

## The influence of adhesin protein from *Aggregatibacter actinomycetemcomitans* on IL-8 and MMP-8 titre in aggressive periodontitis

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### ABSTRACT

**Background:** Adhesion can actually be considered as a part of both a powerful survival mechanism and a virulence mechanism for bacterial pathogens. Bacterial adhesin is an instrument for bacteria to do invasion to host. Bacterial adhesin depends on ligand interaction as a signaling mediator that will influence invasion and increase pro and anti-inflammatory because of the influence of the receptors of innate immune response. *Aggregatibacter actinomycetemcomitans* has fimbriae included in type IV pili containing mostly with protein weighed 6.5 kDa and at least with protein weighed 54 kDa. **Purpose:** The purpose of this research is to analyze the influence of the induction of adhesin protein derived from *A. actinomycetemcomitans* on IL-8 and MMP-8 titre of Wistar rats. **Methods:** Adhesin protein derived from *A. actinomycetemcomitans* weighed 24 kDa was induced on the maxillary first molar sulcus of Wistar rats to prove that adhesin protein could affect IL-8 and MMP-8 titre. Next, to determine its influence, Elisa technique was conducted. **Results:** It is known that the levels of IL-8 and MMP-8 titre were increased in the group induced with adhesin protein derived from *A. actinomycetemcomitans* compared with the control group. **Conclusion:** It can be concluded that adhesin protein derived from *A. actinomycetemcomitans* can cause alveolar bone damage through the increasing levels of IL-8 and MMP-8 in aggressive periodontitis.

**Keywords:** *A. actinomycetemcomitans* adhesin; IL-8; MMP-8; aggressive periodontitis

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### INTRODUCTION

Aggressive periodontitis is a disease found on tissues supporting teeth, characterized by rapid deterioration in periodontal ligament and alveolar bone. Aggressive periodontitis is also known as a process of tissue attachment loss and gingival recession four times faster than chronic periodontitis.<sup>1,2</sup> This disease is more commonly found on patients aged under 30 years old. However, aggressive periodontitis found on young patients is still a problem in dentistry.

The pathogenesis of periodontitis is affected by the interaction between host and bacteria dominated by *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*). The presence of the bacteria in dental plaque can

be associated with the aggressiveness of periodontal tissue damage and exacerbated by both genetic and environmental factors.<sup>3</sup> The direct contact between an infectious agent and a host cell actually begins with adhesion (attachment). Therefore, the increasing of IL-8 in periodontal tissues and GCF is related to the severity of periodontitis. In vitro chemokines, such as IL-8, can be produced by gingival fibroblasts cells, gingival epithelial cells, and endothelial cells. IL-8 produced by osteoblasts can be induced by bacterial products, inflammatory mediators, dentin protein, and debris.<sup>4</sup> IL-8 is the body's first defense by enhancing phagocytosis, killing bacteria, as well as secreting lysosomal enzymes and superoxide anion.

Matrix metalloproteinases (MMPs) are major proteinase enzymes involved in periodontal tissue destruction with

the degradation of extracellular matrix molecules. MMPs are also known as a group of proteolytic enzymes found in neutrophils, macrophages, fibroblasts, epithelial cells, osteoblasts, and osteoclasts, destroying the extracellular matrix molecules, such as collagen, gelatin and elastin. Both MMP-8 and MMP-1 are actually collagenase. MMP-8 is expressed because of the infiltration of neutrophils, whereas MMP-1 is expressed by fibroblasts, monocytes/macrophages, and epithelial cells.

In addition, it is also known that MMPs are produced by bacterial pathogens causing periodontitis, such as *P. gingivalis* and *A. actinomycetemcomitans* although they are not the major factors in the aggressiveness of this disease.<sup>5</sup> This study is aimed to know how the induction of adhesin protein derived from *A. actinomycetemcomitans* can affect the levels of IL-8 and MMP-8 in Wistar rats as an indicator of tissue and bone damage in aggressive periodontitis.

## MATERIALS AND METHOD

The culture of *A. actinomycetemcomitans* was made of bacteria that had been isolated from patients with aggressive periodontitis in Periodonsia Clinic, Faculty of Dental Medicine, Universitas Airlangga. However, those patients had to fill informed concern forms as approval first. This research was approved based on ethical test conducted at Faculty of Dentistry, Universitas Airlangga.

