Higher Prevalence of \textit{HLA-DQB1*0301} in Non-Diabetic Mestee Subjects with Chronic Periodontitis

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Abstract

Objective: The aim of this study was to investigate and to compare the distribution of HLA alleles, genotypes and haplotypes among diabetic subjects with or without severe chronic periodontal disease and non-diabetic subjects with severe chronic periodontal disease.

Materials and Methods: 91 subjects were tested and divided into three groups: DP was formed by 31 diabetic subjects with severe chronic periodontal disease; DWP consisted of 30 diabetic subjects without periodontitis; and NDP was formed by 30 metabolically healthy patients with severe chronic periodontal disease. The polymorphisms studied were \textit{HLA-DRB1}, \textit{DRB3/4/5} and \textit{DQB1}. The data were transferred for a Generic HLA Class II DNA Typing Tray map to determine allele, genotype and haplotype results.

Results: No statistically significant differences were found for allele and genotypic frequencies on \textit{HLA-DRB1} and \textit{HLA-DRB3/4/5} genes. Furthermore, no statistically significant differences were found for haplotypic frequencies among the three groups. The geometric frequency of \textit{HLA-DQB1*0301} was found at a significantly higher level in NDP patients than in the DP group ($p = 0.0402$).

Conclusions: In metabolically healthy mestee patients with chronic periodontal disease from Bahia, Brazil, there is a significant association with \textit{HLA-DQB1*0301}. Further studies with a bigger population are warranted to confirm these results.

Keywords: Chronic Periodontitis; Human Leukocyte Antigens; Diabetes Mellitus; Polymorphism

Introduction

Periodontal disease is a nosological entity of infectious nature, characterized by the triggering of a potent inflammatory reaction responsible for the destruction of the supporting tissue of dental units. The study of Borrel and Papapanou [1] suggested that the microorganisms present in the biofilm are the etiological agents responsible for the disease. According to Fox [2] the response of each individual to the bacterial aggression is singular; striking differences occurring even within sites of the same individual.

Susceptibility to periodontitis involves a triad formed by bacteria, host and environmental factors [3]. The habit of smoking and diabetes are risk factors for the development of periodontal disease, as they raise the probability of occurrence of this infectious illness. However, neither the habit of smoking or diabetes are determining for the development of periodontitis [1].

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Michalowicz, et al. [4] suggested that the uniqueness of the immunological response facing the microbial challenge can be induced by genetic risk factors. Evidence of genetic influence on the pathogenesis of periodontitis was published in some articles of the last decade [5-7]. Van Der Velden, et al. [8] concluded that genetic factors are responsible for the familiar aggregation observed in periodontitis.

Loos, et al. [9] estimated that periodontitis has 10 to 20 gene modifiers of the disease. These contribute to the susceptibility and severity of periodontitis; however, their presence does not determine its development, requiring the simultaneous presence of other factors such as the pathogenic microbiota to initiate it [10].

The polymorphism of cytokine genes and HLA are hereditary risk factors. The polymorphism can result in different sequences of amino acid residues on the linking surfaces between HLA and the T lymphocyte receptor, modifying the pattern of immune response to the microbial challenge [11].

Many authors [12-14] observed a positive association between periodontitis and some HLA antigens such as HLA-A24, -DR4 and -A9; HLA-DQB1*0503, *0602 and *0603, HLA-DRB1*0401, *0404, *0405 and *0408 and HLA-A*03 and -B*14 alleles were also determined. On the other hand, Machulla, et al. [15] observed association between a significantly lower frequency of the allele HLA-A*31 and the genotype HLA-A*30/*31 and the incidence of periodontitis.

Alley, et al. [16] studied the associations among periodontitis, type 1 diabetes, phenotype HLA-DR/DQ and reactive T lymphocytes to bacterial antigens of Porphyromonas gingivalis and Capnocytophaga spuigena. The authors concluded that the presence of the molecules of HLA-DR4 and -DQ3 increases the risk of developing periodontitis. Type 1 diabetes is considered to be a polygenic nosology which has 20 groups of genes associated to susceptibility to it; of these, 13 show evidence of significant association [17]. Motala, et al. [18] concluded that there is significant association among the genotypes HLA-DRB1 and HLA-DQB1 and type 2 diabetes mellitus in Bahraini carriers of type 2 diabetes.

