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Association of Human Leukocyte Antigen with Periodontitis by Next Generation Sequencing – A Cross Sectional Study

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Abstract:
Human leukocyte antigen is known as transplantation antigens which helps in regulating immune response. It acts as a susceptibility factor for periodontitis which differs for different population. In this cross-sectional study a total of 10 periodontitis patients were selected. All their blood samples were analysed by complete locus sequencing by next generation sequencing NGS. Data was analysed statistically by MIA FORA 3.0 software (Immucor, USA) using an updated IMGT/HLA (Release: 3.38.0) database. HLA typing studied includes A; B; C; DRB1; DRB3,4,5; DQB1; DQA; DPB1; and DPA. It is found that NGS based HLA analyses showed that HLA typing and periodontitis are not related.

Keywords: *Human leukocyte antigen, HLA antigen, next generation sequencing and periodontitis.*

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I. Introduction

The term "Metagenomics" was coined by Jon Clardy and Robert M.Goodman, who published their data in 1998. It is defined as the genomic analysis of Microorganism by Direct extraction and cloning DNA from an assemblage microorganism. In Greek, "Meta means Transcenent" (Combination of separate analysis). Genomics refers to the study of genome (The Haploid set of chromosomes in a gamete or in each cell of a multicellular organism)[1,2.]

It is contrasting to the traditional genomic segment in many ways. For instance, met genomic techniques require greater attention to sampling and assessing the diversity of sample and extracting the appropriate nucleic acid from the sample is challenging. HLA typing is performed by next generation sequencing (NGS) and/or sequence specific probe hybridization (SSOP).

In addition, it is seen that genetic makeup of an individual has a pronounced effect on both immunity and manifestation of any disease. In this perspective, not every individual responds similarly to the given bacterial insult. Hence, there is an irrefutable need to link oral flora to some genetic factor. While an infection is fought by leucocytes, the genetic makeup manifesting in leucocyte makes or breaks the success. Hence, variations in leucocyte antigens can potentially have a role in microbial colonization.

However, there is a constant patrolling of leucocytes in the periodontium. Despite this defensive mechanism, infection sets in, implying that there is a failure in defense, thereby pointing out the inefficacy of leucocyte secretions and cellular recognitions. While it may not be possible to evaluate all genes and relate it to oral flora, one potential aspect that can have an influence is the HLA antigen.[3] Also, if there is a relation between HLA and periodontal flora, it enables the clinician to select the treatment plan accordingly, which would lead to better prognosis.

During the 1993 European Workshop, classification was simplified, consisting of adult and early onset periodontitis.[4] In 1999 classification of periodontitis reached finer details and is being used till now.[5] Periodontitis was reclassified as chronic, aggressive (localized and generalized), necrotizing and as a manifestation of systemic disease. Recent classification of periodontal diseases is based upon better alignment with pathophysiology.[6] Hence, it was classified as periodontitis, necrotizing periodontitis and periodontitis as a manifestation of systemic diseases.

Though the causative association of initial classification was well proven and treatments done according to such classifications have been clinically effective. But, when a classification is changed, organization and categorization of the causative organisms is essential to provide proper care to the patient.

This study relates periodontitis to genetic by means of HLA antigen. By this methodology, it would become possible to elaborate the direct link of bacterial species involved in the pathogenesis of periodontitis and to appraise and compare the role of microbiome and genetic factors among the three groups (aggressive, chronic

and health).

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II. Materials And Methods:

A total of 10 participants from South Indian population was screened from the outpatient department of periodontology, Sree Balaji dental college and hospital. Ethical Committee approval was obtained from the university's Ethical Board. 10 periodontitis patients were included in the study. The participants were well informed about the study, and written consent was obtained. All participants were assessed accordance with the previous classification system of periodontal diseases [AAP 1999]. Study participants include healthy individuals, chronic periodontitis and aggressive periodontitis. Inclusion criteria consists of patients with periodontitis having >5 mm of pocket depth and clinical attachment loss >2mm.

Exclusion criteria included pregnant patients, having taken antibiotics or undergone periodontal therapy in last 6 months; usage of anti-inflammatory drugs; smokers or former smokers; any known systemic illness, 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.

Human leukocyte antigen typing

Patients who reported to the OP of periodontics department were taken for the study. After careful subtraction of supragingival plaque with sterile cotton roll, subgingival plaque will be composed from deep sites in each quadrant using a sterile gracey curette. The subgingival plaque will be assembled in a sterile eppendorf tube covering 1ml of phosphate buffered saline. The illustrations will be deposited at -20 C till assay.

SAMPLE COLLECTION:

DNA extraction:

The DNA was extracted from 10 whole blood samples by using QIAamp® Blood Mini Kit (Qiagen, Germany), as per manufacture instruction. The quality and quantity of the extracted DNA was analysed using NanoDrop (Thermo scientific, USA).

HLA sequencing Protocol:

Long-range PCR targeting HLA genes was performed for the all 10 samples using MIA FORA NGS FLEX HLA Typing Kit (Immucor, USA). The amplification was performed for class I (A, B, C) whole gene, class II (DRB1) whole gene except intron 1, class II (DQB1) up to Exon 5, and class II (DPB1) Exon 2 to Exon 4. All amplicons were purified, size selected and library prepared and sequenced using Illumina Mini Seq NGS platform.

