Association of human leukocyte antigens Class I and Class II antigens with chronic periodontitis in East India

Mona Chowdhury, Neeraj Agrawal, Debabrata Kundu, Nitubroto Biswas

Abstract:

Context: Human leukocyte antigens (HLAs) have an important role in the determination of susceptibility and resistance to periodontal diseases in humans, which may vary from population to population. Aims: The aim of this study was to find out the association of HLA Classes I and II genes with chronic periodontitis in East Indian population. Materials and Methods: In a cross-sectional study design, a total of sixty participants of chronic periodontitis (CP) (mean age: 44.12 ± 5.85) and sixty subjects of periodontal disease-free controls (NP) (mean age: 41.85 ± 7.71) were analyzed for their various HLA combinations using serologic (microlymphocytotoxicity test) method. The results are further compared with the HLA profile of 100 samples of blood donors for which periodontal status was unknown. All the data were statistically analyzed by applying Chi-square test, Results: HLA-B7 (P = 0.003), DR7 (P = 0.001), DR53 (P = 0.001), and DQ3 (P = 0.001) were identified as susceptible phenotypes to CP, whereas HLA-A1 (P = 0.010), A3 (P = 0.001), and Cw4 (P = 0.001) phenotypes were identified to be associated with disease resistance. Conclusion: The HLA-B7, DR7, DR53, and DQ3 alleles may represent as risk factors for CP in Eastern Population of India, whereas HLA-A1, A3, and Cw4 may indicate to protective factors for CP of the same.

Key words:
Chronic periodontitis, gene polymorphism, human leukocyte antigen and periodontitis and India

INTRODUCTION

It has been observed that progression of gingivitis into periodontitis depends not only on the local ecological system of the gingival sulcus, which harbors periodontopathic bacteria but also on the host response. Genetic predisposition has now been convincingly demonstrated to be responsible for susceptibility to periodontal diseases in part. It has been observed that 50% of the population variance for periodontitis may be attributed to genetic factors. A number of genetic polymorphisms have been linked with risk for chronic periodontitis (CP) in various populations.

Human leukocyte antigens (HLAs) have an important role in host immunity by binding to the some of the processed antigens and present them to the surface of antigen-presenting cells. Each individual has different genes encoding for HLA molecules for binding of peptides of endogenous or exogenous pathogens, and each gene is polymorph with different alleles. As a consequence of this, there are different peptide-binding grooves in HLA molecules, which are responsible for the susceptibility of an individual to infectious, autoimmune diseases, or cancer. There are two types of HLA Class I and Class II molecules present and both of them have been reported to be associated with periodontal diseases. Most of the studies on the association of HLA antigens to periodontitis were done on Caucasian populations. The association between HLA genes and the susceptibility to CP was showed in the allele frequencies HLA-Cw*08; B*; DRB1*04 in the study done by Stein et al. in 2003. Although in a meta-analysis by Stein et al. in 2008, in patients with CP, slight deviations of the frequency of particular HLA antigens (A*9, A*29, B*15, A*2, B*5) were detected, but overall, it was statistically insignificant. This meta-analysis was done on Caucasian population, and ethnic variation cannot be denied on this association. In India, although there are few studies done in the past, those studies were different in terms of (i) geographical area, (ii) culture, (iii) ethnicity, and (iv) number of HLA antigens investigated.

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Hence, there is a need for further research on this association. Therefore, the present study was planned and designed to be conducted on Bengali population of Eastern part of India to know the status of the association of various HLA molecules with generalized CP.

MATERIALS AND METHODS

Study population and clinical investigations
A total of 152 Bengali participants were screened from the Outpatient Department of Periodontology. Ethical Committee approval was obtained from the university’s Ethical Board. The study was conducted on 75 participants of CP (Group I) and 77 participants of periodontitis-free controls (NP) (Group II) could be included in the study. The participants were well informed about the study, and written consent was obtained. Furthermore, a group of 100 unrelated Bengali Indian blood donors (Group III) were included in the study (periodontal status was unknown), representing the distribution of HLA markers in the same geographical region with the same ethnicity.

All the participants were assessed accordance with the latest classification system of periodontal diseases. The following criteria were crucial for the selection of cases and controls. Age of all the selected participants should be between 35 and 55 years. All the selected participants should not have general diseases, for which an association to periodontitis is known. Patients with CP were selected, if they showed clinical attachment loss (AL) ≥ 4 mm in 30% of the sites with a minimum probing depth (PD) = 4 mm.

Periodontitis-free individuals were included only when they did not have any AL, i.e., PD ≤ 3.5 mm and no gingival recession attributable to periodontitis. AL attributable to some other predisposing factors, i.e., overhanging restoration, traumatic toothbrushing, and endodontic lesion on a single tooth did not lead to exclusion, if all of the other teeth had no signs of periodontitis. All the selected individuals were unrelated Bengali population of Eastern part of India.

