	163 (56.6)	Reference	
			G	167 (46.9)	137 (43.4)	.35	0.86 (0.63–1.17)	179 (46.6)	125 (43.4)	.40	0.87 (0.64–1.19)

Bold forms means statistical significance difference ($p < .05$).

Table 5. Multivariate analysis using caries and gingivitis as co-variant.

Gene	SNP	Reference genotype	Genotype	Healthy versus dental caries ($ICDAS_0$ × $ICDAS_{1-6}$)			Healthy and non-cavitated caries lesion versus cavitated caries lesion ($ICDAS_{0-2}$ × $ICDAS_{3-6}$)			Healthy versus gingivitis		
				Coefficient	OR (CI 95%)	*p* Value	Coefficient	OR (CI 95%)	*p* Value	Coefficient	OR (CI 95%)	*p* Value
IL-6	rs1800795	GG	CG	−0.09	0.90 (0.58–1.42)	.67	0.02	1.01 (0.66–1.59)	.93	0.39	1.48 (0.94–2.34)	.09
			CC	−0.81	0.44 (0.17–1.15)	.09	0.33	1.40 (0.51–3.82)	.51	0.13	1.13 (0.46–2.84)	.78
IL-1β	rs1143627	AA	AG	0.01	0.98 (0.45–2.18)	.97	−0.30	0.73 (0.20–2.69)	.64	−0.06	0.93 (0.42–2.09)	.87
			GG	1.01	0.44 (0.17–1.12)	.08	−1.39	0.24 (0.05–1.00)	**.05**	0.13	1.14 (0.45–2.92)	.78
	rs1143629	AA	GA	0.38	1.47 (0.67–3.25)	.33	0.64	1.90 (0.52–6.87)	.32	0.10	1.11 (0.50–2.47)	.79
			GG	0.27	1.31 (0.52–3.36)	.56	0.79	2.22 (0.52–9.40)	.27	−0.37	0.68 (0.26–1.79)	.44

Logistic regression analysis adjusted by biofilm presence. Bold forms means statistical significance difference ($p < .05$).

95%=0.35–1.00) and in ($ICDAS_{0-2}$ versus $ICDAS_{3-6}$: $p = .03$; OR = 0.71; CI 95%= 0.52–0.97).

The analysis of rs1143629 in *IL-1β* demonstrated that the genotype GG decrease the risk of dental caries in the model recessive model ($ICDAS_0$ versus $ICDAS_{1-6}$: $p = .05$; OR: 0.60; CI 95%=0.35–1.00). The details are shown in Table 4.

Multivariate analysis is in Table 5. In the statistical analysis after adjustment for the potential cofounder, the GG in rs1143627 was associated with low risk of cavitated caries lesion ($ICDAS_{0-2}$ versus $ICDAS_{3-6}$: $p = .05$; OR = 0.24; CI 95%= 0.05–1.00). Multivariate analysis was performed also to analyze SNP-SNP interaction, but did not show significant results (data no shown).

Haplotype analysis is demonstrated in Table 6. The haplotype A-G in *IL-1β* (rs1143627-rs1143629) was statistically associated with gingivitis ($p = .04$).

Discussion

The association of cytokines, that are small proteins, and environmental influences in the health-disease process contribute to the understanding, prevention and cure of oral diseases. Cytokines are a category of biomarkers that have specific effect on the interactions and communications between cells and they are involved in the progress of the caries and periodontal diseases. Cytokines can have their functions altered when their decoding genes have a mutation or SNP [26], thus, this study aimed to assess if SNPs in *IL-6* and *IL-1β* are involved in the susceptibility or resistance to dental caries and gingivitis during childhood and could be possible markers for these conditions.

IL-6 has been widely studied in oral diseases, mainly in periodontal diseases [8,14–16]. IL-6 is synthesized as an immune and inflammatory response. However, IL-6 is considered a pleiotropic interleukin, which can assimilate a pro or anti-inflammatory property, in addition to being involved in osteoclastic differentiation and induction of angiogenesis [27]. Interestingly, individual variability can modulate specific characteristics of IL-6 in the host, and the changes in IL-6 synthesis can define whether it will have an anti- or pro-inflammatory property [28]. The SNP rs1800795 in *IL-6* gene, evaluated in the present study, demonstrated to act as a protective factor against gingivitis. This corroborates with one previous study that evaluated the association between this SNP and gingivitis [8]. However, the association between periodontitis and rs1800795 was subject of several meta-analysis studies [9–11,18] and the results remain inconclusive. In the last meta-analysis, the authors note that, globally, the effect of this SNP on periodontitis is still inconclusive, but the C allele is significantly more frequent in the healthy Brazilian population. A possible explanation for this phenomenon is that the CC genotype has already been associated with a drop in IL-6 production [29].