The culture of *A. actinomycetemcomitans* made was about 200 mL for two groups. It means that the culture of *A. actinomycetemcomitans* in each group consisting of 20 rats was at least about 5 ml at a density of  $10^8$ . *A. actinomycetemcomitans* was induced for 7 days of treatment. Furthermore, there were four groups, each of which consisted of ten rats. The first group was the negative control group induced with 0.9% NaCl. The second group was induced with *adhesin*, while the third group was induced with *adhesin* and *whole cell A. actinomycetemcomitans*. And, the fourth group was the positive control group induced with *whole cell A. actinomycetemcomitans*. *Adhesin* induction in those rats was conducted by giving *adhesin A. actinomycetemcomitans* and *whole cell A. actinomycetemcomitans* about 200 ml for each with 200 pg/ml of protein levels, while the concentration of *A. actinomycetemcomitans* given for 7 days was  $10^8$  in order to get the real symptoms of aggressive periodontitis.<sup>6</sup> The induction was conducted in the pockets of M1 upper right tooth based on “Dumitrescu” method.<sup>7</sup>

Finally, IL-8 and MMP-8 were measured by using Elisa technique, namely Elisa Kit, BG-RAT11692 (Novateinbio). The basic principle of Elisa Kit is the use of a double-antibody sandwich ELISA to analyze the levels of IL-8 and MMP-8 in the samples of those alveolar bones. Put standards and samples into the wells that had been coated with the rats’ osteocalcin antibody. They were added with osteocalcin antibody HRP conjugates to bind the analyte, and then incubated and washed based on the procedure to

remove unbound materials. They were added with HRP substrate, and then incubated for detection. If the colour appeared is blue, it means that there is a reaction. The color will change to yellow when the reaction is stopped after they are added with Stopping Solution (acid solution). The intensity of the yellow color can indicate the concentration of IL-8 or MMP-8 in those rats.

## RESULTS

The results of ELISA test on IL-8 level in the control group, in the group induced with *adhesin*, induced with *adhesin + A. actinomycetemcomitans*, and induced with *A. actinomycetemcomitans* can be seen in Table 1. Based on the results of Kolmogorov-Smirnov test, it is known that the distribution of IL-8 level in the alveolar bones of those four groups was normal ( $p > 0.05$ ,  $p = 0.195$ ). Based on the results of Anova test, it is also known that there was a significant difference among the treatment groups ( $p < 0.05$ ,  $p = 0.001$ ). Based on the results of LSD test, moreover, it can be seen that there was a significant difference between the control group and all the treatment groups.

Furthermore, it is known that IL-8 level in the group induced with *A. actinomycetemcomitans* was  $8.1 \mu\text{g/ml}$  ( $8.10091 \pm 0.621$ ), while that in the group induced with *A. actinomycetemcomitans + adhesin* was  $5.8 \mu\text{g/ml}$  ( $5.80317 \pm 0.68502$ ). IL-8 level in the group induced with *adhesin* was  $2.6 \mu\text{g/ml}$  ( $2.57854 \pm 0.91238$ ), while that in the control group amounted was  $0.00017 \mu\text{g/ml}$  ( $0.00017 \pm 0.00013$ ). In other words, the highest level of IL-8 was in the group induced with *A. actinomycetemcomitans* compared to the other treatment groups. The average levels of IL-8 can be seen in Figure 1.

On the other hand, the results of Elisa test on MMP-8 level in the control group, in the group induced with *adhesin*, induced with *adhesin + A. actinomycetemcomitans*, and induced with *A. actinomycetemcomitans* can be seen in Table 2. Based on the results of Kolmogorov-Smirnov test, it is known that the distribution of MMP-8 level in the alveolar bones of those four groups was normal ( $p > 0.05$ ,  $p = 0.195$ ). Based on the results of Anova test, there was a significant difference among the treatment groups ( $p < 0.05$ ,

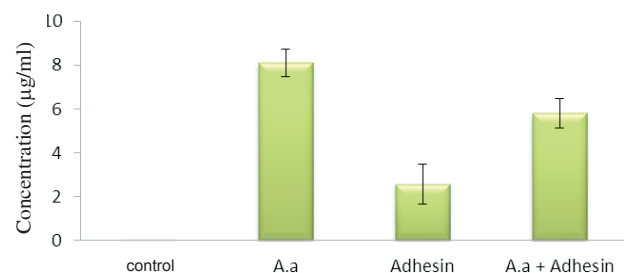
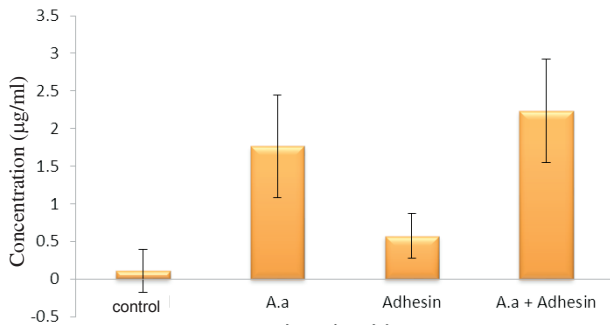


Figure 1. The mean of the levels of IL-8 in alveolar bone.



**Figure 2.** The mean of the levels of MMP-8 in alveolar bone.

$p=0.001$ ). Based on the results of LSD test, moreover, it can be seen that there was a significant difference between the control group and all the treatment groups.