HLA sequence Analysis and Typing:

The HLA sequences of all 10 samples were typed by MIA FORA 3.0 software (Immucor, USA) using an updated IMGT/HLA (Release: 3.38.0) database.

III. Results:

The present study emphasis the current NGS approaches to capture, sequence and analyse HLA genes and loci. The impact of this new methodology has various applications including HLA typing, population genetics and disease association studies.

The table on HLA typing (Table 1) shows various presentations of periodontitis has revealed no association of HLA typing and periodontal disease. The HLA typing has been related to chronic and aggressive presentations of periodontitis. From the results it has been observed that HLA typing and periodontics are not related. Further, the earlier concept of specific bacteria like Aa or Pg involved in specific presentations has also been found to be not relevant.

TABLE 1: HLA TYPING

SAMPLE	HLA-A		HLA-B		HLA-C		HLA-DRB1		HLA-DQB1		HLA-DPB1	
	LOCUS 1	LOCUS 2	LOCUS 1	LOCUS 2	LOCUS 1	LOCUS 2	LOCUS 1	LOCUS 2	LOCUS 1	LOCUS 2	LOCUS 1	LOCUS 2
A4	01:01:01	24:02:01	08:01:01	51:01:01	07:02:01	15:02:01	01:01:01	03:01:01	02:01:01	05:01:01	02:01:02	04:01:01
A5	02:11:01	33:03:01	40:01:02	44:03:02	03:04:01	07:06:01	07:01:01	08:03:02	02:02:01	03:01:01	01:01:01	02:01:02
A11	24:02:01	24:07:01	27:05:02	40:01:02	02:02:02	03:04:01	01:01:01	11:01:01	03:01:01	05:01:01	04:01:01	849:01
A12	11:01:01	24:02:01	15:02:01	35:03:01	04:01:01	08:01:01	12.02:01	15:02:02	03:01:01	06:01:01	02:01:02	13:01:01
C1	01:01:01	24:02:01	39:06:02	57:01:01	06:02:01	07:02:01	04:03:01	10:01:01	03:02:01	05:01:01	03:01:01	04:01:01
C2	01:01:01	24:02:01	55:01:01	57:01:01	01:02:01	06:02:01	10:01:01	13:01:01	05:01:01	06:03:01	02:01:02	04:01:01
C3	30:01:01	68:01:02	07:02:01	35:03:01	04:01:01	07:02:01	04:03:01	04:03:01	03:02:01	03:02:01	02:01:02	04:01:01
H1	01:01:01	32:01:01	49:01:01	52:01:01	07:01:01	12:02:02	13:01:01	14:04:01	05:03:01	06:03:01	02:01:02	04:01:01
H2	02:11.01	24:02.01	13:01:01	51:01:05	04:03:01	14:02:01	12:02:01	14:04:01	03:01:01	05:03:01	04:01:01	04:01:01
H3	01:01:01	31:01:02	40:01:02	58:01:01	03:02:02	12:03:01	03:01:01	14:04:01	02:01:01	05:03:01	09:01:01	26:01:02

IV. Discussion:

Sippert et al., (2015)[7] have suggested that Human leukocyte antigens (HLA), due to their key role in immune response may have its implication of periodontal diseases. They have attempted to find the genetic basis or predisposition to CP using the circulation of HLA alleles in Brazilian population. The significant results showed a positive association of the A∗02/HLA-B∗ 40 haplotype with CP and a lower frequency of HLA-B∗ 15/HLA-DRB1∗ 11 haplotype in CP compared to panels. In summary, the HLA-A∗02/B∗40 haplotype may show the development of CP, while the HLA-B∗15/DRB1∗11 haplotype may show resistance to disease amid Brazilians. This implies the existence of immune component in periodontitis.

Jazi et al., (2013)[8] have investigated dissimilarities in allele and haplotype frequencies of HLA class II antigens in a set of Iranian patients with aggressive periodontitis and compared then with a healthy control group. The frequencies of HLA-DQA1*03:01, HLA-DQB1*03:02 and HLA-DQB1*03:05 alleles and HLA-DRB1*04:01 alleles were significantly higher in aggressive periodontitis subjects, when compared with control subjects. On the contrary, the frequency of the HLA- DQB1*0603 allele was considerably lower in aggressive periodontitis subjects. While considering haplotype association, a significantly higher frequency of two haplotypes

- HLA-DRB1*04:01/HLA-DQA1*03:01/HLA-DQB1*03:02 and HLA-DRB1*16:01/HLA-DQA1*01:03/HLA-DQB1*05:01was clearly seen in subjects with aggressive periodontitis. Their results show that class II HLA polymorphisms in the DQ locus, are associated with higher susceptibility to aggressive periodontitis.

