Int J Hum Genet, 4(2): 137-140 (2004)
PRINT: ISSN 0972-3757 ONLINE: 2456-6360 **DOI: 10.31901/24566330.2004/04.02.07**

Association of HLA -A*9 and A*10 with Aggressive Periodontitis in South India

Rasmi Thomas[1], Resmi C. Nair[2], K. Nandakumar[2] and Moinak Banerjee[1]

1. Human Molecular Genetics Laboratory, Rajiv Gandhi Centre for Biotechnology, Thycaud P.O., Trivandrum 695014, Kerala, India
2. Department of Periodontics, Government Dental College, Trivandrum 695020, Kerala, India

KEYWORDS Periodontitis; aggressive periodontitis; South India; HLA

ABSTRACT Periodontal diseases are essentially infectious in origin, their outcome depending on interaction between the pathogenic challenge and host response. Nevertheless the presence of an underlying genetic predisposition cannot be overlooked. HLA antigens have been considered as risk factors for periodontitis. The aim of the present study was to investigate the HLA-A*9 and HLA-A*10 association with aggressive periodontitis in the south Indian population. A significant increase of the HLA-A*24 antigen was observed in the patient group compared to the control samples. A positive association of HLA-A*24 with aggressive periodontitis was also noticed. HLA-A*10 did not vary significantly in both the study groups and is not having an association with disease. HLA typing was carried out using polymerase chain reactions with sequence specific primers (PCR-SSP).

INTRODUCTION

Human periodontitis is considered as a multifactorial disease, comprising of a heterogeneous group of infectious diseases characterized by the complex host-microorganism infections in the dental periodontium. The American Academy of Periodontology (Wiebe and Putnins 2000) classifies periodontitis as aggressive periodontitis (AP), chronic periodontitis and periodontitis associated with systemic disease (Table 1). Periodontal disease may differ with respect to bacterial etiology, host response and clinical disease progression. However the evidences suggest that underlying host susceptibility factors play a significant role in disease manifestation. Hereditary factors are suggested to play an important role in comparison to environmental factors in manifesting the early onset of periodontitis. Immunogenetic mechanisms may determine individual host susceptibility and thus result in the onset of periodontal disease, or individual predisposition to mild or aggressive severe forms of periodontitis. Several studies have demonstrated a high prevalence of infection in siblings of affected individuals (Boughman et al. 1987; Marazita et al. 1994; Long et al. 1987). With particular regard to possible genetic factors, the significance of various HLA (Human Leukocyte Antigen) markers have been investigated in several studies determining individual susceptibility factors for localized juvenile periodontitis, rapidly progressive periodontitis and adult periodontitis. From bacterial mimicry with HLA (Ebringer et al. 1983) and HLA dependent immunoreactivity to bacterial antigens (Buckley et al. 1973) an association with periodontopathic bacteria can be assumed. The most striking associations with periodontitis were found for HLA-A24 (Amer et al. 1988; Firatli et al.1996; Klouda et al. 1986). Negative associations with periodontitis have been reported for HLA-A10 (Amer et al. 1988; Klouda et al. 1986). Shapira et al. (1994) found no significant difference in HLA-A, B, C, DR and DQ antigens between patient and control group.

In the present study we are attempting to check the genetic predisposition of HLA-A9 and HLA-A10 with aggressive periodontitis (AP) in the south Indian population. Ethnicity and genetic predisposition is correlated wherein the association between HLA antigens and periodontal disease could be correspondingly different among various races. Previous studies have all been restricted to mainly the Caucasian (Amer et al. 1988), Negroid (Cullinan et al. 1980), Turkish (Firatli et al. 1996) and Japanese (Ashikaga et al. 1984) populations.

Table 1: Classification of periodontitis (American Academy of Periodontology, 2000)

Classification Strata	*Description*
Agressive Periodontitis	An otherwise clinically healthy patient -Rapid attachment loss and bone destruction -Amount of microbial deposits inconsistent with disease severity -Familial aggregation of diseased individuals
Chronic periodontitis	Amount of destruction consistent with local factors -Slow to moderate rate of progression -prevalent in adults but can occur in children-Possibly modified by systemic diseases(diabetes),environmental factors(cigarette smoking)
Periodontitis associated with systemic disease	-Hematologic disorders Acquired neutropenia Leukemias -Genetic disorders Down syndrome Papillon-Lefevre syndrome Chediak-Higashi syndrome Familial and cyclic neutropenia Leukocyte adhesion deficiency syndrome

