OUTER MEMBRANE PROTEIN OF PORPHYROMONAS GINGIVALIS AS BASIC OF DIAGNOSTIC TOOL IN AGGRESSIVE PERIODONTITIS

(PROTEIN MEMBRAN BAGIAN LUAR PORPHYROMONAS GINGIVALIS SEBAGAI ALAT DIAGNOSTIK PERIODONTITIS AGRESIF)

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Abstract

Porphyromonas gingivalis is a gram-negative anaerobic bacteria and has been shown previously to be one of the major pathogens in aggressive periodontitis. Outer membrane protein is the major virulence factor of *P. gingivalis* and plays role in the host immune response impair againts *P. gingivalis*, which in turns, causing tissue destruction and bone resorption. This study was aimed to investigate the isolation and characteristic outer membrane protein of *P. gingivalis*. Protein of OMP P. gingivalis ATCC 33277 was isolated using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and than continued by hemaglutination test. The result showed that protein profil in SDS-PAGE of OMP protein was 40 kDa molecular weight dan hemaglutination test was positive in titer of hemaglutination 1/8.In conclusion, outer membrane protein molecular mass of 40kDa produced by *P. gingivalis* is a key virulence factor involved in the co-aggregation activity of *P. gingivalis*

Key words: polyclonal antibody, *Porphyromonas. gingivalis*, outer membrane protein

INTRODUCTION

Periodontitis is a chronic immunoinflammatory disease of the periodontium that results a progressive loss of gingival tissue, the periodontal ligament, and adjacent supporting alveolar bone. Chronic inflammation of the periodontium is initiated by complex subgingival biofilms containing several likely periodontal pathogens. The biofilm generally contains a portion of the gram negative anaerobic commensal microbiota as well as opportunistic pathogens of the oral cavity, including *Porphyromonas gingivalis.*[1]

Black-pigmented anaerobe is a pathogen bacteria group in oral cavity and related with gingivitis, periodontitis, endodontic infection and odontogenic abscess. *P. gingivalis* and *P. intermedia* are one of the *black-pigmented* anaerobe bacteria. They are pathogen bacteria in early and advanced periodontal disease.[2]

Outer membrane protein (OMP) bacteria of *P. gingivalis* caused the increasing of humoral immune response, so it can stimulate inflammatory cytokine expression, such as TNF-α, IL-1β, and IL-6 in monocyte and fibroblast of gingiva. It also induced bone resorption activity.[3]

Imai et al. reseach[4] showed outer membrane protein found in negative gram bacteria is RagA, RagB and OMPA-*like protein*. RagB has the strongest virulent ability in periradicular lession subject. Whole cell of *P. gingivalis* bacteria, cell extract or OMP immunization can reduce periodontal tissue destruction that caused by *P. gingivalis* bacteria. Rat's study showed that 40 kDa OMP antibody is potential to kill *P. gingivalis* bacteria.[5]

The aim of this study was to investigate the isolation and characteristic outer membrane protein of *P. gingivalis* bacteria as the making dot enzyme immunoassay (EIA) material to examine aggressive periodontitis.

MATERIALS AND METHODS

This study was done at the Microbiology Laboratory of Dental Faculty, Jember University and

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physiology Laboratory of Medicine Faculty, Brawijaya University. *P. gingivalis* bacteria culture in phosphate buffer saline (PBS) was soaked by *fortex mixer* for 10 seconds, then 0.5 mL. The mixture was taken and cultured on blood medium. Blood medium composition were 2 mL *brucella broth*, 0.4 µl/ml K_1 vitamin, and 5 µl/ml hemin and 10% *sheep blood*. Medium and bacteria were put in *anaerobic jars* then were incubated in 37°C room condition that contained 10% CO_2 by *GasPack* CO_2 *generating sachet* for 7-14 days. Then it was cultured in biphasic medium (BHI/TCG).

Before OMP isolation, the pili was cut. Bacterial collection result was added with trichlor-acetic-acid (TCA) and centrifuged 6000 rpm for 10 minutes in 4°C. Pellet was taken and resuspended with pH 7.4 PBS and the ratio was 1:10. Bacteria was blend by mixer. Pili in the top part was taken. OMP isolation used Evan's modification method. Sample modification was the top sediment of pili cutting treatment in the last centrifugation, pellet was resuspended in pH 7,4 PBS until its volume reached 15 times, then added with n-octyl B-D-glucopyranoside (NOG) until its concentration reached 0.5%. Supernatant fluid was taken and dialyzed. The fluid was dialyzed on the first 24 hours using H_20 and on the second 24 hours using pH 7.4 PBS.

Monitoring of molecule weight used sodium dodecyl sulphate polyacrylamide gel electrophoresis. Protein samples were heated 100°C for 5 minutes in buffer solution that contained 5 mM Tris HCI pH 6,8-2 mercapto etanol 5%, w/v sodium dodecyl sulfate 2,5%, v/v gliserol 10% with detection color bromophenol blue. Selected mini-slab gel of 12.5% with 4% tracking gel used 125 mV voltages. Material used *coomasive brilliant blue* material and standard molecule sigma *low range marker*.

The results of SDS-PAGE gel straight cut at 40 kDa were collected and included in the dialysis membrane and performed electroelusion and dialysis. Dilution of samples were prepared with a concentration 1/2 on microplate V, the volume of each well 50µl. Each of the wells murine blood concentration of 0.5% was placed in room temperature until 1 hour. The amount of titer was determined by observation of blood agglutination.

