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ORIGINAL ARTICLE

Examining Caries Risk With the Characteristic of HLA-DRB1 Gene

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ABSTRACT

Introduction: HLA-DRB1 alleles were derived from MHC class II molecules. These alleles encoded IgA secretion which contribute as a barrier to *S. mutans* colonization. HLA-DRB1 was known as genes with high mutations causing differences in peptide bond, thus affecting the progression and vulnerability to a disease. **The purpose of this study was to determine the effect of mutations in HLA-DRB1 alleles in different dental caries risk.** **Methods:** In this study, we extracted the genomic DNA from whole blood samples of 30 patients with low level dental caries (indeks deft < 2) as control group and high level dental caries (deft > 3) as case group. HLA-DRB1 variants were studied through genomic DNA isolation for PCR-RFLP. RFLP is analyzed through BseRI, BsaJI, RsaI, and Sau96I restriction enzymes was used in this assay. **Results:** The PCR-RFLP typing method was evaluated on 60 genomic DNA samples, result found that all samples were divided into 5 groups of variants, two variants in the control group and three variants in the case group. **Conclusion:** PCR-RFLP was shown to be a sensitive method for the detection of mutation in HLA-DRB1 alleles caused a dental caries level differences. HLA-DRB mutations caused changes in signal transduction and therefore contributes to imunogenetik pathway of dental caries.

Keywords: Caries risk, HLA-DRB1, Gene mutation

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INTRODUCTION

The Major Histocompatibility Complex (MHC) molecule is a membrane protein in Antigen-Presenting Cell (APC) that presents antigen peptides to be recognized by T lymphocyte cells. MHC molecule is a genetic locus that plays a major role in tissue grafting between individuals. The physiological function of MHC molecules is to present peptides derived from APC. The human MHC protein is known as Human Leukocyte Antigens (HLA) and there is an HLA locus in the gene that codes for this molecule (1,2).

Extracellular microbes or microbial proteins is recognized by APC, then processed and presented by MHC class II molecules. APC molecules recognize this protein through various mechanisms, namely microbial phagocytosis or protein pinocytosis. After the introduction of APC with the various pathways above, microbial proteins enter intracellular vesicles called endosomes or phagosomes to be fused together with the HLA molecule, the name for MHC in humans. This superlocus contains a large number of genes that are related to the function of the

immune system in humans. HLA-DRB1 was a gene group that located on chromosome 6. It was a locus of 266 bp, located at nucleotides 136-402 in the HLA gene (gene bank Reference Sequence: NM_002124.2) (2,3).

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The loci diversity in MHC class I and II is very extensive with thousands of loci that have been discovered. HLA-DRB1 mutations occur in a population through balance selection, especially in the selection of pathogenicity patterns in heterozygote development. This shows that HLA-related diseases differ in each ethnic group. Research in several different ethnic groups concludes that the HLA-DRB1 locus is a marker of the susceptibility of a disease in an ethnic group to another ethnic group (4).

HLA class II is a locus with high mutation in various mammals. This mutation causes differences in peptide bonds, thereby affecting the functional progression and susceptibility of a disease. HLA-DRB1 presents antigens from APC to CD4 + T cells. CD4 cell count is a major clinical indicator of immune competence and plays a role in determining diagnosis and therapy.

Mutation of HLA-DRB1 exon 2 region is related to the pathogenesis of dental caries, because changes in the amino acid composition in the exon 2 region of the HLA-DRB1 locus cause abnormalities in the

presentation of antigen peptides to T cell receptors. There is a correlation between HLA locus mutations with CD4 counts as indicators of infection and defense humoral. Mutation in HLA-DRB1 is one of the genomic factors of dental caries that has an important role in the immunogenetic pathway in the pathogenesis of dental caries (5,6). This study was conducted to predict the response of mucosal immunity to cariogenic bacteria by tracking the mutation of HLA-DRB1 alleles in the Javanese population in Surabaya Indonesia.

MATERIALS AND METHODS

Samples

The type of this research is case-control, with matching cases and controls in terms of age, ethnicity and gender. The study population was elementary school students from Javanese, aged 6-9 years, in the Surabaya area. The case population are 30 students with low sIgA (<300ng / ml) and the control population are 30 students with high sIgA (> 300ng / ml) as described in previous studies (7). The approval and ethical clearance were obtained from Health Research Ethical Clearance Commission, Universitas Airlangga Faculty of Dental Medicine 307/HERCC.FODM/XII/2018.

HLA-DRB1 Locus amplification

Two cc of blood from the cubital vein were collected in EDTA vacutainer, separated by serum and buffy coat. buffy coat is used for HLA-DRB1 examination. genomic DNA analysis is carried out through DNA extraction. DNA amplification was performed on exon 2 HLA-DRB1 of 266bp. The results of DNA isolation were examined for the purity and concentration of DNA. The results of this examination are then used as a reference for determining the amount of DNA in the HLA-DRB1 locus amplification via PCR using Forward primer DRB3: 5 'CAC GTT TCT TGG AGT ACT CT 3' and Reverse primer AmpB: 5 'CCG CAC TGT GAA GCT CT 3' .