The aim of this study was to investigate and compare the distribution of the alleles, genotypes and haplotypes of class II HLA (HLA-DRB1, -DRB3, -DRB4, -DRB5 and -DQB1) among diabetic subjects with or without severe chronic periodontal disease and non-diabetic subjects with severe chronic periodontal disease.

**Material and Methods**

**Population**

The sample of convenience was composed of 61 unrelated mestizo individuals according to the criteria of Azevedo [19], with ages between 40 and 70 years, recruited from patients treated in the clinic of Cooperfeira in Feira de Santana, Bahia, and distributed into two groups: DP – 31 type 2 diabetic individuals with severe chronic periodontal disease; and DWP – 30 type 2 diabetic individuals who did not show periodontitis at the periodontal clinical exam. A third group formed by 30 metabolically healthy individuals with severe chronic periodontal disease, screened by the Bahiana Foundation for Development of Science, was called ND, closing the sample at 91 individuals. The individuals who composed the sample signed a consent form. The criteria of severity were described by Gomes-Filho, et al [20].

Individuals who had already undergone previous periodontal treatment, individuals who had oral infection with drainage indication or who were smokers were not included in the sample. Also excluded were individuals who had been prescribed antibiotics, anti-inflammatory drugs, corticosteroids, antidepressants, hormones other than insulin and anticonvulsants in the four months preceding the start of this study.

**Data Collection**

After screening, the selected individuals from the three groups were submitted to periodontal clinical evaluation: O’Leary plaque index described by Baderstein, et al. [21]; percentage of bleeding faces to probing [22]; measure of the probing pocket depth of the sul-
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Collection of periodontal clinical data

The individuals in the sample attended a first consultation, during which their dental summary data were registered, referring to the clinical periodontal parameters: O’Leary plaque index described by Baderstein, et al. [21]; percentage of bleeding faces to probing [22]; measure of the probing pocket depth of the sulcus/pocket [23] and measurement of clinical attachment level [24].

The clinical periodontal parameters were determined with the aid of a calibrated standardized Williams XP23/UNC15 periodontal probe (Hu-Friedy). Six sites per dental unit were investigated: midvestibular, mesiovestibular, distovestibular, midlingual, distolingual and mesiolingual, as described by Pihlstrom, et al [23].

All periodontal evaluation parameters were measured by a single researcher, a specialist, which provided an intra-examiner agreement average of 92.68% for the present longitudinal study. For this, ten patients were examined at two different times, with a 21-day interval. A total of 1,104 faces from 184 dental units were examined.

HLA-DRB1, -DRB3, -DRB4, -DRB5 and -DQB1 genotyping

The genomic DNA was purified from peripheral blood leukocytes using a GFX™ Kit and Genomic Blood DNA Purification Kit (Amersham Pharmacia Biotech). The concentration and purity of the obtained DNA was determined by reading optical density at 260 and 280 nm, and later on the samples were frozen to -20°C (4°F) until use.

Determination of the class II HLA genotypes (HLA-DR, -DQ) was done by the PCR-SSP method using a Micro SSP™ SSP2L DNA Typing Kit (One Lambda, Inc., Canoga Park, CA, USA, Lote 05 A) according to the manufacturer’s instructions. The PCR products were separated in a 2.5% agarose gel containing 10μL of ethidium bromide; electrophoresis was accomplished between 140 and 150 V, with 40 mA at the beginning of the migration. Then, the bands were visualized using UV light, photo documented and the band pattern obtained was transcribed to the map of its own typing kit.

Statistical analysis

For comparison of the frequencies among the groups a chi-square or Fisher’s exact test was used.