OMP 40 kDa protein in wistar rats was injected by intra-peritoneal injection. The first injection was mixed with complete *Freund's adjuvant* (Booster I). The second and further were mixed with incomplete *Freund's adjuvant* for 1 week (Booster II). 40 kDa protein antigen injection *P.gingivalis* until booster V. After the wistar rats were injected ketamine, blood was collected from the heart. Blood disentifuse 1500 rpm for 15 minutes. Serum taken from the supernatant, obtained polyclonal antibodies anti-40 kDa OMP of *P. gingivalis* serum from wistar rats.

RESULTS

After culturing in blood medium, we obtained the result of colony of *P. gingivalis* bacteria. Figure 1 showed the results of bacterial culture isolation of *P. gingivalis* ATCC 33277 on day 14, which on this day the bacterial harvesting of isolated OMP. Colonies of these bacteria have a characteristic brown or black pigment on blood agar.

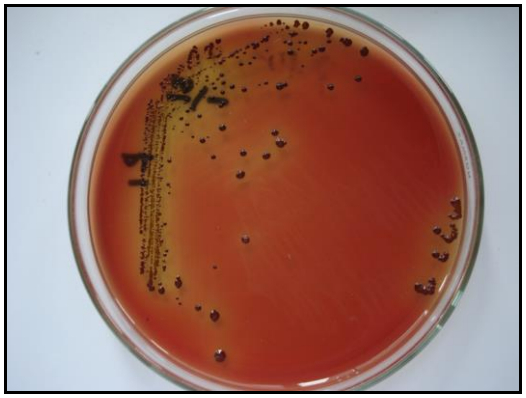

Figure 1. Bacterial colony of *P. gingivalis* ATCC 33277 on the 14th day

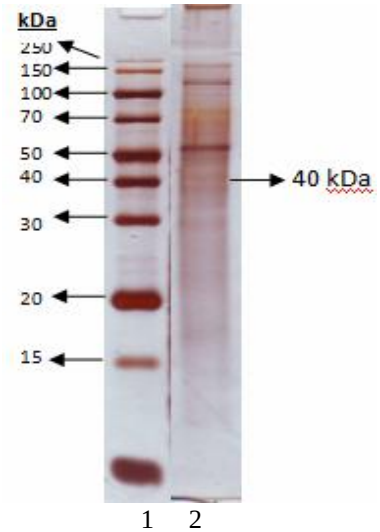

Figure 2. Result of SDS-PAGE of OMP *P. gingivalis* 1. Protein marker, 2. OMP *P. gingivalis*

Protein profile on SDS-PAGE of OMP *P. gingivalis* showed 40 kDa protein. Lanes 1 is a protein marker and lanes 2 is the OMP *P.gingivalis* with protein band is 40 kDa. Hemaglutination test results used eritrosit of mice can be seen in Table 1.

Table1. Hemaglutination test 40 kDa OMP protein of P. gingivalis ATCC 33277

Molecular Weight Proteins (kDa)	Dilution										
	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	K(-)
40	+	+	+	-	-	-	-	-	-	-	-

(+): No Coagulation, (-): Coagulation, K(-): Control negative

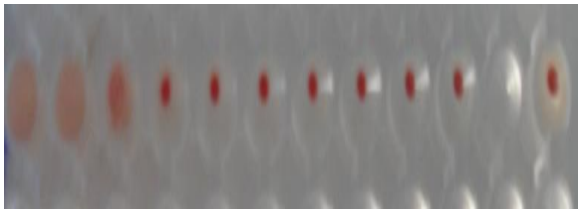

Figure 3. The result hemaglutination test against 40 kDa OMP *P. gingivalis*

Table 1 and Figure 3 Showed that protein with a 40 kDa molecular weight provide the highest hemagglutinin titer of 1/8. Hemaglutination protein is considered as one of the virulence factors of pathogenic bacteria and as a basis for making antibodies.

DISCUSSION

Porphyromonas species was an anaerobic *black-pigmented* bacteria that is pathogen and was related specifically with advanced periodontitis lesion. *P. gingivalis* was a virulent bacteria in vivo and in vitro, and almost found in subgingival plaque of periodontitis subject. This species had brown or black pigmented characteristic in blood medium.[6]

The amount of anaerobic *black-pigmented* bacteria in aggressive periodontitis subject and healthy subject showed that was significant different in both of the groups. The amount of *black-pigmented* bacteria in aggressive periodontitis subject was higher than healthy subject.[7] Subsequently, amount of *black-pigmented* bacteria in aggressive periodontitis subject had strong positive relationship with pocket depth, that deepest pocket had higher amount *black-pigmented* bacteria.[8]

Porphyromonas gingivalis had virulent factors such as lipopolysccharide (LPS), fimbriae, toxic metabolic product, and prothease. Virulent factors can cause disease directly or not by host cell activation to release inflammatory mediators.[9] *Porphyromonas gingivalis* can produce protease extracellular, such as arginine-specific *protease* and lysine-specific protease, both of them were coded as polyprotein that contained protease and adhesin domain. Both of the proteases had C-terminal adesin domain that was involved in attachment of host protein.[10]

Forty kDa molecule weight of outer membran protein of *P. gingivalis* was virulent factor for co-aggregation. Forty kDa outer membran protein was found in some strains of *P. gingivalis*. It also described that anti opsonisation of 40 kDa-OMP antibody of *P. gingivalis* was as phagocyte target of neutrophil cell, so several studies indicated that 40 kDa OMP can be used as candidate of anti vaccine of periodontitis.[11] It can be concluded that outer membran protein of *P. gingivalis* bacteria can be used as polyclonal antibody of making diagnostic tool of aggressive periodontitis in 40 kDa molecule weight.

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