MATERIALS AND METHODS

Aggressive Periodontitis: The clinical assessment for AP included the following parameters: plaque index (PI) and gingival index (GI). Both the maximum clinical probing depth (PD) and maximum clinical attachment loss (AL) for each tooth was derived by measuring six sites around each tooth and recording the maximum values. Alveolar bone loss on interproximal tooth surfaces was estimated by radiographs. The AP patients were selected on the following criteria: age of onset of periodontitis below 35 years, at least eight teeth with an attachment loss of 4mm or more, at least three affected teeth more than molars or incisors, more vertical than horizontal approximal bone loss in the affected sites, minimal accumulation of mineralized plaque in comparison to chronic periodontitis, bleeding on probing, increased mobility of certain teeth, rapid course and no systemic diseases.

Sample Collection: Seventy-eight healthy unrelated individuals belonging to the south Indian population served as ethnically and geographically matched controls. None of the persons among the control group were investigated for the presence of periodontal disease. The group of patients consisted of 20 unrelated AP samples of south Indian origin. They were undergoing treatment at the Dental College, Trivandrum, Kerala. The patient group included 17 females and 3 males.

DNA Isolation: 5 ml of venous blood was collected in vials containing the anti-coagulant EDTA. Genomic DNA isolation from the lymphocytes was carried out by the routine organic extraction method (Sambrook et al. 1998). Quality of DNA was checked in a spectrophotometer based on the 260/280 ratios.

HLA Typing: HLA-A9 and HLA-A10 typing was carried out by polymerase chain reactions using sequence specific primers (PCR-SSP) according to Tonks et al. (1997), following the guidelines of the 12th International Histocompatability Workshop. Internal controls amplifying a 796bp fragment from the third intron of HLA-DRB1 was used in all sample PCRs. Amplification conditions were established on a MJ Research PTC 225 machine with the previously said cycling parameters. The amplification products were visualized on 1.2% agarose gels containing 0.5 ìg/ml ethidium bromide after the addition of 5ìl loading dye consisting of 0.25% Orange G, 30% v/v glycerol and 0.5x TBE buffer. Orange G is an excellent marker as it migrates with the primer band on gel electrophoresis. The gel was documented in a Fluor-S ™ multi-imager (Biorad, USA).

Statistical Analysis: Statistical comparison observed to be more prevalent in females, which constitutes 85 percent of the patient population. No correlation between HLA alleles and gender was observed in the present study.

DISCUSSION

In the present study, the patients with AP were compared with a group of systemically healthy samples. From initial investigations in the AP samples of south India, it is clear that HLA-A24 is having a clear positive association with the disease. More than seventy percent of the patient group is HLA-A24 positive. The statistical significance is also very high indicating the high level of association of HLA-A24 allele with AP in the south Indian population. HLA-A23 is completely absent in the study group indicating that the association is with the A24 split in the south Indian population. (Table 2)

Table 2: HLA allele frequencies (in %) in patients and controls

HLA	*Patients (N=20)*	*Controls (N=78)*	*P/RR*
A*9	70	30	0.001#/2.63
A*23	0	0	-
A*24	70	30	0.001#/2.63
A*10	10	6.4	n.s

Increased HLA-A9 frequency was observed in early onset periodontitis (Firatli et al. 1996), juvenile periodontitis (Reinholdt et al. 1977; Kaslick et al. 1980; Cullinan et al. 1980, Marggraf et al. 1993), in rapidly progressive periodontitis (Kloude et al. 1986; Shapira et al. 1994) and in adult periodontitis (Reinholdt et al. 1977; Kaslick et al. 1980, Amer et al. 1988). The suggestion by Amer et al. (1988) regarding HLA-A10 as a marker for resistance does not hold good in the present study. The frequency of HLA-A10 was not significantly different in both the groups. The earlier study was in a Caucasian population while the present study is in a Dravidian population group and hence differences in HLA predisposition to diseases differ between racial groups.

In the present investigation, 85 percent of the patients were females out of which 75 percent were HLA-A24 positive. Out of the three male AP patients two were positive for HLA-A24. A recent study by Reichert et al. (2003) in German population reports that gender is a confounding variable in AP, and significant HLA deviations in relationship to AP and chronic periodontitis is present. Such a conclusion cannot be formulated from the study in the south Indian population since the majority of the AP patients were females.