Results

The distribution of the individuals according to age was similar in the DP and DWP groups and significantly different in the NDP group when compared to both groups (p = 0.0489 and p = 0.0452, respectively). The time since the diagnosis of the diabetes was significantly higher in the DWP group than the DP group (p = 0.002). The gender distribution did not present a significant statistical difference between the groups (p = 0.2411) (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>X</th>
<th>SD</th>
<th>M</th>
<th>F</th>
<th>X</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>DWP</td>
<td>30</td>
<td>54.83 ±</td>
<td>1.65</td>
<td>34</td>
<td>66</td>
<td>13.13</td>
<td>1.13</td>
</tr>
<tr>
<td>DP</td>
<td>31</td>
<td>56.54 ±</td>
<td>1.59</td>
<td>55</td>
<td>45</td>
<td>7.87</td>
<td>1.18</td>
</tr>
<tr>
<td>NDP</td>
<td>30</td>
<td>47.00 ±</td>
<td>1.38</td>
<td>42</td>
<td>58</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Descriptive statistics of the demographic variables.

DP: Diabetic Subjects with Severe Chronic Periodontal Disease; DWP: Diabetic Individuals without Periodontitis; NDP: Non-Diabetic Subjects with Severe Chronic Periodontitis; X: Mean Value; SD: Standard Deviation of the Mean; M: Male Gender; F: Female Gender.

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Concerning the plaque index and the bleeding index to probing, a significant statistical difference was observed when comparing the indices of the DP and NDP groups to the indices of the DWP group. On the other hand, when comparing both the bleeding index and the plaque index of both DP and NDP groups, no significant differences were observed. As to the percentage of the faces framed in the three subgroups in regard to the probing depth, considering the probing depth values as shallow (0 to 3 mm), moderate (3 to 6 mm) or profound (greater than or equal to 7 mm), when comparing the three subgroups among the DP and NDP groups, no significant statistical differences were observed. On the other hand, when we compared the three subgroups of both DP and NDP to the three subgroups of the DWP group, statistical differences were observed in all comparisons.

The percentage of framed faces according to the criteria described by Armitage regarding clinical attachment loss can be seen in table 2. It can be observed that the data have been divided into slight (light) attachment loss – L – (1 to 2 mm), moderate – M (2 to 3 mm) and severe – S (greater than or equal to 5 mm). Also, a fourth subgroup was described, composed by the faces which did not have clinical attachment loss (NAL). There were no significant statistical differences when comparing the three subgroups of the DP and NDP groups. However, when comparing the four subgroups of both the DP and NDP groups to the four subgroups of the DWP group, significant statistical differences were observed in all comparisons.

<table>
<thead>
<tr>
<th>Group</th>
<th>PI</th>
<th>BOP</th>
<th>PD</th>
<th>AL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>SD</td>
<td>X</td>
<td>SD</td>
</tr>
<tr>
<td>DP</td>
<td>85.1</td>
<td>5.28</td>
<td>77.6</td>
<td>7.74</td>
</tr>
<tr>
<td>DWP</td>
<td>57.1</td>
<td>10.39</td>
<td>47.9</td>
<td>6.47</td>
</tr>
<tr>
<td>NDP</td>
<td>8.3</td>
<td>6.11</td>
<td>81.9</td>
<td>6.68</td>
</tr>
</tbody>
</table>

Table 2: Distribution of the plaque index, percentage of bleeding on probing, percentage of tooth faces according to the three probing depth levels and percentage of tooth faces according to the loss of attachment levels between the three study groups.

DP: Diabetic Subjects with Severe Chronic Periodontal Disease; DWP: Diabetic Individuals without Periodontitis; NDP: Non-Diabetic Subjects with Severe Chronic Periodontis; PI: Plaque Index; BOP: Bleeding on Probing; PD: Probing Depth; SPD: Shallow Probing Depth; MPD: Moderate Probing Depth; PPD: Profound Probing Depth; AL: Attachment Loss; NAL: No Attachment Loss; SAL: Slight Attachment Loss; MAL: Moderate Attachment Loss; SEAL: Severe Attachment Loss.

