Association of Dental Caries with HLA Class II Allele in Brazilian Adolescents

N. Valarini[a]   S.M. Maciel[a, b]   S.K. Moura[a]   R.C. Poli-Frederico[c]

[a]North Paraná University (UNOPAR), Londrina, [b]Dental School, Maringa State University (UEM), Maringá, and [c]Dental School, North Paraná University (UNOPAR), Londrina, Brazil

Abstract

The purpose of this study was to evaluate the association between the frequencies of DR and DQ HLA class II alleles and the prevalence of dental caries in Brazilian adolescents. The study sample consisted of 164 adolescents aged 15–19 years. For the assessment of caries experience the DMFT index was used, according to World Health Organization criteria. DNA samples of the adolescents were extracted from oral mucosa cells. The amplification of the alleles HLA-DR and HLA-DQ was carried out by polymerase chain reaction with sequence-specific primers (PCR-SSP). The products of PCR-SSP were electrophoresed and analyzed in agarose gel. Statistical analysis was performed using simple and multiple logistic regression to test the association between the adolescents' caries experience and the presence of HLA-DR and -DQ alleles. Sociodemographic covariates were included in the model. The significance level for all analyses was $p < 0.05$. The prevalence of caries was 60.4% and the mean DMFT was 2.41 ± 2.53. Adolescents positive for HLA-DQ2 allele were less likely to have dental caries than their counterparts who were negative for this allele (OR = 0.33, CI 0.16–0.66), and those whose mothers presented low levels of education were 2.01 (CI 1.02–3.97) times more likely to have dental caries. There was no statistical difference between HLA-DR4, -DQ4, -DQ5, -DQ6 and dental caries. Our data provide evidence that genes within the MHC, especially the DQ2 group, may influence susceptibility to dental caries in Brazilian adolescents.

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Despite the general decline in caries disease, it is still a significant problem in many countries [Petersen et al., 2005]. It has also a considerable impact on individuals and communities, since it causes pain, suffering, impairment of function and reduced quality of life [World Health Organization, 2003]. Moreover, traditional treatment of caries is extremely costly, representing the fourth most expensive disease to treat in most of the industrialized world, and the access to appropriate treatment is difficult in many low-income countries [Yee and Sheiham, 2002].

Dental caries is a chronic, complex and multifactorial disease. It results from the interaction between the host, the host's diet, and the microflora on the tooth surface bounded by the time factor [Newbrun, 1989]. Although widely known as a disease mostly influenced by environmental factors, there is also increased evidence for a genetic component in caries susceptibility [Werneck et al., 2010; Kang et al., 2011; Wang et al., 2012]. According to Vieira et al. [2008], the factors related to the host may be influenced by genetic components, but environmental factors may overcome the individual's genetic susceptibility to the disease. Socioeconomic conditions, besides

Regina Célia Poli-Frederico
Faculty of Dentistry, University of Northern Parana (UNOPAR)
Rua Marselha 183, Jardim Piza
Londrina, PR, CEP 86041-120 (Brazil)
Tel. +55 43 3371 7820, E-Mail regina.frederico@unopar.br

the biologic factors, may interfere in dental caries prevalence, which affects more extensively and more seriously disadvantaged groups, disclosing a scenario of inequity in the distribution of the disease [Narvai et al., 2006].

Alterations in the immune response to cariogenic bacteria may also be related to dental caries [Yildiz et al., 2009]. Some studies report that there is a relationship between HLA types and an increased incidence of caries [Acton et al., 1999]. The term HLA refers to the human leukocyte antigen system and it is a collection of genes arranged within a long, continuous stretch of DNA on chromosome 6p21.3. The HLA loci are part of the genetic region known as the major histocompatibility complex, which encodes polymorphic class I, II and III molecules that play a major role in antigen processing and presentation to the T-cell receptor, which is crucial for the cell interactions in cell-mediated immunity [Altun et al., 2008]. Therefore, differences in HLA molecules can cause variations in the immune response to microorganisms, such as *Streptococcus mutans*, and may influence the individual's susceptibility to dental caries [Bagherian et al., 2008]. HLA-DR and -DQ gene polymorphism, which results in different amino acid residues, will affect not only the recognition of the peptide by the T-cell receptor but also the interaction between the T-cell receptor and HLA molecules. Furthermore, different HLA alleles may encode HLA molecules with differing affinity for bacterial peptides [Ozawa et al., 2001].

