ANALISIS VARIAN HLA-DRB1 PADA JALUR IMUNOGENETIK SEKRESI sIgA SALIVA SEBAGAI RISIKO KARIES GIGI
(PENELITIAN PADA POPULASI JAWA DI SURABAYA)

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IMMUNOGENETIC; DENTAL CARIES

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RINGKASAN

ANALISIS VARIAN HLA-DRB1 PADA JALUR IMUNOGENETIK SEKRESI sIgA SALIVA SEBAGAI RISIKO KARIES GIGI

Karies gigi merupakan penyakit infeksi yang menimbulkan masalah kesehatan di beberapa negara maju dan berkembang. Karies gigi adalah demineralisasi jaringan gigi yang bersifat kronis. Produksi asam hasil fermentasi karbohidrat oleh kolonisasi bakteri menyebabkan kerusakan kristal hidroksi apatit sehingga komponen enamel dan dentin pada jaringan keras gigi mengalami demineralisasi.

Risiko karies gigi salah satunya dikendalikan oleh saliva karena keberadaan *Secretory Immunoglobulin A* (sIgA) sebagai substansi anti bakteri. Penelitian sebelumnya membuktikan terdapat hubungan antara aspek genetik dan respon imunitas terhadap karies gigi. Penelitian pada manusia dan hewan membuktikan perbedaan genetik menyebabkan penyimpangan imunomodulator terhadap antigen yang berperan pada karies gigi. Eliminasi antigen kariogenik melibatkan respon imun alami dan respon imun adaptif. *Major Histocompatibility Complex* (MHC) berperan dalam imunitas seluler untuk merangsang sistem imunitas melalui presentasi antigen kepada reseptor sel T. MHC pada manusia dikenal dengan *Human Leucocyte Antigen* (HLA). Lokus HLA-DRB1 menyandi rantai beta fungsional dan bersifat sangat polimorfik. Polimorfisme ini menyebabkan perbedaan ikatan peptida sehingga mempengaruhi progresi dan kerentanan suatu penyakit secara fungsional. Variasi genotip HLA-DRB disebabkan oleh mutasi pada ekson 2 gen HLA. Sebagian besar mutasi berupa *Single Nucleotide Polymorphism* (SNP). Mutasi ini menyebabkan perubahan susunan pasangan basa sehingga menimbulkan perubahan asam amino. Berbagai penyakit genetik yang disebabkan oleh mutasi titik diketahui menimbulkan modifikasi atau inaktifasi produk gen. Mutasi titik di regio ekson 2 HLA-DRB terkait erat dalam patogenesis karies. Hal ini diduga terjadi karena perubahan susunan pasangan basa regio ekson 2 pada gen ini menyebabkan perbedaan ikatan peptida antigen. Berbagai sitokin bekerja di jalur ini yaitu IL-2, IL-4, IL-10 dan TGF-β. TCR dan ko-reseptor bekerja secara simultan untuk inisiasi respon sel T agar terjadi aktifasi sel B sehingga menghasilkan sekresi sIgA pada permukaan mukosa. Penelitian ini bertujuan untuk mengetahui pengaruh mutasi pada lokus HLA-DRB1 terhadap perbedaan kadar sIgA sebagai risiko karies gigi serta peran polimorfisme HLA-DRB1 terhadap perbedaan jumlah CD4 dan ekspresi tgf-β1 sebagai regulator *switching isotype* pada jalur imunogenetik sekresi sIgA. Metode penelitian meliputi analisa DNA pada kelompok sampel dengan kadar sIgA rendah dan tinggi berdasar uji