In addition, MMP-8 level in the group induced with *A. actinomycetemcomitans* + adhesin was 2.2 µg/ml ( $2.2339 \pm 0.6846$ ), while that in the group induced with *A. actinomycetemcomitans* was 1.7 µg/ml ( $1.7654 \pm 0.6821$ ). IL-8 level in the group induced with adhesin was 0.6 µg/ml ( $0.5735 \pm 0.2947$ ), while that in the control group was 0.1 µg/ml ( $0.1081 \pm 0.2828$ ). In other words, the highest level of MMP-8 was in the group induced with *A. actinomycetemcomitans* + adhesin compared to the other treatment groups. The average levels of MMP-8 can be seen in Figure 2.

## DISCUSSION

Based on the results, there was a significant difference of IL-8 level in the alveolar bones between in the group induced with *A. actinomycetemcomitans*, in the group induced with *A. actinomycetemcomitans* + adhesin, in the group induced with adhesin, and in the control group. IL-8 level in the group induced with *A. actinomycetemcomitans*

was significantly increased compared to that in the group induced with *A. actinomycetemcomitans* + adhesin, in the group induced with adhesin, and in the control group. It indicates that the presence of adhesin can make the colonization and invasion of *A. actinomycetemcomitans* in periodontal tissues stimulate the activation of proinflammatory cytokines, one of which is IL-8 serving as chemokines. IL-8 will attract neutrophils, causing neutrophil degradation. The neutrophil degradation then can cause the expression of elastase and lactoferrin. Elastase can usually cause damage to periodontal tissues and alveolar bone.

The induction of *A. actinomycetemcomitans* + adhesin can cause IL-8 level lower than the induction of *A. actinomycetemcomitans* only, but, the induction of adhesin can cause IL-8 level higher than in the control group. It is because adhesin induced along with *A. actinomycetemcomitans* can serve as an inhibitory role in the process of *adhesion*, so the colonization and invasion of the host, *A. actinomycetemcomitans*, will be reduced. This condition has been confirmed in an adhesion test of *A. actinomycetemcomitans* on HeLa cells. The test result shows that the adhesion of *A. actinomycetemcomitans* adhesin protein with receptors can be found in HeLa cells. The result also shows that this *adhesin* protein can inhibit the attachment of *A. actinomycetemcomitans* on HeLa cell surface as indicated by the declining number of *A. actinomycetemcomitans* attached to the HeLa cells when administered with the increasing doses of adhesin protein derived from *A. actinomycetemcomitans*.<sup>8</sup>

MMP-8 level in the group induced with *A. actinomycetemcomitans* + adhesin was significantly increased compared to that in the group induced with *A. actinomycetemcomitans*, in the group induced with *adhesion*, and in the control group. It indicates that the ability of *A. actinomycetemcomitans* and adhesin protein derived from *A. actinomycetemcomitans* to perform attachment to the receptors of the host, so *A. actinomycetemcomitans* can do

**Table 1.** The mean and standard deviation of the levels of IL-8 in alveolar bone

Group	$\bar{X}$	SD	Min	Max	Anova
Control	0.00017	0.00013	0.0000156	0.0003498	F= 300.5
<i>A. actinomycetemcomitans</i>	8.10091	0.621	6.8367827	8,9837638	p= 0.001
Adhesin	2.57854	0.91238	1.2672389	3.9748729	
<i>A. actinomycetemcomitans</i> + adhesin	5.80317	0.68502	4.8738794	6.8738987	

**Table 2** The mean and standard deviation of the levels of MMP-8 in alveolar bone

Group	$\bar{X}$	SD	Min	Max	Anova
Control	0.1081	0.2828	0.0007	0.9087	F= 35.97
<i>A. actinomycetemcomitans</i>	1.7654	0.6821	1.0571	2.9387	p= 0.001
Adhesin	0.5735	0.2947	0.2538	0.8376	
<i>A. actinomycetemcomitans</i> + adhesin	2.2339	0.6846	1.2839	3.0981	

colonization and invasion. The high level of *MMP-8* caused by the induction of *A. actinomycetemcomitans* + adhesin will stimulate proinflammatory cytokines, namely IL-8 expressed by monocytes, keratinocytes, endothelial cells and fibroblasts which then will stimulate the expression of *MMP-8* by neutrophils.

*MMP-8* is considered as the potential collagenase-2 playing an important role in the degradation of connective tissue in inflammation area. It means that the increasing of *MMP-8* will result in damage to periodontal tissues and alveolar bone. *A. actinomycetemcomitans* is a powerful stimulator of *MMP-8* expression, notably by LtxA as one of virulence factors.<sup>8</sup> The situation is also in accordance with the statement of Kiili<sup>10</sup> stating that *MMP-8* plays a role in inflammation and tissue destruction diseases, so the activation of *MMP-8* in periodontitis has been reported to reflect the level of disease severity and activity. *MMP* group, such as *MMP* 1, 3, 7, 8, 9, 13, 25 and 26 has been widely studied in gingival tissue and gingival crevicular fluid (GCF) of patients with different periodontal diseases, and then *MMPs* are associated with the development and progression of periodontal disease.<sup>11</sup> It can be concluded that adhesin protein derived from *A. actinomycetemcomitans* with a molecular weight of 24 kDa has an ability to increase the levels of IL-8 and *MMP-8* in the Wistar rats' alveolar bone.

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