Associations between dental caries and the HLA region were obtained in both animal [Niiyama et al., 1987; Kurihara et al., 1991; Suzuki et al., 1998] and human studies [Lehner et al., 1981; Acton et al., 1999; Altun et al., 2008; Bagherian et al., 2008; Yildiz et al., 2009]. In the study conducted by Lehner et al. [1981] the genetic predisposition towards caries was found to be positively associated with HLA-DR4 antigens and negatively with HLA-DRw6-related antigens. However, de Vries et al. [1985] did not replicate these findings in a similar study, in which there was no association between HLA-DR types and caries experience. Recent studies have shown a relationship between HLA and increased risk for dental caries and higher levels of cariogenic bacteria in individuals [Acton et al., 1999; Altun et al., 2008; Yildiz et al., 2009]. Bagherian et al. [2008] recommended HLA-DRB1*04 detection as a molecular marker for early diagnosis of early childhood caries.

The aim of this study was to evaluate the association between allelic frequencies for HLA-DR4, -DQ2, -DQ4, -DQ5 and -DQ6 and the experience of dental caries in Brazilian adolescents.

Subjects and Methods

Study Subjects

For this cross-sectional study, all students who were attending the 2nd year of high school classes from a public school located in the central area in the city of Londrina, Parana, Brazil, were initially eligible (n = 192). This school was chosen because it presented a greater number of students attending high school. All of the students were invited to participate in the study and the inclusion criterion was age-related and students should be between 15 and 19 years of age, while the exclusion criterion was met if written informed consent was not signed by legal guardians. A total of 164 adolescents participated in the research (positive response rate of 85.2%).

The aim and details of the experiments were explained, and informed consent forms were obtained from parents or legal guardians of the adolescents prior to the beginning of the experimental procedures. The research was approved by the Ethics Committee of the University of North of Parana and by the local health and education authorities.

Demographic and social characteristics were obtained through personal interviews with the adolescents using a semistructured questionnaire previously tested in the pilot study. The demographic variables were the adolescent's age (15, 16, 17–19 years), gender (male, female) and skin color/race (white and nonwhite). The social characteristics studied were maternal educational level in years of study (≤8 years and >8 years) and monthly family income calculated in Brazilian currency – Reais (≤BRL 1,245.00, BRL 1,246.00–2,075.00, ≥BRL 2,076.00).

The prevalence of dental caries was determined by the DMFT index (number of decayed, missing, and filled permanent teeth), according to the criteria defined by the World Health Organization [World Health Organization, 1997]. To analyze the relationship between the experience of caries and the independent variables, the DMFT was dichotomized into DMFT = 0 (adolescents without caries experience) and DMFT ≥1 (adolescents with caries experience).

The clinical examination was performed in school settings, by the same examiner, under indirect natural illumination, using clinical mirrors and CPI probes, with both the examiner and the patient seated. Prior to examination, the teeth were cleaned with gauze. Revised infection control guidelines were followed to protect the research team and patients. The data were written down by trained recorders in individualized patient forms.

Intraexaminer agreement for caries was considered excellent, with a kappa statistic of 0.97.

DNA Extraction

Genomic DNA was obtained from epithelial cells from epithelial buccal cells as previously described [Aidar and Line, 2007]. The subjects were asked to vigorously rinse their mouths with a 5-ml solution of sucrose (3%) for 60 s, and each individual mouthwash was collected in a 15-ml centrifuge tube. In each tube, 3 ml of TNE solution (17 mM Tris-HCl, pH 8.0; 50 mM NaCl; 7 mM EDTA) was diluted in 66% ethanol. The epithelial buccal cells were centrifuged for 10 min at 3,000 rpm and resuspended in 1 ml of a solution containing 10 mM Tris (pH 8.0), 0.5% SDS, 5 mM EDTA and proteinase K (20 mg/ml), followed by an overnight incubation at 55°C. After incubation, proteins and other contaminants were removed by adding a solution containing 8 M ammo-

Table 1. Oligonucleotide primers used in the PCR-SSP for HLA-DR and HLA-DQ allelic typing (n = 164)