Restriction Fragment Length Mutation (RFLP) analysis

RFLP was performed on DNA amplicon using the enzymes BseRI, BsaJI, RsaI and Sau 961. BseRI is a restriction enzyme that will cut the PCR amplicons from the sequence of GAGGAGn. BsaJI cuts the PCR results from the sequence C'CnnGG. RsaI is a restriction enzyme that will cut the PCR amplicons from the GT'AC recognition sequence. Sau961 cuts the PCR results from the G'GnCC sequence identifier. These four restriction enzymes will each cut the PCR amplicons into 2 bands (5,8).

RESULTS

RFLP analysis for exploits variations in DNA sequences

RFLP analysis was performed in the 30 control subjects with high level sIgA and the 30 case subjects with low level sIgA. HLA-DRB mutation examination is done through DNA isolation from blood samples. The

results of DNA isolation were examined for purity and DNA concentration, then this examination was used as a reference for determining the amount of DNA used in DNA amplification via PCR. Electrophoresis was performed to determine the location of the HLA-DRB amplicon at 266 bp. DNA amplicons are analyzed through RFLP to determine the restriction of nucleotides which causes mutation.

Restriction fragments of BseRI and RsaI was positive on 25 patients with high level sIgA. Restriction fragments of BsaJI and Sau961 was positive on 9 patients with low level sIgA. Restriction fragments of BsaJI only was positive on 10 patients with low level sIgA. Restriction fragments of BsaJI, RsaI and Sau961 was positive on 11 patients with low level sIg. There were five subjects from high level sIgA which is not digested by four restriction enzymes that used in this study. From the RFLP results, all samples are divided into 5 variant groups, two variants in the control group and three variants in the case group. The RFLP data is shown in table I.

Table I: RFLP data used BseRI, BsaJI, RsaI, and Sau961 enzymes in 5 sample variants

Group	Variant Group	BseRI	BsaJI	RsaI	Sau961
High level sIgA	1	-	-	-	-
	2	+	-	+	-
Low level sIgA	3	-	+	-	+
	4	-	+	-	-
	5	-	+	+	+

Sample distribution for each HLA-DRB1 variant based on PCR-RFLP analysis

The low level sIgA group consisted of three HLA-DRB1 variants and the control sample group with high level sIgA consisted of two HLA-DRB1 variants. The frequencies of restriction fragments distribution on two groups of variant group 1-5 are summarised in table II.

Table II: Distribution of samples in each HLA-DRB1 variant group based on PCR-RFLP analysis

Variant Group	Low level sIgA	High level sIgA	Σ
1	0	5	5
2	0	25	25
3	9	0	9
4	10	0	10
5	11	0	11
Σ	30	30	60

DISCUSSION

sIgA secretion from gingival crevicular fluid and the presence of sIgA in saliva play a role in the pathogenesis of dental caries. Salivary gland hypofunction influences salivary flow rate and also affects the development of

dental caries (9).

Saliva secretion in saliva is controlled by the HLA-DRB1 locus, a fragment in the HLA class II gene. Multiplicity loci in heavy chains (α) and light chains (β) against antigens cause high diversity of HLA class II molecular combinations in humans. Increased susceptibility of some diseases is known to be associated with the HLA locus, because this locus is associated with an increase in immunology. Research shows IgA deficiency is inherited and is associated with HLA. The pathogenesis of IgA reduction is generally caused by transcription errors when DNA rearrangement in cell B. IgA levels in some individuals will increase with age due to decreased IgA suppressor factor in B cell differentiation (10).

The strength of antigen peptide presentation by HLA-DRB1 to T cell receptors is related to the formation of Th2 and activation of B cells that lead to the level of sIgA secretion. Based on the explanation related to HLA and disease associations, it is clear that the HLA system collaborates with other factors that are not related to genes, and environmental factors have an influence on the process of occurrence of a disease (11). Differences in HLA-DR in encoding patterns of immunity in several different races have been reported in various studies. Idiopathic membranous glomerulonephritis in caucasians is coded by DR3, whereas in Japan it is coded by DR2. Some diseases such as hyperthyroidism, rheumatoid arthritis and type I diabetes mellitus, also show a different relationship between each race with HLA-DRB variants (12).

5 In this study we determine the effect of mutations in HLA-DRB1 alleles to find out why there are some differences in dental caries risk. We speculated that there is heterogeneity in dental caries process and that the sIgA plays a part in only some patients. It is possible that dental caries index may differ depending on HLA-DRB1 mutation. HLA-DRB1 variants further influence the HLA-DRB1 transduction signal to T cells, this affects the production of antibodies that appear through sIgA levels. Furthermore sIgA level influences the blockade of the initiation of bacteria that cause dental caries. From this hypothesis it can be concluded that the mutation in HLA-DRB affects the dental caries index.

CONCLUSION

From recent study we concluded, differences in restriction patterns affect sIgA levels. At the low level of sIgA, there are three enzyme restriction, BsaJI, RsaI and Sau96, that are thought would influence the HLA-DRB1 signal transduction and cause a decrease in sIgA level. HLA-DRB mutations caused changes in signal transduction and therefore contributes to imunogenetik pathway of dental caries. There may be many genetic factors in the pathogenesis pathway of low level sIgA and it is possible that HLA-DRB1 is only one of them. Further research is needed to be conducted regarding

the requirements for HLA data collection from various populations.

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