ELISA. Polimorfisme HLA-DRB1 diteliti melalui isolasi DNA untuk PCR-RFLP dilanjutkan dengan sekuensing. Hasil sekuensing digunakan untuk penentuan tata-nama varian HLA-DRB1 dan analisis homologi melalui bioinformatik. Jalur imunogenetik sekresi sIgA diteliti melalui hubungan varian HLA-DRB1 terhadap jumlah CD4 dan ekspresi TGF-β1. Uji statistik dilakukan untuk menganalisis perbedaan kadar sIgA, jumlah CD4 dan ekspresi TGF-β1 pada tiap varian HLA-DRB1. Uji korelasi *Pearson* untuk menganalisis korelasi antara sIgA,CD4 dan TGF-β1 serta korelasi antara sIgA dan indeks *def-t*. Analisis jalur digunakan untuk menganalisis hubungan antara sIgA,CD4 dan TGF-β1. Hasil penelitian diketahui varian HLA-DRB1 dengan nomenklatur DRB*1209(2), DRB*1209(3) dan DRB*1209(4) berhubungan dengan kadar sIgA rendah dan resiko karies tinggi. Varian DRB*1209dan DRB*1209(1) berhubungan dengan kadar sIgA tinggi dan resiko karies rendah. Penyebab peningkatan resiko karies ini adalah penurunan kekuatan presentasi bakteria karies oleh HLA-DRB1 kepada reseptor sel T. Penurunan kekuatan presentasi pada DRB*1209(2), DRB*1209(3) dan DRB*1209(4) secara kuat berkaitan dengan delesi nukleotida sehingga menyebabkan *frameshift* dan posisi stop kodon maju ke posisi kodon ke 61 pada DRB*1209(2) dan DRB*1209(3) serta kodon ke 79 pada DRB*1209(4), sehingga menyebabkan translasi berhenti lebih awal. *Premature Termination Codons* (PTC) adalah kodon stop yang terbentuk sebelum akhir translasi. Hal ini menyebabkan polipeptida lebih pendek sehingga presentasi peptida antigen ke sel T reseptor tidak sempurna. Uji korelasi Pearson menunjukkan jumlah CD4, ekspresi TGF-β1 dan kadar sIgA pada seluruh sampel terdapat korelasi kuat dengan p=0,000. Korelasi sIgA dan indeks *def-t* pada seluruh sampel menunjukkan hubungan yang berlawanan dimana semakin tinggi nilai *def-t* maka kadar sIgA semakin rendah, demikian pula sebaliknya. Hasil analisis jalur diperoleh CD4 mempengaruhi sIgA melalui TGF-β1, karena TGF-β1 berpengaruh langsung terhadap sIgA dan CD4 berpengaruh terhadap TGF-β1. Pada penelitian ini disimpulkan bahwa mutasi pada lokus HLA-DRB1 menyebabkan perubahan kadar sIgA sehingga berpengaruh terhadap risiko karies gigi. Polimorfisme HLA-DRB1 menyebabkan perubahan jumlah CD4 dan perubahan ekspresi tgf-β1 pada jalur imunogenetik sekresi sIgA.

SUMMARY

VARIANCE ANALYSIS OF HLA-DRB1 ON IMMUNOGENETIC PATHWAY OF SALIVARY sIgA SECRETION AS RISK IN DENTAL CARIES

Dental caries is an infectious disease that causes health problems in some developed and developing countries. Dental caries is a demineralization of tooth tissue that is chronic. Production of acids from fermentation of carbohydrates by bacterial colonization cause damage of hydroxy apatite crystals and demineralization of enamel and dentin components on dental hard tissues. Dental caries risk is controlled by the saliva because of the presence of secretory immunoglobulin A (sIgA) as an anti-bacterial substance. Previous research has shown there is a relationship between immune response and genetic aspects of dental caries. Research in humans and animals show genetic differences cause immunomodulator deviations against antigens that play a role in dental caries. Elimination of cariogenic antigen involving innate immune responses and adaptive immune responses. Major Histocompatibility Complex (MHC) play a role in cellular immunity to stimulate the immune system through antigen presentation to T cell receptor.