Allele	Genotype	Sequence (5′ to 3′)	Amplicon size, bp
HLA-DQ2	0201	GTG-CGT-CTT-GTG-AGC-AGA-AG TGC-AAG-GTC-GTG-CGG-AGC-T	206
HLA-DQ4	0401–0402	GTG-CTA-CTT-CAC-CAA-CGG-GAC-C CTG-GTA-GTT-GTG-TCT-GCA-TAC-G	211
HLA-DQ5	0501–0504	GAC-GGA-GCG-CGT-CCG-GGG TGC-AGG-ATC-CCG-CGG-TAC-G GGG-ACG-GAG-CGC-GTG-CGT-TA	216
HLA-DQ6	0601–0609	GGG-ACG-GAG-CGC-GTG-CGT-CT CTG-CAA-GAT-CCC-GCG-GAA-CG TGC-AGG-ATC-CCG-CGG-TAC-C	218–219
HLA-DR4	DR4	CGT-TTC-TTG-GAG-CAG-GTT-AAA-CA CTC-GCC-GCT-GCA-CTG-TG	213
HGHg	HGHg	GCC-TTC-CCA-ACC-ATT-CCC-TTA TCA-CGG-ATT-TCT-GTT-GTG-TTT-C	360

nium acetate and 1 mM EDTA, followed by vortexing at high speed for 5 s and centrifuging at 17,000 *g* for 10 min. The DNA pellet was washed with ethanol and resuspended in a TE solution (10 mM Tris, pH 7.8; 1 mM EDTA). DNA then was conditioned at –4°C, prior to the PCR analysis.

Genetic Polymorphism Analysis by PCR-SSP

The presence of HLA-DR4, -DQ2, -DQ4, -DQ5 and -DQ6 alleles was determined by polymerase chain reaction amplification of genomic DNA with sequence-specific primers (PCR-SSP). For DNA amplification, the primer sequences, PCR reaction profiles and expected product sizes for the DR and DQ alleles have previously been reported by Olerup and Zetterquist [1992] and Aldener-Cannavá and Olerup [1994], respectively (table 1). To check the PCR conditions, the internal positive control primer pairs that amplify the human growth hormone gene (HGHg) segments were used, and as a negative control, ultrapure water was used.

The PCR master mix for DR4 alleles contained 1× buffer, 1.5 mM $MgCl_2$, 200 μM dNTP, 1 μM HLA-DRB1*04, 0.2 μM HGHg and 1 U Taq DNA polymerase (Invitrogen Life Technologies®, São Paulo, Brazil). PCR amplifications were carried out in a thermal cycler (Endurance TC-512 Techne™, Burlington, N.J., USA). After an initial denaturation at 94°C for 5 min, a total of 30 PCR cycles were performed; each cycle consisted of denaturation at 94°C for 20 s, annealing at 50°C for 50 s, extension at 72°C for 20 s and a final extension for 5 min at 72°C.

For the DQ2, DQ4, DQ5 and DQ6 alleles, the PCR master mix contained 1× buffer, 1.5 μM $MgCl_2$, 200 μM dNTP, 0.4 μM HLA-DRB1*02, 04, 05 and 06, 0.1 μM HGHg, and 1 U Taq DNA polymerase (Invitrogen Life Technologies). After an initial denaturation at 94°C for 2 min the DNAs were amplified by 30 amplification cycles. The first 10 cycles consisted of denaturation at 94°C for 10 s and a combined annealing-extension step at 65°C for 60 s. The 20 following cycles consisted of denaturation at 94°C for 10 s, annealing at 61°C for 50 s and extension at 72°C for 30 s.

Visualization of Amplifications by Agarose Gel Electrophoresis

Following amplification, the products of PCR-SSP were electrophoresed on a 2% agarose gel. A 100-bp DNA ladder was included on each gel. The agarose gel was stained with 5 μl of Sybr Safe (Invitrogen Life Technologies) and visualized under UV illumination. The reading and interpretation of the agarose gels were made with the LabImage L-PIX (H.E.) 1D-L340 program (Loccus Biotecnologia, Brazil).

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences. Simple and multiple logistic regression analyses were used to test the association between the adolescents' caries experience and the presence of HLA-DR4, -DQ2, -DQ4, -DQ5 and -DQ6 alleles. Sociodemographic covariates were included in the unadjusted model. The significance level for all analyses was p < 0.05; χ^2 tests determined Hardy-Weinberg equilibrium for DR and DQ HLA class II alleles to compare the observed allelic frequency with those expected under Hardy-Weinberg equilibrium. Linkage disequilibrium (LD) was determined between all alleles using ARLEQUIN 3.1 [Excoffier et al., 2007].