This study is of particular importance as it is a first attempt to study the correlation of HLA alleles with aggressive periodontitis in India. Further studies have to be carried out in order to identify the various HLA haplotypes present in the AP patients. This would prove to be an important prophylactic step towards dental hygiene, since preventive measures could be instituted prior to the onset of disease.

ACKNOWLEDGEMENT

RT acknowledges the Council of Scientific and Industrial Research (CSIR) fellowship.

REFERENCES

Amer A, Singh G, Darke C, Dolby AE 1988. Association between HLA antigens and periodontal disease. *Tissue Antigens*, **31**: 53-58.

Ashikaga T 1984. Association between HLA antigens and periodontal disease. *Bell Josai Dent Univ*, **13**: 275-284.

Boughman JA, Astemborski JA, Suzuki JB 1992. Phenotypic assessment of early onset periodontitis in sibships. *Journal of Clinical Periodontology*, **26**:77-84.

Buckley CE, Dorsey FC, Corley RR, Ralph WB, Woodbury MA and Amos DB 1973. HL-A-linked human immune resoponse genes. *Proceedings of the National Academy of Sciences, USA*, **70**: 2157-2161.

Cullinan MP, Sachs J, Wolf E and Seymour GJ 1980. The distribution of HLA-A and –B antigens in patients and their families with periodontitis. *Journal of Periodontal Research*, **15**: 177-184.

Ebringer A 1983. Ankylosing spondylarthritis, HLA B27 and the theory of crossed tolerance. *Revue du Rhumatisme et des Maladies Osteo- Articulaires*, **50**: 763-769.

Firatli E, Kantarci A, Cebeci I 1996. Assocaiation between HLA antigens and early onset periodontitis. .*Journal of Clinical Periodontology*, **23**: 563-566.

Kaslick RS, West TL, Chasens AI 1980. Association between ABO blood groups, HLA-A antigens and periodontal diseases in young adults: A follow up study. *Journal of Periodontology*, **51**: 339-342.

Klouda PT, Porter SR, Scully C, Corbin SA, Bradley BA, Smith R, Davies RM 1986. Association between HLA-A9 and rapidly progressive periodontitis. *Tissue Antigens*, **28**: 146-149.

Long JC, Nance WE, Waring P, Burmeister JA, Ranney RR 1987. Early onset Periodontitis: a comparison and evaluation of two proposed methods of inheritance. *Genetic Epidemiology*, **4**:13 24.

Marazita ML, Burmeister JA, Gunsolley JC, Koertge TE, Lake K, Schenkein HA 1994. Evidence for autosomal dominant inheritance and race-specific heterogeneity in early-onset peridontis. *Journal of Periodontology*, **65**: 623-630.

Marggraf E, Von Keyserlingk-Eberius HJ, Komischke B,

Wollert N 1983. Die Assoziation von Histokompatibilitatsantigen (HLA-Antigene) mit profunden Parodontopathien. *Dtsch Zahnarztl Z*, **38**: 585-589.

Reichert S, Stein J, Gautsch A, Langner J, Schaller HG, Machulla HKG 2002. Gender differences in HLA phenotype frequencies found in German patients with generalized aggressive periodontitis and chronic periodontitis *Oral Microbiol Immunol*, **17**: 360-368.

Rheinholdt T, Bay I, Svejgaard A 1977. Association between HLA- antigens and periodontal disease. *Journal of Dental Research*, **56**: 1261-1263.

Sambrook J, Fritsch EF, Maniatis T 1989. *Molecular Cloning. A Laboratory Manual*. New York: Cold Spring Harbour Laboratory Press.

Shapira L, Eizenberg S, Sela MN, Soskolne A, Brautbar H 1994. HLA- A9 and B15 are associated with the generalized form, but not the localized form, of early-onset periodontal diseases. *Journal of Periodontology*, **65**: 219-223.

Tonks S, Marsh SGE, Bunce M, Bodmer JG 1999. Molecular typing for the HLA class I using ARMS-PCR: Further developments following the 12th International Histocompatability Workshop. *Tissue Antigens*, **53**:175-183.

Wiebe CB and Putnins EE 2000. The periodontal disease classification system of the American Academy of Periodontology—an update. *Journal of the Dental Canadian Association*, **66**:594-597.