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ANTI-ADHERENCE POTENTIAL OF IMMUNOGLOBULIN Y *AGGREGATIBACTER ACTINOMYCETEMCOMITANS* AGAINST *AGGREGATIBACTER ACTINOMYCETEMCOMITANS* ADHESION

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ABSTRACT : ***Aggregatibacter actinomycetemcomitans*** **is the main cause of aggressive periodontitis. Immunoglobulin Y (Ig Y) is the main antibody in poultry, reptiles, lungfish and can be found in chicken egg yolk. IgY has been proven effective to prevent against several pathogens that harm towards animals and humans. This study purpose is to investigate that Ig Y*A. actinomycetemcomitans*** **have an anti-adherence potential against *A. actinomycetemcomitans*** **adherence on epithelial cell as an alternative prevention of periodontitis. The sample group was divided into 8 groups, 1 control group and 7 treatment groups. The control group consisted of a control group of *A. actinomycetemcomitans*. The number of bacteria attached to 100 enterocyte cells was calculated to determine the adhesion index. IgY in egg yolk can significantly reduce the adhesion index of *A. actinomycetemcomitans*** **bacteria in each concentration group. IgY A. *actinomycetemcomitans*** **have an anti-adherence potential against *A. actinomycetemcomitans*** **adherence in epithelial cell.**

Key words : Immunoglobulin Y, egg yolk, *Aggregatibacter actinomycetemcomitans*, periodontitis, medicine.

INTRODUCTION

Periodontal disease is one of the dental and oral health problems that has a high prevalence in the community (Larindy *et al*, 2015). The prevalence of periodontal disease in Indonesia is quite high at 96.58% (Nandya *et al*, 2012). According to the World Health Organization (WHO), since 2004 periodontal disease is the top ten diseases in the world that causes death (Larindy *et al*, 2015). Indonesia has a prevalence of aggressive periodontitis which is between 3% and 10% (Widjaja *et al*, 2013). Based on data obtained from research at the periodontics clinic of the Faculty of Dentistry, Airlangga University, it is known that the prevalence of aggressive periodontitis in 1991 was 9% and in 2002 it increased to 13%, this shows that the prevalence of patients with aggressive periodontitis increases every year (Setyari *et al*, 2014).

Aggressive periodontitis is periodontal disease with characteristic of early onset, which generally attacks individuals under 30 years old, although sometimes, it also attacks individuals over 30 years old (Widjaja *et al*, 2013). The pattern of bone loss and attachment around the teeth is very rapid, including very significant bone damage and loss of attachment in a short time. Aggressive periodontitis is classified into two, namely Localized Aggressive Periodontitis (LAP) and Generalized Aggressive Periodontitis (GAP) (Sasmita *et al*, 2014).

Major bacteria found in aggressive periodontitis is *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*). *A. actinomycetemcomitans* is an anaerobic gram-negative bacterium known that is found in the human oral cavity such as in the gingival plaque, subgingival plaque, saliva, gingiva, tongue, and tonsils, also attached in gingival crevicular epithelium (Baik *et al*, 2013; Raja *et al*, 2014). *A. actinomycetemcomitans* has various virulence factors to colonize and surive in the oral cavity which are divided into factors that support colonization, factors that damage host tissues, and factors that interfere with host defense (Velusamy*et al*, 2016; Gholizadeh *et al*, 2017).

The ability of various bacteria to attach to the host surface is an important characteristic for colonizing and is the initial stage of the infection process (Kachlany *et al*, 2001; Kundera *et al*, 2014). The mechanism of bacterial adhesion consists of two stages, non-specific adhesion and specific adhesion. Non-specific adhesion is

reversible adhesion of bacteria to the eukaryotic surface, while specific adhesion is irreversible adhesion from bacteria to the host surface. In general, non-specific adhesion occur before specific adhesion. Specific adhesion involves the formation of many specific lock-and-key bonds between adhesives with receptors on the host cell surface. Examples of specific adhesion factors are chemical components of capsules, cell walls, fimbriae. Non-specific adhesion consists of hydrophobic interactions, electrostatic forces and brown motion (Thomsen *et al*, 2016).

