RISK FACTORS OF MUTATION NUCLEOTIDE OLIGOMERAZATION DOMAIN 2 GENE TO CHRONIC PERIODONTITIS

(MUTASI GEN NUCLEOTIDE OLIGOMERAZATION DOMAIN 2 (NOD2) SEBAGAI FAKTOR RISIKO TERHADAP TERJADINYA PERIODONTITIS KRONIS)

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Abstract

Chronic periodontitis is a chronic inflammatory disease that causes a damage of tooth supporting tissues. The incidence of chronic periodontitis disease is highly reported in Indonesia.Survey in Java, Bali and Sulawesi reported the incidence of chronic periodontitis for 35-44 years age group was 88.67%. This disease is a major cause of tooth loss for 35 years or more age group. The results of various studies was found many chronic periodontitis diseases are common in young age, therefore the genetic role in diseases is susceptible for chronic periodontitis. The aim study was to know if the mutation of the gene Nucleotide-Binding Oligomerization Domain 2 (NOD2) is a risk factors for chronic periodontitis. Total sample was 162 subject, 81 samples with chronic periodontitis and 81 without periodontal disease as control group. The results showed that there was 12,3 % of samples that had been mutation of NOD2 gene, and in the control group only 1,2 %, and statistically there was a significant difference (p=0,005). In conclusion, mutation NOD2 gen is a risk factor to chronic periodontitis.

Key words: mutation, nucleotide-binding oligomerization domain 2 gene, chronic periodontitis

INTRODUCTION

Periodontal disease is initiated with microbial plaque that is accumulated on gingival cervical region and cause inflammatory response. This condition is marked by the presence of chronic gingivitis and continued to be destructive chronic swelling.[1] Beside microbial and environment factors, gene is believed become an important factors in periodontal disease and supported with the finding of some strong evidences. [2]

Clinical and scientific fact showed that genetic factor is an important determinant of periodontitis susceptibility and the pathogenesis. It is supported by the study in human and animal that showed influence of genetic factors inflamematory response, immune system generally, and periodontitis specifically. Individual responses differently toward environmental change and those different response is influence by the individual genetic profile. Various gene form (alel varian) could produce variation of tissue structure (acquired immune), antibody response (adaptive immune), and mediator inflamematory (non-specific inflammatory). Alel that is found in different gene locus may influence the susceptibility of periodontitis. [3]

The most prominent evidence on genetic risk in chronic periodontitis is the occurrence of chronic periodontitis is not getting along with the increase of age, but some studies showed the younger age. It shows that chronic periodontitis is correlated with the genetic role in the susceptibility of chronic periodontitis incidence.[4,5]

Genetically, human body could detect the infection through nucleotide-binding oligomerization domain 2 (NOD 2). NOD 2 is the initial protein that function in bacterial detection dan recognize the present of lipopolysacharide protein whether it is a negative gram or positive gram through introduction of peptide muramil in the leucinrich bacteria that is repeat on NOD 2.[6,7] The binding of peptide muramil and NOD 2 will activate nuclear factor kB (Nf-kB) and induce apoptosis, whereas this factor is one of transcription factor that is highly effective in in-

flamatory support cytokines secretion. NOD 2 mutation will reduce body's capability to eliminate pathogen bacteria.[8-10] NOD 2 frameshift mutation play a role in the apoptosis management, physiology of cell's death that caused by the caspase that is important in the pathogenesis of periodontitis.[11]

MATERIALS AND METHODS

This research was performed in the Periodontology Department of Oral and Dental Hospital, Dentistry Faculty, Hasanuddin University, Makasar. Total sample was 162 subjects, consisted of 81 samples with chronic periodontitis, and 81 samples without chronic periodontitis as control group. The age range of subject was 30-60 years old. The age group is a reason to minimalize the influence of age on the predisposition of individual genotip. Written and oral informed consent was obtained from all subject based on the Agreement of Ethical Committee Medical Faculty Hasanuddin University.

Boom methods were used in the extraction and DNA purity. A total 2-3 cc of venous blood sample was added into the eppendorf tube with 900 µl Buffer lisis L6.[12] The blood sample was rotated in Gerhardt for 24 hours in a lying down position then centrifugated for 15 minutes and added 40 µl of cellite suspension [diatoms] which already homogenized in the vortex shaker. This solution was rotated in the Gerhardt at 100 rpm and 12.000 rpm for 15 second, respectively.