No significant statistical differences were observed either in the allelic or genotypic frequencies of the genes HLA-DRB1, -DRB3, -DRB4 and -DRB5 among the three groups. In the DP group, the most frequent alleles were HLA-DRB1*07, -DRB1*13, HLA-DRB1*01 and -DRB1*11; the alleles which presented the lowest frequency were HLA-DRB1*12, -DRB1*14 and -DRB1*16. In the DWP group, the most frequent alleles were HLA-DRB1*07, -DRB1*0301 and -DRB1*04; the least frequent were HLA-DRB1*10 and -DRB1*12. In the NDP group the most frequent alleles were HLA-DRB1*07, -DRB1*13, HLA-DRB1*11 and -DRB1*15; the least frequent were HLA-DRB1*09 and -DRB1*16. The alleles HLA-DRB1*0302, -DRB1*12 and -DRB1*14 were not observed in the NDP group. In all three groups a higher frequency of the allele HLA-DRB3* was observed in comparison to the alleles -DRB4* and -DRB5* (Table 3).
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Table 3: Distribution of the allelic frequency of HLA-DRB1, DRB3*, DRB4* and DRB5*.

<table>
<thead>
<tr>
<th>Group/ allele</th>
<th>DP n: 31</th>
<th>DWP n: 30</th>
<th>NDP n: 30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>DRB1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Blank</td>
<td>5 (8.1)</td>
<td>3 (5.0)</td>
<td>5 (8.3)</td>
</tr>
<tr>
<td>*01</td>
<td>7 (11.3)</td>
<td>3 (5.0)</td>
<td>4 (6.7)</td>
</tr>
<tr>
<td>*0301</td>
<td>5 (8.7)</td>
<td>8 (3.3)</td>
<td>4 (6.7)</td>
</tr>
<tr>
<td>*0302</td>
<td>2 (3.2)</td>
<td>3 (5.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>*04</td>
<td>3 (4.8)</td>
<td>6 (10.0)</td>
<td>3 (5.0)</td>
</tr>
<tr>
<td>*07</td>
<td>9 (14.5)</td>
<td>8 (13.3)</td>
<td>10 (16.7)</td>
</tr>
<tr>
<td>*08</td>
<td>2 (3.2)</td>
<td>5 (8.3)</td>
<td>4 (6.7)</td>
</tr>
<tr>
<td>*09</td>
<td>2 (3.2)</td>
<td>3 (5.0)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>*10</td>
<td>3 (4.8)</td>
<td>1 (1.7)</td>
<td>2 (3.7)</td>
</tr>
<tr>
<td>*11</td>
<td>6 (9.7)</td>
<td>5 (8.3)</td>
<td>8 (13.7)</td>
</tr>
<tr>
<td>*12</td>
<td>1 (1.6)</td>
<td>1 (1.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>*13</td>
<td>9 (14.5)</td>
<td>5 (8.3)</td>
<td>10 (16.7)</td>
</tr>
<tr>
<td>*14</td>
<td>1 (1.6)</td>
<td>3 (5.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>*15</td>
<td>6 (9.7)</td>
<td>4 (6.7)</td>
<td>8 (13.3)</td>
</tr>
<tr>
<td>*16</td>
<td>1 (1.6)</td>
<td>3 (5.0)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>DRB3*</td>
<td>24 (38.7)</td>
<td>25 (41.7)</td>
<td>22 (36.7)</td>
</tr>
<tr>
<td>DRB4*</td>
<td>13 (21.0)</td>
<td>16 (26.7)</td>
<td>14 (23.3)</td>
</tr>
<tr>
<td>DRB5*</td>
<td>7 (11.3)</td>
<td>7 (11.7)</td>
<td>9 (16.0)</td>
</tr>
</tbody>
</table>

Table 4 shows the frequency distribution of the allele HLA-DQB1* in the three groups. In the DP group the most frequent alleles were HLA-DQB1*02, -DQB1*05 and -DQB1*06; the alleles HLA-DQB1*0303, -DQB1*0302 and -DQB1*0301 were the least frequent. In the DWP group the most frequent alleles were HLA-DQB1*02, -DQB1*05 and -DQB1*06; the least frequent alleles were HLA-DQB1*0303 and -DQB1*0302. In the NDP group the alleles HLA-DQB1*02, -DQB1*0301 and -DQB1*06 were the most frequent; the alleles HLA-DQB1*0303 and -DQB1*0302 were the least frequent.