Immunoglobulin Y (IgY) is a polyclonal antibody obtained from chicken yolk that received immunization. IgY has high specificity to bind and deactivate foreign substances therefore IgY can be used for diagnosis and therapy (Grando *et al*, 2017). Immunoglobulin Y (IgY) is the main antibody in poultry, reptiles and lungfish (Dubie *et al*, 2015). Passive immunization using IgY is proven effective in preventive efforts against several pathogens toward animals and humans (Zorriehzahra *et al*, 2016). The most effective way for passive immunization for IgY is to use IgY produced in response to specific bacterial antigens (Müller *et al*, 2015). For the production of IgY, chicken were immunized by intramuscularly injecting specific antigens (Xia *et al*, 2017).

IgY has many advantages compared to mammalian antibodies, such as: similar function to mammalian IgG and IgE and no cross reaction with mammalian IgG detected (Grando *et al*, 2017). These antibodies effectively prevent gastrointestinal tract infections caused by *E. coli* and dental caries caused by *Streptococcus mutans* (Al- Adwani *et al*, 2013). The mechanism of action of IgY in protecting the host is through inhibition of bacterial enzyme activity, neutralization of toxins, and inhibiting cell adhesion of microorganisms (Müller *et al*, 2015). IgY increases the phagocytic activity of macrophages and inhibits bacterial growth and colonization. IgY is stable in saliva so that it can be used to treat local infections associated with the oral mucosa and can be an alternative antibiotic treatment to treat antibiotic-resistant microbial pathogens (Zorriehzahra *et al*, 2016). Clinical use of IgY has proven effective in preventing airway colonization of *Pseudomonas aeruginosa* in cystic fibrosis patients and is effective for immunotherapy in long-term care without causing side effects. The use of IgY in human has been shown to prevent *Helicobacter pylori* infection by inhibiting bacterial adhesion and growth (Dubie *et al*, 2015).

Based on previous researches, there has been no research on the anti-adherence activity of IgY against bacteria that cause periodontitis namely *A. actinomycetemcomitans*. Therefore, researchers conducted a study using IgY *A. actinomycetemcomitans* produced from egg yolk to determine the anti-adherence activity of IgY *A. actinomycetemcomitans* for adhesion of *A. actinomycetemcomitans* bacteria to epithelial cells.

MATERIALS AND METHODS

Isolation of Epithelial cells

Isolation of epithelial cells was taken from enterocyte cells in the intestines of rats using the Weisler method (Nagayama *et al*, 1995). The mice used were healthy mice weighing around 135 g. Mice were sacrificed using chloroform solution then intestinal tissue was taken and separated from the surrounding tissue. The intestine was cut to ± 10cm then the intestinal lumen was opened by cross-cutting and washed with solution A (PBS pH 7.4 + 1 mM DTT) in one to three times in the petri dish. After the intestinal tissue was clean, the intestinal tissue was put into solution B (PBS pH 7.3) in the falcon tube and the water bath was stirred for ± 30 minutes at 37°C. Next, intestinal tissue was inserted in solution C (PBS pH 7.4 + 1.5 mM EDTA + 0.5 mM DTT) and shaked for ± 30 minutes at 37°C until enterocyte cells are separated from the intestinal tissue of mice. The tube containing the sample was then centrifuged at a speed of 1000 rpm for 10 minutes at 4°C. The liquid was removed the enterocyte cell deposits were added with solution B and centrifuged at 1000 rpm for 10 minutes at 4°C. The fluid was taken slowly then the enterocyte cells were stored in a sterile place. Enterocyte cells contained in suspension fluids were examined with leukocyte count rooms. The concentration of enterocyte cells used was 10^6/ml.