The formed supernatant were separated with micropipe and rinsed, it was added with 1 ml Buffer L2, homogenized and centrifugated at 12.000 rpm for 15 seconds. The rinsing process which using Buffer L2 was done twice. Then, 1 ml etanol 70% was added, homogenized and centrifugated at 12.000 rpm for 15 seconds. Rinse with etanol 70% twice.

The supernatant was mixed with 1 ml acetone, homogenized and centrifugated. The supernatant was removed and the tube was left opened. Then the tube was placed in the oven of 55ºC for 10 minutes until it is dried, and added with 80 µl Buffer TE solution, homogenized [up-down] for 15 times, centrifugated for 30 seconds to tied the DNA. The samples was homogenized with vortex shaker [up-down], centrifugated for 30 seconds. The formed supernatant was placed in the eppendorf tube and labeled.

The amplification of NOD 2 gene DNA was done using restriction fragment enzyme lenght polymorphism-polymerase chain reaction (RFLP-PCR) method. The samples were placed in the PCR tube which contained 100 mg DNA genome mixed with PCR buffer (10mM Tris-HCl pH 8,3; 50 mM KCl; 1,5 mM $MgCl_2$), 200 uM dNTP, 1,25 enzyme DNA polymeraze and 10 pmol of primary pairs (which is, sense: 5'CCCAGCTCCTCCCTCTTC3' and Antisense 5' AAGTCTGTAATGTAAAG-CCAC 3'). The samples were incubated in a PCR machine, initial denaturations were done at 95ºC for 5 minutes, followed with 35 PCR cycles which included denaturation at 95ºC for 45 seconds, annealing at 53ºC for 40 seconds, and extension at 72ºC for 30 seconds.

After the additional cycle completed, the same volume of fenol-chloroform solutions was added into the sample, mixed in the tube for 10 seconds, and centrifuged at 14.000 rpm for 10 minutes. The formed supernatant was aspirated and placed in a new tube.

To recognized if the DNA target amplificated in the correct way, the PCR product was placed in a 2% agarose gel which already added with TAE 10x solutions mixed with ethium bromide. Two µl product samples of loading buffer (0,25% of bromophenol blue, 40% b/v sucrose) and 4 µl dH2O were mixed with 2 µL PCR product samples. This solutions were mixed homogenically and aspirated 8 µl to be placed in the agarose gel slot. The electrophoresis process started at 220 volt for 40 to 45 minutes. The visualized DNA ribbons which marked by et-hium bromide under the UV lights were documented and recorded. Then, 5 µl PCR amplification results and 2 µl buffer loading were mixed and added into the 1.5 agarose gel and ethium bromide All data was analyzed using a Chi square test. Subject was assessed based on clinical parameter: probing pocket depth (PPD), and attachment destruction/clinical attachment loss (CAL) on 6 surfaces of teeth (mesiobuccal, mesiolingual/palatal, distobuccal, distolingual /palatal, buccal and palatal/lingual (Table 2).

RESULTS

The mean age of chronic periodontitis samples was 38.90 years old and the control group without chronic periodontitis was 37,61 years old.

Table 1. Characteristic of the subjects

Characteristic	Sample	Mean ± SD	Control	Mean ± SD
Age (year)	81	38,90 ±9,24	81	37,61 ±11,42
OHI-S	81	1,63 ± 0,96	81	2,29 ± 0 69
PPD (mm)	81	4,54 ± 1,27	81	-
CAL (mm)	81	3,02 ± 1,48	81	-
Edentulous	81	2,48 ± 2,69	81	1,06 ± 1,07
Caries	81	2,17 ± 2,30	81	2,04 ± 1,69

The RFLP- PCR description in restriction sample with enzyme BamH1. The results showed that were gen mutations in slot 1, slot 15, slot 27,slot 28, slot 30, slot 64, slot 68, slot 69, slot 72, slot 77 with 187 bp, 104 bp, 83 bp, and the other slot did not show any mutation at all . Mutation from RFLP-PCR control group test was only in slot 63 and the other slot did not show any mutation at all. (Fig. 1, 2)