A significant statistical difference was observed when comparing the prevalence of the allele HLA-DQB1*0301 among the NDP and DP groups (p = 0.0402). Also, a tendency of individuals of the DWP group to present higher allelic frequency of HLA-DQB1*0301 was observed in comparison to the observation in the DP group (p = 0.00561).

In the DP group a tendency to a higher frequency of the allele HLA-DQB1*05 was observed in comparison to the NDP group (p = 0.073).

**Discussion**

The aim of this study was to evaluate and compare the frequency of the alleles HLA-DRB1, -DRB3, -DRB4, -DRB5 and -DQB1 in 91 patients distributed in three groups: the DP group – composed by 31 type 2 diabetic individuals, uncontrolled metabolically, with severe, chronic generalized periodontitis; and the DWP group – 30 type 2 diabetic individuals, uncontrolled metabolically, who presented no periodontitis, which makes up an adequate control group according to Gomes-Filho., et al [20]. The NDP group was composed by 30 non-diabetic individuals with severe, chronic generalized periodontitis.

Comparison of the frequencies of HLA-DQB1 alleles among the three groups and among diabetics and non-diabetics, revealed a significant difference only in the prevalence of the allele HLA-DQB1*0301 among the DP and NDP groups (p = 0.040). The non-diabetic patients with chronic periodontitis had a prevalence of this allele about three times greater than that observed in the group of diabetic patients with chronic periodontitis. No association of HLA-DQB1*0301 and periodontitis or metabolic health was found in the literature. In the DP group, a tendency for a higher frequency of the allele HLA-DQB1*05 was observed in comparison with the NDP group (p = 0.073).

Emery, et al [26] and Cerna., et al [27] associated the allele HLA-DQB1*0302 with type 1 diabetes. This same allele was associated with type 2 diabetes by Haiyan., et al [28]. Reichert., et al. [29] observed a significantly higher statistical frequency of the allele HLA-DQB1*06 among periodontal individuals when compared to a control group. In the present study there was no association of this allele to chronic periodontitis. Perhaps this difference is a consequence of the different ethnic origins in the studied population, as the study of Reichert., et al. [29] evaluated Caucasian individuals and the present study researched a population characterized by inter-relationship among distinct people, resulting in a genetically hybrid ethnicity. This population presents a tri-racial origin: Whites, Blacks and Indians, resulting in a wide variety of mixed multiracial phenotypes (mestees) [19].

In this study the distribution of the allelic frequency of HLA-DRB1 among the mestees of Feira de Santana, Bahia, revealed that in the DP group the alleles HLA-DRB1*07, *13 and *01 were the most prevalent, and the alleles HLA-DRB1*12 and *14 were the least prevalent, whereas in the DWP group the most frequent alleles were HLA-DRB1*0301 and *07, while the rarest were HLA-DRB1*0303, *10 and *12. In the NDP group the most frequent alleles were HLA-DRB1*07 and *13.

Onengut-Gumuscu., et al [17] reported that HLA-DRB1*04 is in linkage disequilibrium with HLA-DQB1*0302, occurring in high frequency among type 1 diabetic subjects; these data were confirmed by Emery., et al [26], Cerna., et al [27] and Horton., et al [30]. Mimura., et al [31] observed a higher frequency of the antigens HLA-Cw4 and DR4 among Japanese individuals with type 2 diabetes mellitus and Motala., et al [18] observed that the allelic groups HLA-DRB1*04, *07 and *15 and HLA-DQB1*0302, *05, *06 and *02 were the most frequent among type 2 diabetic individuals. In the present study it was observed that one of most frequent allelic groups among type 2 diabetics was HLA-DRB1*07.