Exclusion criteria were pregnancy; the use of antibiotics or a periodontal therapy during the past 6 months; chronic usage of anti-inflammatory drugs; smokers or former smokers; drug-induced gingival enlargement; any kind of immunodeficient conditions; any kind of viral infection having oral manifestations; desquamative gingival conditions; any known systemic diseases, for example, diabetes, cardiac of renal diseases; and diseases with known associations to HLA alleles such as rheumatic diseases, systemic lupus erythematosus, birdshot retinopathy, or narcolepsy.

The clinical assessment included the determination of bleeding on probing, PD (in mm) as a distance between the gingival margin and the bottom of the pocket, and clinical AL (in mm) as a distance between the cement-enamel junction and the bottom of the pocket [Table 1].

Human leukocyte antigen typing
For all the recruited participants, the HLA typing was done in the Department of Pathology, Institute of Post-graduate Medical Education and Research, Kolkata, India. HLA types were determined by serology, i.e., by microlymphocytotoxicity using the 72 well tray with predropped anti-HLA-ABC and HLA DR/DQ reagents (Bio-Rad Medical Diagnostics GmbH, Dreieich, Germany). For assessment, 20 ml of blood samples was taken from all the participants by venepuncture from the antecubital fossa. Heparin (7 ml) was added as an anticoagulant in the samples. The blood was layered on to the liquid cushion, Lymphoprep. Lymphocytes were isolated by centrifuging (Remi-R8C, Vasai, India) the blood for 20 min at 3000 rpm. As being less dense, mononuclear leukocytes were found as a white ring at the boundary between the plasma and the Lymphoprep. Lymphocytes were taken in a separate test tube, and x1 phosphate-buffered solution was added and further passed into nylon wool column to separate T- and B-cells. Then the lymphocytes were placed in 72 well tray with predropped anti-HLA reagents. The cells were first incubated with antibody then complement was added, and tray was allowed to incubate at room temperature. Antibody binding was detected by complement-dependent cytotoxicity. The cell death was detected by adding a dye eosin, which have the ability to stain the interior of the cells whose membrane had been damaged. The cells were then examined by inverted phase microscope Olympus IX70, and pattern of the reaction was noted. The vital lymphocyte appeared bright and luminous (negative reaction), and lysed lymphocyte appeared somewhat larger and stained dark (positive reaction). The total number of lysed lymphocytes compared with the total number of lymphocytes was quoted as a score value in each well, and the result was plotted in a worksheet provided with the test kit.

Statistical analysis
The frequency of HLA Class I including 25 types of HLA-A, 49 types of HLA-B, and 7 types of HLA-C, and HLA Class II comprising 21 types of HLA-DR and 9 types of HLA-DQ antigen were examined in each of the study group. The antigen frequencies of CP were compared with the NP using Chi-square test (2×2 contingency table) [Table 2]. The significant values [Table 3] were further compared with the values of Group III to compare the distribution with a normal population [Table 4].

RESULTS AND OBSERVATIONS
In a given period of time, a total of sixty patients (out of 75) of Group I (CP) and 60 patients (out of 77) of Group II (NP) were analyzed. The reason for rest of the participants, who were screened, but not completed the study was either they did not report on the day of blood collection or refused for blood withdrawal. The percentage of dropout was 21%.

Table 1: Clinical and demographic characteristics of the participants

| Variables | CP Group I (n=60) | NP Group II (n=60) | Random population Group III (n=100) |
|-----------|------------------|--------------------|-----------------------------------|
| Age (years), mean±SD | 44.12±5.85 | 41.85±7.71 | 40.08±8.79 |
| Gender (male/female) (%) | 53.3/46.7 | 48.3/51.7 | 56.2/43.8 |
| BOP (%), mean±SD | 100 | 11 | - |
| PD (mm), mean±SD | 5.07±0.53 | 1.65±0.45 | - |
| AL (mm), mean±SD | 4.7±0.57 | 1.5±0.87 | - |

BOP – Bleeding on probing; PD – Probing depth; AL – Clinical attachment loss; CP – Chronic periodontitis; SD – Standard deviation; NP – Periodontitis free controls; n – Number of subjects
Table 2: Distribution of human leukocyte antigens-A, B, C, and DR alleles among chronic periodontitis patients and control individuals