Concerning to dental caries, previous studies has been shown that exist an increase of production of salivary IL-6 in individuals with this condition [4,5], however, no previous study has evaluated the effect of any SNP in *IL-6* gene in dental caries. In our study, there were no significant results

Table 6. Haplotype analysis among the groups in the haplotype order *rs1143627–rs1143629*.

	$ICDAS_0$ versus $ICDAS_{1-6}$			$ICDAS_{0-2}$ versus $ICDAS_{3-6}$			Healthy versus gingivitis		
Haplotype	Fa[a]	Fu[b]	*p* Value	Fa[a]	Fu[b]	*p* Value	Fa[a]	Fu[b]	*p* Value
G–G	0.40	0.46	.08	0.39	0.46	.09	0.42	0.44	.52
A–G	0.02	0.00	.14	0.02	0.00	.07	**0.00**	**0.02**	**.04**
G–A	0.03	0.04	.61	0.02	0.04	.20	0.05	0.03	.19
A–A	0.53	0.47	.12	0.54	0.47	.08	0.52	0.49	.52

Bold forms means statistical significance difference ($p < .05$).
[a]Frequency in cases.
[b]Frequency in controls.

associating *IL-6* and dental caries but we strongly recommend further investigations with the rs1800795 and others SNPs in *IL-6* gene to investigate the association between dental caries and IL-6, perhaps changing the genetic model used.

IL-1β is a member of the IL-1 family [30]. Endogenous or exogenous signs of inflammation or microbial infection induce the activation of caspases, which promote the production of IL-1β by several types of cells. Basically, IL-1β acts as an amplifier of immune reactions. However, mutations/SNPs observed in the *IL-1β* gene can contribute to an excessive or decreased production of the protein and deregulate the host's response to a disease [30], for this reason, we decided to explore SNPs *IL-1β* with dental caries and gingivitis. To the best of available knowledge, only three studies evaluated the association between SNPs in *IL-1β* with dental caries, as follows: Hu et al. [13] associated the SNP rs1143627 with low risk of dental caries. We also observed such association in the univariate analysis. However, it is still difficult to clearly elucidate the effects of rs1143627 on the *IL-1β*. It is assumed that the mutant allele is a pro-inflammatory allele, which would increase the transcription of IL-1β and decrease the risk of the disease [31]. Our study found an association between the SNP rs1143629, also in *IL-1β*, with low risk of dental caries. There are only few studies about rs1143629 and its function is not fully understood to suggest a possible explanation for its effect on dental caries. However, this SNP can be a candidate for further studies and, thus, contribute to a possible understanding of its function.

IL-1β is a cytokine largely studied in periodontal diseases and a significant increase in salivary levels of this cytokine is observed in patients with periodontal diseases [7]. This study is the first that assesses rs1143627 and rs1143629 SNPs association with gingivitis and, in the haplotype analysis, both were associated with the disease. However, it is not possible to conclude about the effects of these SNPs on periodontal diseases. Only few studies evaluated the effects of these SNPs on periodontal diseases and, also, the racial and geographical heterogeneity among the existing studies do not allow a conclusion on the behaviour of these SNPs [32]. Brodzikowska et al. [32] also recommend that more studies should be carried out with these SNPs in different populations so that more reliable results are obtained.

It is important to emphasize that our results may or may not be generalizable to other populations. Therefore, replication efforts in other racial/ethnic groups will enhances generalizability of the present results. Future studies design should also be aware that a possible limitation of our study was that levels of interleukins in oral fluids were not obtained. This would allow to investigate if the SNPs rs1143629, rs1143627 and rs1800795 are involved in variations of interleukins levels in saliva and/or in the subgingival fluid. However, our study suggests that SNPs in *IL-6* could be a biomarker for gingivitis in children and *IL-1β* could be a biomarker for both gingivitis and dental caries.

Conclusion

The rs1800795 SNP in *IL-6* gene was associated as a protective factor for gingivitis, while the SNPs rs1143627 and rs1143629 in *IL-1β* gene were associated both low risk of dental caries and gingivitis. The also, the haplotype in *IL-1β* gene was associated with gingivitis.

Author Contributions

E.C.K, D.C.L and D.S.B.O designed the epidemiological study and coordinate sample collection. M.C.F.B, B.M.S.M.M. and C.L.B.R performed the data collection. M.C.F.B performed the DNA extraction. F.B.F e J.A B funding support e laboratory experiments design and coordination. C.L.B.R and S.S.P.B performed PCR laboratorial analysis. E.C.K., D.S.B.O and C.L.B.R analyzed and interpreted the data and wrote the article. All authors contributed to the final version of the article.

Disclosure statement

The authors declared that they have no conflicts of interest to this work.

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