Results

Among the adolescents there was a predominance of females (69.5%), white skin color/race (68.9%), age of 15 years (45.7%), and mothers with low levels of education (53.0%, table 2). The mean monthly family income was about BRL 1,583.27.
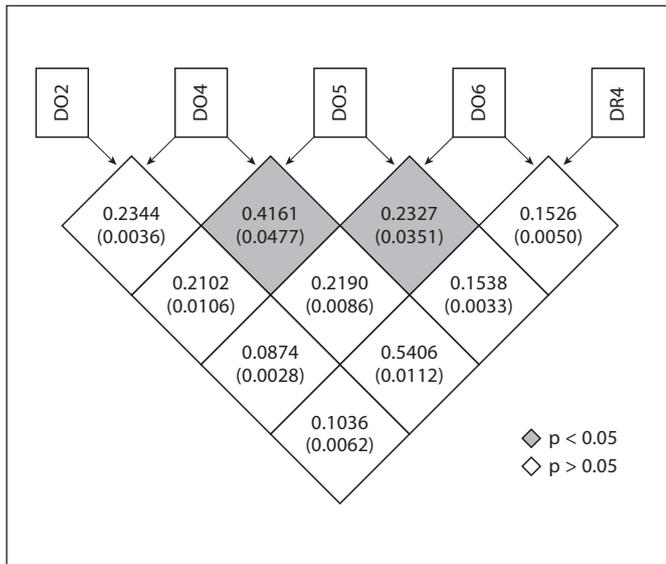
Fig. 1. Schematic representation of LD between the five HLA alleles that were analyzed. D' for LD between each marker is reported; r^2 values between markers are reported in parentheses.

Table 2. Characterization of the adolescents according to sociodemographic and caries experience (n = 164)

Characteristics	n	%
Adolescent gender		
Male	50	30.5
Female	114	69.5
Adolescent age		
15 years	75	45.7
16 years	48	29.3
17–19 years	41	25.0
Skin color/race		
White	113	68.9
Nonwhite	51	31.1
Maternal educational level		
≤8 years	87	53.0
>8 years	77	47.0
Family income, BRL		
≤1,245.00	61	37.2
1,246.00–2,075.00	39	23.8
≥2,076.00	64	39.0
Dental caries experience		
With caries	99	60.4
Without caries	65	39.6

Table 3. Frequency of the HLA-DR4 and HLA-DQ2, -DQ4, -DQ5 and -DQ6 alleles in adolescents (n = 164)

Allele	n	%
HLA-DR4	33	20.1
HLA-DQ2	51	31.1
HLA-DQ4	21	12.8
HLA-DQ5	57	34.8
HLA-DQ6	74	45.1

Dental caries prevalence was high. Only 39.6% of the adolescents studied were caries-free (table 2). The mean DMFT was 2.41 ± 2.53.

The results of genetic polymorphism analysis regarding the presence of HLA class II alleles in the adolescents showed a higher frequency of DQ6 allele (45.1%), followed by DQ5 (34.8%), DQ2 (31.1%), DR4 (20.1%) and DQ4 (12.8%) alleles (table 3). All the alleles tested were in Hardy-Weinberg equilibrium. None of the alleles were in perfect linkage disequilibrium. Significant LD was found only between DQ5/DQ4 and DQ5/DQ6 alleles (fig. 1).

Results from simple logistic regression (table 4) showed that the presence of HLA-DQ2 allele was associated with the adolescents' caries experience. Adolescents positive for this allele were less likely to experience dental caries than those who were negative (p = 0.003). Maternal educational level was also significantly associated with the outcome variable (p = 0.042). None of the other variables were significant.

In the multiple logistic regression (table 5), the presence of HLA-DQ2 allele (p = 0.002) and the maternal level of education (p = 0.043) remained significant, indicating that both these variables were independently related to the adolescents' caries experience. Adolescents positive for HLA-DQ2 allele were less likely to have dental caries than their counterparts, who were negative for

this allele, the odds ratio being 0.33 (CI 0.16–0.66). In addition, adolescents whose mothers presented low levels of education were 2.01 times more likely to have dental caries.

Discussion

Although caries formation depends on many other factors in addition to levels of cariogenic organisms and immune responsiveness, such as tooth anatomy, diet, and overall health [Caufield, 1997], this study's main purpose was to investigate a possible association between HLA-DR and HLA-DQ alleles and dental caries.