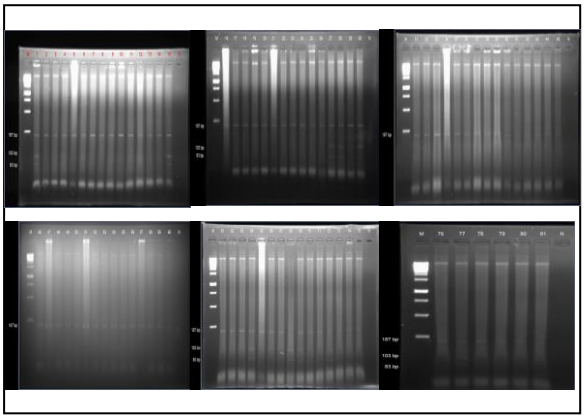

Figure1. Test result of RFLP-PCR in chronic periodontitis patient

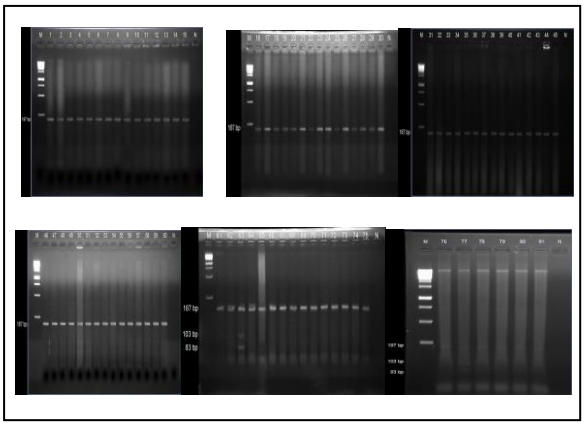

Figure 2. Test result of RFLP-PCR in control group without chronic periodontitis

Table 2 showed there was 12,3 % of the periodontitis subject that had been mutated and in the control group only 1,2 %, statistically was a significan difference (p=0,005).

Table 2. Statistical analysis of Nod2 gene mutation in chronic periodontitis and control group

NOD2 Gene	Sample		Control		*p*
	n	%	n	%	
Not Mutation	71	87,7	80	98,8	
Mutation	10	12,3	1	1,2	0,005
Total	81	100	81	100	

DISCUSSION

The chronic periodontitis subject had OHI-s, PPD, CAL and edentulous and caries. This is relevant with the previous research that showed the role of OHI-s and caries toward the occurence of chronic periodontitis. Edentulous showed the tooth loss history also reflect high caries or poor oral hygiene. Haake suggest that the primary cause of periodontal disease is bacterial iritation that is caused by plaque.[4] Most of inflammatory periodontal disease is caused by bacterial infection. Major cause of periodontal disease is colonized microorganisms on teeth surface (bacterial plaque and their product). Plaque accumulation is associated with the increase of bacterial amount. But, the present of bacterial is not enough to initiate the disease. The susceptibility of host immunity against periodontal disease plays a role in the occurence of periodontal tissue inflammation.[13]

The mean age of chronic periodontitis subject was 38.90 years old and consisted of more woman than man. It was contrast with the longitudinal study about the risk factor of periodontitis by Taize[14,] that showed the higher frequency of this disease in man (35% higher). In some literature, it was mentioned that the frequency of chronic periodontitis was higher in man than woman, and smoking habit was a predisposition factor of periodontitis. According to Manson, the risc of periodontal disease in smoking woman at the age of 20-39 years old and smoking man at the age of 30-59 was two times higher than those who did not smoke at all.

Some authors have made hypothesis that smoking could cause vasoconstriction of gingival because nicotine or another component of cigarette. Clarke et al[15], studied the direct effect of nicotine to the gingival microvascular and reported that perifer vasoconstriction lead to nicotine absorption in the gingival blood flow. So, the difference of this research result possibly caused by the exclusion of smokers in the sample requirement and patient that come to the Hospital of Oral and Dental Dentistry Faculty Hasanuddin University is dominated by woman

The result of this study provide a relation between gene mutation NOD 2 with chronic periodontitis insidence. Chronic periodontitis is a multifactorial inflamatory disease, resulted from complex interaction between bacterial and specific host. Several study at least ten years ago support the hypothesis that immune host reaction,quality and quatity of response to inflamation is controlled by gene. [16]