Reichert et al., (2002)[9] have studied the incidence of "gender-dependent HLA associations" in a sample of 50 patients with generalized aggressive periodontitis (AP) and another sample of 102 patients with chronic periodontitis (CP), both in comparison to 102 probands without any sort of attachment loss caused by periodontitis. Female Aggressive Periodontitis patients displayed both increase in frequency of HLA-A*68/69 and decrease in the frequency of DRBblank* (non- DRB3/4/5*) and DQB1*05-positive probands. Also, in female Chronic Periodontitis patients HLA-DQB1*0303 was absent and HLA-DQB1*06 homozygosity significantly increased. They strongly suggest that gender is a major confounding variable, and hence should be considered in future studies of HLA relating to periodontitis.

Takashiba et al., (1999)[10] have reviewed the biology of early onset periodontitis and HLA genetics. The HLA-DRB1*1501-DQB1*0602 genotype was found with higher frequency in Early Onset Periodontitis patients. The T cell response against the Ag53- outer membrane protein of P.gingivalis was examined through this HLA genotype. T cell response was inhibited partially by anti-DR antibody but not anti-DQ antibody. Host and bacterial peptides that are capable of binding DRB1*1501 were elucidated while the peptide sequence was compared to gene and protein databases. They strongly suggest that patients with HLA-DRB1*1501-DQB1*0602 genotype may be having an accelerated T cell response to certain bacteria in hyperimmune reactions and consequent increased susceptibility to Early Onset Periodontitis.

Stein et al., (2003)[11] have analyzed the incidence of HLA and estimated haplotypes in German Caucasian groups with generalized aggressive and chronic periodontitis and compared it to control probands without periodontitis. They have elaborated a variety of HLA associations and found the difficulty in assigning single HLA markers to any periodontal disease. Susceptibility of both periodontites may be influenced by certain HLA marker combinations. The variation of associations in aggressive and chronic periodontitis may indicate variation in susceptibility or resistance factors for both diseases.
Goteiner et al (1984)[12] reported that the frequency of HLA-A, -B,
-C haplotypes in patients, who are resistant to chronic periodontitis. They also attempted to determine any association between specific HLA genes and periodontal health. 25 high resistance healthy individuals were matched to 25 subjects, who had chronic periodontitis, and also to a periodontal undiagnosed population (22,000 individuals). A significant correlation was seen for HLA-A28 in blacks and HLA-B5 in whites. They have opined that HLA-A28 and HLA-B5 individuals may be resistant to progression of chronic periodontitis.

Ohyama et al., (1996)[13] have performed DNA typing on Japanese patients with early- onset periodontitis (N=24). Their results have shown that DQB1 molecule has a key role in the onset and progression of EOP. Further, susceptibility to EOP may be found by the binding ability of the peptide and HLA-DQ antigens.

Roshna et al., (2006)[14] had reported a study which assessed the association of HLA- A*9 & HLA-B*15 with generalized aggressive periodontitis. Also, they evaluated their role in influencing the severity of such conditions in a South Indian population of 40 cases and 80 healthy subjects (as controls). HLA-B*15 was identified as an important risk factor and was seen to be positively correlated with the disease severity. However, HLA-A*9 had no significant association with the disease. Therefore, haplotype of HLA-A*9:B*15 did not bestow additional risk for patients. They also have concluded that the underlying pathophysiology and association being "causal" or "casual" is yet to be investigated.

Zhang et al., (2004)[15] have investigated the association of HLA-DRB1*1501 polymorphism and susceptibility to severe chronic periodontitis in Chinese Han nationality. In their study, 134 subjects with severe chronic periodontitis and 81 healthy subjects (Control) were involved in the study. The allele frequencies of DRB1*1501 was detected in higher frequency in severe CP patients. The 1501/1501 genotype frequencies were considerably increased in subjects compared with the given reference population. Hence, they suggest that HLA-DRB1*1501 allele may be a risk indicator for the susceptibility to severe CP in Han nationality. The HLA- DRB1*1501 homozygote genotype was associated significantly with severe CP.

Okada et al., (2002)[16] have reported "microflora, immunological profiles of host defense functions, and human leukocyte antigen (HLA) findings for a mother, son and daughter who were diagnosed as having 'periodontitis as a manifestation of systemic diseases, associated with hematological disorders'". It was observed that members of this family showed a similar prediliction to periodontitis and also with regard to certain host defense functions. Authors have suggested that the depressed neutrophil chemotaxis could be a risk factor for this kind of familial periodontitis.
HLA typing of 10 samples was done using whole blood by complete locus sequencing by NGS.

V. Conclusions:

Within the limitations of the study, it is concluded that there is no relation between HLA antigen and the pathogenesis of periodontitis.

ETHICAL CLEARANCE:
Institutional ethical clearance from Bharath institute of science and technology were obtained.

FUNDING:
No funding was obtained.

CONFLICT OF INTEREST:
It was found that there was no conflict of interest regarding the study.

CONTRIBUTION:
Author 1: Contributed to conception, design, data acquisition and interpretation and critically revised the manuscript Author 2: Performed all statistical analyses, drafted and critically revised the manuscript Author 3: Performed Drafting, critically revised the manuscript 4: Critically revised the manuscript

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