Solution dilution

Dilution of the solution was made in concentrations of 1/10, 1/20, 1/40, 1/80, 1/160, 1/320 and 1/640. A PBS solution of 90 µl was inserted into the first tube and as much as 50µl to the second tube until the seventh tube. A 10ìl of *A. actinomycetemcomitans* IgY was inserted into the first tube for 1/10 dilution and homogenisation was carried out with shaking. Then a 50ìl solution was taken from the first tube using a micropipette and put into the second tube for 1/20 dilution and then homogenized with shaking. Dilution to a concentration of 1/640 was carried out. Then as much as 50µl of the solution from the 1/640 concentration tube was removed, leaving only 50µl of solution in each tube.

Adhesion test

Each tube containing PBS and IgY *A. actinomycetemcomitans* was given a 50 µl of prepared enterocyte cell suspension. Shaking was carried out for ± 30 minutes at 37°C at the waterbath, respectively. Each

tube containing PBS, *A. actinomycetemcomitans* and enterocyte cell suspension, added a bacterial suspension (10^8CFU / ml) of 50 µl. The next step was centrifugation at 1500 rpm, at 4°C for 3 minutes. The deposits were taken from each tube and smeared on the glass object and Gram staining was carried out. Preparations were observed under a microscope with 1000x magnification and counted the number of bacteria attached to the enterocytes. The adhesion index value was obtained from the average number of bacteria attached to enterocytes, calculated for each observation of 100 enterocytes.

RESULTS

The results of observations of the adhesion index of *A. actinomycetemcomitans* bacteria obtained results as listed in Table 1.

Table 1 : Adhesion index of *A. actinomycetemcomitans* IgY toward *A. actinomycetemcomitans.*

Concentration of IgY Aa	Adhesion index Aa
1/10	3.33
1/20	3.85
1/40	5.22
1/80	5.17
1/160	5.52
1/320	5.18
1/640	6.68
Control	7.57

Data from the calculation of the adhesion index in the bacterial group were tested statistically to prove the research hypothesis. In the normality test, Shapiro Wilks test was used and the results of the adhesion index calculation in the group of *A. actinomycetemcomitans* were normally distributed ($p > 0.05$).For the homogeneity test, Levene's test was used and the calculation of the adhesion index in the group *A. actinomycetemcomitans* have $p < 0.05$, which means that the variation in data is not homogeneous.

Due to normal data distribution and not homogeneous data variants, non-parametric tests were used. The non-parametric test was ANOVA Welch Test with $p<0.05$, indicating that the variable adhesion index of *A. actinomycetemcomitans* bacteria in the control group and treatment group had significant differences. To analyze the differences in each group, a comparative test using Multiple Comparisons Tukey HSD was conducted to obtain a p-value< 0.05, indicating that the variable intensity index of *A. actinomycetemcomitans* in the control group and the treatment group had significant differences.

DISCUSSION

Based on the results of the research obtained, the results of the adhesion index of *A. actinomycetemcomitans* bacteria in all groups were obtained according to the theory, which was higher in the control group than in the dilution group. This is because in the control group, bacteria were not given treatment therefore the bacterial adhesion process took place without any obstacles and in the dilution group, IgY would interfere with the virulence factors possessed by the *A. actinomycetemcomitans* bacteria associated with bacterial adhesion to the host cell. IgY *A. actinomycetemcomitans* interacting with *A. actinomycetemcomitans* will inhibit the action of virulence factors possessed in the form of fimbriae, thus inhibiting the stimulation of adhesion to host cells. IgY *A. actinomycetemcomitans* can also inhibit the action of bacterial vesicles *A. actinomycetemcomitans* thus inhibiting the release of bacterial toxic substances. IgY *A. actinomycetemcomitans* can also inhibit bacteriocin activity in reducing ecological pressure, hence there is no reduction in ecological pressure and competition with other organisms for nutrition occurs (Xia *et al*, 2017; Zorriehzahra *et al*, 2016).

CONCLUSION

From this study, it can be concluded that IgY *A. actinomycetemcomitans* has anti-adherence activity to the adhesion between *A. actinomycetemcomitans* and epithelial cells.

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