| HLA markers | Study Group I (n=60) | Study Group II (n=60) | Test of significance (Chi-square test) | P |
|-------------|----------------------|-----------------------|--------------------------------------|---|
| n | Pf | n | Pf |
| A*1 | 8 | 13.3 | 20 | 33.3 | 6.71 | 0.010* |
| A*2 | 12 | 20 | 24 | 26.7 | 0.745 | 0.389 |
| A*3 | 8 | 13.3 | 16 | 40 | 10.9 | 0.001** |
| A*9 | 20 | 33.3 | 12 | 20 | 2.73 | 0.099 |
| A*10 | 4 | 6.7 | - | - | 4.14 | 0.042* |
| A*19 | 32 | 53.3 | 28 | 46.7 | 0.533 | 0.465 |
| A*24 | 16 | 26.7 | 12 | 20 | 0.745 | 0.388 |
| A*28 | 4 | 6.7 | - | - | 4.14 | 0.042* |
| A*30 | 4 | 6.7 | 4 | 6.7 | 0.00 | 1.000 |
| A*33 | 28 | 46.7 | 20 | 33.3 | 2.22 | 0.136 |
| B*5 | 16 | 26.7 | 16 | 26.7 | 0.00 | 1.000 |
| B*7 | 16 | 26.7 | 4 | 6.7 | 8.64 | 0.003** |
| B*12 | 12 | 20 | 10 | 16.67 | 0.223 | 0.637 |
| B*15 | 12 | 20 | 8 | 13.3 | 0.960 | 0.327 |
| B*22 | 8 | 13.3 | 4 | 6.7 | 1.48 | 0.220 |
| B*35 | 15 | 25.0 | 20 | 33.3 | 1.008 | 0.315 |
| B*44 | 20 | 33.3 | 8 | 13.3 | 3.33 | 0.068 |
| Bw*4 | 44 | 73.3 | 52 | 86.7 | 0.635 | 0.426 |
| Bw*6 | 40 | 66.7 | 44 | 73.3 | 0.635 | 0.426 |
| Cw*1 | 8 | 13.3 | 4 | 6.7 | 1.48 | 0.224 |
| Cw*4 | 4 | 6.7 | 20 | 33.3 | 13.3 | 0.001** |
| Cw*6 | 12 | 20 | 12 | 20 | 0.00 | 1.000 |
| DR*1 | 4 | 6.7 | 8 | 13.3 | 1.481 | 0.223 |
| DR*4 | 8 | 13.3 | 8 | 13.3 | 0.00 | 1.000 |
| DR*5 | 8 | 13.3 | 12 | 20 | 0.960 | 0.372 |
| DR*7 | 36 | 60.0 | 32 | 53.3 | 20.0 | 0.001** |
| DR*10 | 16 | 26.7 | 8 | 13.3 | 3.33 | 0.068 |
| DR*12 | 4 | 6.7 | 1 | 0.02 | 1.878 | 0.170 |
| DR*52 | 8 | 13.3 | 16 | 26.7 | 3.33 | 0.068 |
| DR*53 | 40 | 66.7 | 20 | 33.3 | 13.3 | 0.001** |
| DQ*1 | 40 | 66.7 | 44 | 73.3 | 0.635 | 0.426 |
| DQ*2 | 33 | 55 | 25 | 41.7 | 2.136 | 0.143 |
| DQ*3 | 36 | 60 | 16 | 26.7 | 13.6 | 0.001** |

*Significant; **Highly significant. Pf – Phenotype frequency; HLA – Human leukocyte antigens; P – Probability; n – Number of subjects; p value is significant at 0.05 level

Table 3: Various positive human leukocyte antigens markers among the Group I and II

| Disease | HLA-A | HLA-B | HLA-C | HLA-D |
|---------|-------|-------|-------|-------|
| Group I (CP) | A*10, A*28 | B*7 | DR*7, DR*53, DQ*3 |
| Group II (NP) | A*1, A*3 | Cw*4 |

CP – Chronic periodontitis; HLA – Human leukocyte antigens; NP – Periodontitis free controls

Table 4: Comparison of significantly associated human leukocyte antigens markers with random population (Group III)

| HLA | Study Group I (n=60) | Random population (n=100) | Test of significance (Chi-square test) | P |
|-----|----------------------|--------------------------|--------------------------------------|---|
| n | Pf | |
| A*1 | 8 | 13.3 | 11 | 0.195 | 0.6587 |
| A*3 | 24 | 40 | 12 | 16.860 | 0.000** |
| A*10 | 4 | 6.7 | 13 | 1.584 | 0.2082 |
| A*28 | 4 | 6.7 | 15 | 2.488 | 0.1147 |
| B*7 | 16 | 26.7 | 8 | 10.248 | 0.001** |
| Cw*4 | 4 | 6.7 | 18 | 4.061 | 0.04* |
| DR*7 | 36 | 53.3 | 20 | 26.376 | 0.000** |
| DR*53 | 40 | 66.7 | 34 | 16.097 | 0.000** |
| DQ*3 | 36 | 60 | 16 | 26.7 | 13.6 | 0.001** |

*Significant; **Highly significant. Pf – Phenotype frequency; HLA – Human leukocyte antigens; P – Probability; n – Number of subjects; p value is significant at 0.05 level

DISCUSSION

The HLA types differ throughout the world, and each major race is characterized by a high or low frequency of a specific HLA.8,16,27 Hence, the study of the association of HLA needs to be carried out in each population. Most of the studies done in the past on association of CP and HLA were done in the Caucasian population, and results of those studies are still inconclusive.25 In India, as mentioned earlier, studies are limited,26-28 and this is a first study from the eastern part of the country.