MHC in humans known as Human Leucocyte Antigen (HLA). HLA-DRB1 locus encodes a functional beta chain and are highly polymorphic. This polymorphism causes differences in peptide bond and thus functionally affects the susceptibility and progression of a disease. HLA-DRB1 genotype variation is caused by mutations in exon 2. Most of the mutations are single nucleotide Polymorphism (SNP). These mutations cause changes in the composition of base pairs, giving rise to amino acid changes. A variety of genetic diseases caused by point mutations known to cause modification or inactivation of gene product. Point mutations in the region of exon 2 of HLA-DRB1 locus closely linked in the pathogenesis of caries. Thought this is occur because of changes in the composition of base pair region of exon 2 in this gene cause differences in antigen peptide bond. Various cytokines work in this line are IL-2, IL-4, IL-10 and TGF-β1. TCR and co-receptors work simultaneously for the initiation of T cell response for B cell activation resulting in secretion of sIgA at mucosal surfaces. The aims of this study was determine the effect of mutations in HLA-DRB1 locus to the difference levels of sIgA as the risk of dental caries and the role of HLA-DRB1 polymorphism on the difference of CD4 count and expression of TGF-β1 as a regulator of isotype switching in immunogenetic pathway of sIgA secretion. Methods of this research include DNA analysis on samples with low and high levels of sIgA by ELISA. HLA-DRB1 polymorphisms studied through the isolation of DNA for PCR-RFLP followed by sequencing. Sequencing is used for the determination of nomenclature variants of HLA-DRB1 and homology analysis through Bioinformatics. Immunogenetic pathway of sIgA secretion studied through the relationship of HLA-DRB1 variant of the CD4 cell count and expression of TGF-β1. The statistical test performed to analyze differences in sIgA levels, CD4 cell count and expression of TGF-β1 in each variable of HLA-DRB1. Pearson correlation used to analyze the correlation between sIgA, CD4 and TGF-β1 and the correlations between sIgA and index def-t. Path analysis used to analyze the relationship between sIgA, CD4 and TGF-β1. The results of this research are variants of HLA-DRB1 with the nomenclature of DRB * 1209 (2), DRB * 1209 (3) and DRB * 1209 (4) associated with low sIgA levels and high caries risk. Variant DRB * 1209dan DRB * 1209 (1) associated with sIgA levels high and low caries risk. The cause of the increased risk of caries is the declining of caries bacteria presentation by HLA-DRB1 to T cell receptor. Presentation declining of the DRB * 1209 (2), DRB * 1209 (3) and DRB * 1209 (4) are strongly associated with nucleotide deletions resulting in frameshift and earlier stop codon positions at codon 61 on the DRB * 1209 (2) and DRB * 1209 (3) and codon 79 on the DRB * 1209 (4), causing the translation stop early. Premature Termination Codons (PTC) is the stop codon that formed before the end of translation. This leads to a shorter polypeptide so that the antigen peptide presentation to T cell are not going well. Pearson correlation showed CD4 cell count, expression of TGF-β1 and sIgA levels in all samples have a strong correlation with $p = 0.000$. Correlation sIgA and index def-t on the entire sample showed the opposite relationship in which the higher value of def-t the lower sIgA levels, and vice versa. Results obtained by path analysis showed that CD4 affects sIgA via TGF-β1, because TGF-β1 directly influence the sIgA and CD4 effect on TGF-β1. The study has concluded that a mutation in HLA-DRB1 locus cause changes in sIgA levels and therefore contributes to the risk of dental caries. HLA-DRB1 polymorphisms cause changes in CD4 cell count and changes in expression of TGF-β1 on the immunogenetic pathway of sIgA secretion.

ABSTRACT

VARIANCE ANALYSIS OF HLA-DRB1 ON IMMUNOGENETIC PATHWAY OF SALIVARY sIgA SECRETION AS RISK IN DENTAL CARIES

HLA-DRB1 allele was derived from MHC class II molecules and play an important role in the control of antigen peptides presentation to TCR that encoded sIgA secretion which contribute to prevent S. mutans colonization. The purpose of this study were to determine the role of HLA-DRB1 polymorphisms that affect the CD4 count and TGF-β1 expression on immunogenetic pathway of sIgA secretion in saliva and raises the risk of dental caries. Methods of this research were DNA analysis on case and control sample groups with low and high levels of sIgA by ELISA test. HLA-DRB1 polymorphisms were studied through DNA isolation for PCR-RFLP proceeded with sequencing. Immunogenetic pathway of sIgA secretion was studied through the relationship between HLA-DRB1 variant to CD4 count and TGF-β1 expression. Results proved that nomenclature of HLA-DRB1 variant DRB * 1209 (2), DRB * 1209 (3) and DRB * 1209 (4) were associated with low sIgA levels and high caries risk. Whereas DRB * 1209 and DRB * 1209(1) were associated with high sIgA levels and low caries risk. Path analysis showed that CD4 affects sIgA via TGF-β1, because TGF-β1 directly influence the sIgA and CD4 effect on TGF-β1.

The study has concluded that mutation in HLA-DRB1 alleles cause changes in sIgA levels and therefore contributes to the risk of dental caries. HLA-DRB1 polymorphisms contributes to the change in CD4 cell count and changes in expression of TGF-β1 on the immunogenetic path of sIgA secretion.

Keyword: HLA-DRB1, DNA analysis, sIgA level, CD4 count, TGF-β1 expression, dental caries.