Table 4. Simple logistic regression of exploratory variables on the caries experience of Brazilian adolescents (n = 164)

Exploratory variables	With caries n (%)	Unadjusted OR (95% CI)	p values
Adolescent gender			
Female	67 (58.8)	1	
Male	32 (64.0)	0.80 (0.40–1.59)	0.529
Adolescent age			
15 years	44 (58.7)	1	
16 years	30 (62.5)	1.17 (0.56–2.47)	0.672
17–19 years	25 (61.0)	1.10 (0.51–2.40)	0.808
Skin color/race			
White	66 (58.4)	1	
Nonwhite/race	33 (64.7)	1.30 (0.66–2.59)	0.396
Maternal educational level			
>8 years	40 (51.9)	1	
≤8 years	59 (67.8)	1.95 (1.03–3.67)	0.042
Family income, BRL			
≥2,076.00	38 (59.4)	1	
1,246.00–2,075.00	24 (61.5)	1.09 (0.45–2.47)	0.835
≤1,245.00	37 (60.7)	1.05 (0.51–2.16)	0.881
HLA-DR4 allele			
Absence	78 (59.5)	1	
Presence	21 (63.6)	1.19 (0.53–2.62)	0.665
HLA-DQ2 allele			
Absence	77 (68.1)	1	
Presence	22 (43.1)	0.35 (0.18–0.70)	0.003
HLA-DQ4 allele			
Absence	86 (60.1)	1	
Presence	13 (61.9)	1.07 (0.42–2.76)	0.877
HLA-DQ5 allele			
Absence	64 (59.8)	1	
Presence	35 (61.4)	1.07 (0.55–2.06)	0.843
HLA-DQ6 allele			
Absence	54 (60.0)	1	
Presence	45 (60.8)	1.03 (0.55–1.94)	0.916

Table 5. Multiple logistic regression of exploratory variables on the caries experience of Brazilian adolescents (n = 164)

Exploratory variables	Adjusted OR (95% CI)	p values
Maternal educational level		
>8 years	1	
≤8 years	2.01 (1.02–3.97)	0.043
HLA-DQ2 allele		
Absence	1	
Presence	0.33 (0.16–0.66)	0.002

We did not observe an association between HLA-DQ4, -DQ5, -DQ6 and -DR4 alleles with the experience of dental caries. This finding is in line with previous studies that failed to observe an association between dental caries and HLA-DRB1 alleles [de Vries et al., 1985; Acton et al., 1999; Altun et al., 2008].

It must be kept in mind that findings tend to be affected by the methodology used. Thus, comparison with other studies is difficult. The study reported by Lehner et al. [1981] suggested a DR4 caries connection. However, it did not provide high resolution genotyping as the antigens tested were determined by immunological reactions and not direct DNA sequencing.

Bagherian et al. [2008] also reported that the DR4-positive subjects younger than 5 years were 10 times more likely to develop early childhood caries than their negative counterparts. In the same way, Yildiz et al. [2009] found that HLA-DRB1*04 and -DRB1*07 frequencies were significantly higher in the DMFT ≥1 group as compared with the DMFT = 0 group.

Surprisingly the results of this study revealed an association between dental caries and a different allele, specifically HLA-DQ2. Adolescents positive for HLA-DQ2 allele were less likely to have dental caries. We hypothesize that ethnic differences might explain the difference, the immunological genetic repertoire being selected over time as a consequence of exposure to infectious agents [Acton et al., 1999]. One set of authors recently observed that identical diseases might be associated with different HLA-II alleles in different ethnic groups or populations [Bondinas et al., 2007]. Moreover, it is known that many genes that code for molecules involved in the immune response are polymorphic [Yildiz et al., 2009]. Thus, further studies are needed to elucidate the nature of this relationship.

Although the study's main purpose was to investigate a possible relationship between HLA alleles and dental caries, its results confirm the well-known relationship between dental caries and maternal level of education. Adolescents whose mothers had the lowest levels of education were 2.01 times more likely to experience dental caries. These findings are consistent with those described by Nadanovsky and Sheiham [1995], Freire et al. [2001] and Moreira et al. [2006]. Interestingly, the latter noted that the level of maternal education was the only sociodemographic variable that showed significant association with the presence or absence of dental caries, similar to our findings.

Our data provide evidence that having genes within the major histocompatibility complex, specially the DQ2 group, may be one of the factors that influence suscepti-

bility to dental caries in Brazilian adolescents. Considering that little is known about the genetic basis of HLA for dental caries in the Brazilian population, the analysis of genetic polymorphism in a sample from this population represents important information concerning dental caries in Brazil.

Acknowledgments

Disclosure Statement

The authors have no conflict of interest to declare.

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