It is important to take into consideration that the studies of Onengut-Gumuscu., et al [17], Cerna., et al [27] and Emery., et al [26] evaluated type 1 diabetic individuals, who present a varied pathogenesis from that presented by type 2 diabetic individuals. It is also important to consider that in the studies by Mimura., et al [31] and Motala., et al [18] the studied populations were formed by type 2 diabetic individuals; however, they were from Japanese and Arabic origins, respectively.

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Stein, et al. [32] observed a positive association among HLA-DRB1*04, -Cw*08 and -B*14 and chronic periodontitis. Also, Bonfil, et al. (1999) concluded that HLA-DRB1*0401, 0404, 0405 and 0408 are associated with rapid progressive periodontitis. In the present study, twice the frequency, not significant, of the allele HLA-DRB1*04 was observed in the type 2 diabetics without severe periodontitis when compared to the DP group. It is important to emphasize that the sample of this study was composed by individuals with chronic periodontitis, which presents peculiar aspects in its pathogenesis that differ from the pathogenesis observed in aggressive periodontitis.

A tendency was observed in the individuals from the DP group to present a higher frequency of the allele HLA-DRB3* in comparison to the frequency observed among the NDP group (p = 0.079). On the other hand, Stein, et al. [32] while evaluating Caucasian individuals observed a higher frequency, not statistically significant, of HLA-DRB5* among chronic periodontitis carriers and Alley, et al. [16] reported that HLA-DRB4* was significantly more frequent among periodontitic individuals, diabetic or not. These differences could have origin in the different ethnicities which were studied.

Evaluation of the obtained haplotype in the three study groups did not show significant statistical differences; just the NDP group presented a tendency (p = 0.081) for a higher frequency of the haplotype HLA-DRB1*11-DQB1*0301 when compared to DP. The most common haplotypes among the diabetic individuals with periodontitis were: DRB1*01-DQB1*05, DRB1*13-DQB1*06 and DRB1*07-DQB1*02 and the most frequent among the diabetic individuals with a healthy periodontium were: DRB1*07-DQB1*02 and DRB1*0301-DQB1*05.

Motala, et al. [18] observed that the haplotypes DRB1*040101-DQB1*0302, DRB1*070101-DQB1*050101, DRB1*150101-DQB1*060101 and DRB1*070101-DQB1*020101 were the most frequent among type 2 diabetic subjects when compared to non-diabetic subjects, and that the allele DRB1*0701-DQB1*0201 granted susceptibility to type 2 diabetes; this same haplotype was the most frequent among all the evaluated groups in the present study; a result probably determined much more by the characteristics of the population than a susceptibility marker. Also, the haplotype DRB1*1501-DQB1*0601 was positively associated with type 2 diabetes [18]. However, in the present study the same haplotype was one of the most frequent among the non-diabetic subjects, showing twice the frequency of this haplotype when compared to the diabetic groups separately, thus this difference was not significant.

Careful evaluation of the periodontal indices reveals that the DP and NDP groups, carriers of periodontitis, demonstrated a higher plaque index and bleeding faces to probing. In addition, there was a higher percentage of framed faces in the subgroups associated with higher probing depth and greater attachment loss, demonstrating a situation of greater periodontium destruction in both of these groups. Group DWP, on the other hand, showed a lower plaque index and bleeding faces to probing, and there were no faces framed in the subgroups associated with a probe depth greater than 3 mm and moderate and severe attachment loss, demonstrating a scene of healthy periodontium or gingivitis.

The results obtained from this work suggest an association between the allele HLA-DQB1*0301 and metabolically healthy mestee individuals with severe chronic periodontitis. A redesigned study with a significantly bigger sample is necessary to confirm the lower frequency of the allele HLA-DQB1*0301 in type 2 diabetic subjects with severe chronic periodontal disease in comparison to the frequencies observed in type 2 diabetic individuals without periodontitis and in non-diabetic subjects with severe chronic periodontal disease.

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