There were a lot of supportif evidences to claim that gene play a role of the advance of periodontal

disease, genetic factor is important to periodontitis insidence.[17]

NOD 2 gene was identifies as a gene which has a role to increase suspectibility the inflammatory disease. NOD 2 gene coded intracelluler protein that implicated in innate immune response, which follow by bacterial induction in inflammatory response.[16]

NOD 2 gene mutation effect which can,t identify ligand muramyl peptide (MDP), it show the decreasing of microba activity and gradually increase the microflora growth. It causes insentifity to commensal flora and inflammatory.[17]

As conclusion, there is a relation between NOD 2 gene mutation as one of risk factor in chronic periodontitis insidence (p= 0,005).

References

1. Armitage GC. Development of a classification system for periodontal desease and condition. Ann Periodontal 2002; 4: 1-6.
2. Michalowicz BS, Diehl SR, Gunsolley JC, Sparks BS, Brocks CN, Koertge TE, et al. Evidence of a substantial genetic basic for risk of adult periodontitis, J Periodontol 2000; 71: 1699 – 707.
3. Khoury MJ, Mc Cabe LL, Mc cabe ER . Population screening in the age of genomic medicine, N Engl J Med 2003; 348: 50–8.
4. Haake SK, Nisengard RJ, Newmann MG, Miyasaki KT. Microbial interactions with the host in periodontal diseases. In: Editors. Washington: ASM Press. Clinical Periodontology, 9th ed. Philadelphia: WB Saunders Co, 2002: 134-45.
5. Fokkema SJ. The central role of monocytes in systemic immune effects induced by the chronic periodontal infection. In: Editors. Maurizio Tonetti Emeritus. Susceptibility to chronic periodontitis. Wageningen ; Ponsen & Looijen BV, 2002: 104-16.
6. Chamaillard M, Girardin SE. An essential role for NOD1 in host recognition of bacterial peptide-glycan containing diaminopimelic acid. Nat Immunol 2003; 4: 702–7.
7. Girardin SE, Boneca IG . Nod 1 and Nod 2 detects a unique mucopeptida from gram negative bacterial peptidoglycan. Science 2003; 300: 1584–7.
8. Fokkema SJ, Loos BG, de Sleege C, van de Velden U. A Type 2 response in LPS-stimulated whole blood cell cultures from periodontitis kronis patiens. Clin Exp Immunol 2002; 127: 374-8.
9. Akira S, Takeda K. Toll like recuptor signalling. Nat Rev Immunol 2004; 4: 499-511.
10. Fritz J.H, Ferrero, RI, Philpott, DJ. Signal tranduction pathways use by NLR type innate immune reseptors. 2006 http://www. Rsc. Org/ publishing/ journals/ MB/ article. (14 July 2008).
11. Leong RW, Armuzzi A, Ahmad T. NOD2/-CARD15gene polymorphism and chron disesase in the chinese population, Aliment Pharmacol Ther 2003; 17: 1465-70.
12. Hatta M, Smits HL. Detection of Salmonella typhi by nested Polymerase Chain Reaction in blood, urine and stool samples. American J Tropical Medicine Hygiene 2007; 76: 139-43.
13. Novak MJ. Classification of diseases and condition affecting the periodontium. In: Editors. Newman Michael G, Takei Henry, Carranza Fermin A., Klokkevold Perry. Clinical Periodontology. 9th ed. Philadelphia; WB Saunders Co. Toronto 2002; 64-72.
14. Taize MNC, Maria C. *Factors related to periodontal disease in a rural population. Brazilian Oral Res* 2006; 20 (3).
15. Monteiro da Siva AM, Newman HN, Oeklay DA, O' Leaary R. Psychosocial factors, dental plaque levels and smoking in periodontitis chronis patients. J Clin Periodontol 1998; 25: 517-23.
16. Noack B, Gorgens H et al. CARD 15/ NOD2 gene variants in aggressive periodontitis. J Clin Periodontal 2006; 33: 779-83.
17. Hart TC, Kornman KS. Genetic factors in pathogenesis of periodontitis kronis. Periodontol 2000; 14: 202-15.