In this study, a group of 100 unrelated Bengali Indian blood donors (Group III) were included (periodontal status was unknown), representing the distribution of HLA markers in the same geographical region with the same ethnicity. The frequencies of HLA markers in CP were compared with these random populations of blood donors to check any variation with NP.

In the literature, many studies have shown the association of HLA polymorphisms with periodontitis in different population groups and considered as a risk factor for periodontal diseases.10,11,18 However, data are inconsistent because of various HLA antigen combinations and en bloc-inherited HLA alleles.11 By combined analysis of all HLAs, antigen frequencies of the association did not find any significant positive or negative associations with CP. However, only a slight tendency for an increased frequency of HLA-A*9, B*15 and A*29 (A*19) and a decreased occurrence of HLA-A*2, A*3, and B*5 among patients with CP were found.25 However, these findings were limited to the Caucasian population only.

In the present study, which was conducted in the eastern part of the India, increased frequency of HLA-A*10, A*28, B*7, DR*7, DR*53, and DQ*3 were found in patients with CP, whereas increased frequency of HLA-A*1, A*3, and Cw*4 were found with NP.

HLA-A*1 and A*3 were found to be associated with resistance to periodontitis. A similar result was noted by Machulla et al.21 in German population for HLA-A*3.21 Firatli et al. in 1991 also noted that the HLA-A*1 is associated with resistance periodontitis, but they got this association with juvenile periodontitis and rapidly progressing periodontitis.19 Another possible association explanation comes from the study by Stein et al., 2003; HLA-A*3 expression might change in the presence of coexpression of HLA-A*1 and A*2. Hence, coexpression of HLA-A*1 may contribute to higher protection from CP.21
In HLA-B category, only HLAB*7 was found to be significantly associated with CP. No any other study in the literature has found this association till now. Probably, this may be a new allele in this population group. Other studies which have found significant association on different alleles of HLA-B are Shapiro et al. in 1994 (B*151),[22] Marggraf et al. in 1983 (B*351),[23] Goteiner and Goldman 1984 (B*51),[24] and Machulla et al. 2002 (B*141).[25]

HLA-Cw*4 was significantly associated with resistance to the CP. In contrast to this study, only one study has reported a positive association with periodontitis which may be due to the lack of presentation of true cases of periodontitis (cases were not well defined in the study) or geographical variations.[23]

In HLA-DR haplotypes, DR*7, DR*53, and DQ*3 were found to be positively associated with CP, whereas DR*1, DR*5, and DR*52 were associated with NP group. In a similar study by Alley et al. in 1993 and Dyer et al. in 1997 have reported positive association of HLA DR*53 and DQ*3 with adult periodontitis.[25,26] Moses et al. in 1994 have also reported the positive association with HLA-DR*7.[27] Although in studies by Alley et al.[25] and Dyer et al.,[26] DR*4 was found in association with CP, but it was insignificant in the present study.

Thus, significant variations with new alleles were observed in the present study, which were not observed by any of the previous investigator. However, this difference of result can be explained in several ways. Furthermore, the inconsistent findings could be caused by the difference of geographical and/or ethnological origin of populations under study. All the previous observations were made on Jewish, Turkish, Danish, German, British, Japanese, American, French, and Afro-Caribbean population. It is well documented that some HLA phenotype frequencies are characteristically prevalent in particular ethnic groups.[16,17,28] Hence, the result of the studies should be compared only when the studies are done in the same geographical area on a population of the same ethnic group. It is well established that microbes are responsible for the initiation of the inflammation of the periodontal tissues.[11] López et al. (1996) reported that the prevalence of certain bacteria in subgingival plaque in patients from different countries and ethnic groups differs.[20] In addition, researchers have isolated particular bacterial strains or serotypes in racially distinct population.[20] Hence, this can be considered that these factors could have bearing on the different HLA-related responses to periodontal pathogens in different population.

In addition to all, this study was performed in a certain geographical area (east India), where the racial variation was not considered in the study. However, with this limitation, the present study provides an additional insight of the different HLA phenotypes of the Bengali population of Eastern India, involved in pathogenesis of CP. However, these factors can only be considered as one of the factors involved in the disease process. However, this study can add up scientific data to reach at a certain level of definitive conclusion.

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Conflicts of interest
There are no conflicts of interest.

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