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# INCREASED LEVELS OF MALONDIALDEHYDE AND CATHEPSIN C BY *AGGREGATIBACTER ACTINOMYCETEMCOMITANS* IN SALIVA AS AGGRESSIVE PERIODONTITIS BIOMARKERS : A REVIEW

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**ABSTRACT : Aggressive Periodontitis results in rapid destruction of the periodontium and can lead to early tooth loss in the affected individuals if not diagnosed early and treated appropriately. Aggressive periodontitis is an economically important disease as it is time-consuming and expensive to treat. Aggressive periodontitis has a worldwide prevalence of 5–15%, including Indonesia has increased in recent decades. Early detection of aggressive periodontitis plays a crucial role in successful therapy. Early diagnosis and management reduces the severity and possible complications of the aggressive periodontitis. Saliva contains an abundance of protein and nucleic acid molecules that reflects physiological status; however, unlike other bodily fluids, salivary diagnostics offer an easy, inexpensive, safe and non-invasive approach for disease detection. Saliva appear promising for future application to diagnose and prognosticate aggressive periodontitis treatment. *Aggregatibacter actinomycetemcomitans* were highest in aggressive periodontitis could act as an antigen and stimulate polymorphonuclear (PMN) cells to eradicate the antigen. Reactive Oxygen Species (ROS) released by PMN cells could damage the surrounding cells and destroy the healthy periodontal tissue structures around them. Patients with aggressive periodontitis had significantly higher values of Malondialdehyde (MDA) and cathepsin C level in saliva. The purpose of this study is to review MDA and Cathepsin C induced by *A. actinomycetemcomitans* in saliva as aggressive periodontitis biomarker. Journal and other sources were searched with spesific keywords to search the previous studies. MDA and Cathepsin C induced by *A. actinomycetemcomitans* increasing in Saliva. Level of MDA and Catphepsin C in saliva may useful to predict as aggressive periodontitis.**

***Key words :*** Aggresive periodontitis, illness, cathepsin C, malondialdehyde, saliva.

## INTRODUCTION

Periodontitis is an inflammation in the periodontal tissue that affects many people in the world. Periodontitis is a disease with a widespread in society (Yohana, 2011). Periodontal disease is a dental and oral health problem which if left untreated can cause tooth loss, alveolar bone loss and even trigger systemic diseases such as diabetes and heart disease (Yudhaprawira, 2014; Sari *et al*, 2019; Prahasanti *et al*, 2020; Rezkita *et al*, 2020). The incidence of periodontitis varies in various countries in the world and shows an increasing trend. Research conducted in Brazil in 2005 showed the prevalence of aggressive periodontitis at the age of 12-25 years by 6.5% and increased to 9.9% (Slade *et al*, 2007). The periodontal disease ranks second after caries and is still a problem in society. According to the results of a dental and oral health survey in East Java in 1995, the periodontal disease occurred in 459 people out of 1000 population (Melok, 2009).

Periodontitis is an infectious disease in the tissue supporting the teeth, caused by bacteria and causing damage to periodontal ligaments, alveolar bone, forming a pocket, recession, or both (Carranza *et al*, 2002; Nugraha *et al*, 2019a). One classification of periodontitis is aggressive periodontitis. Aggressive periodontitis is more destructive to the periodontal attachment and alveolar bone so that it can cause tooth loss (Nagy*et al*, 2002). Aggressive periodontitis usually appears in a relatively short period of time with minimal accumulation of local factors such as dental plaque and dental calculus. Patients

with aggressive periodontitis often show inadequate or imbalance immune responses against pathogenic organisms. Aggressive periodontitis also shows a family history pattern (Marcuschamer *et al*, 2009). Aggresive periodontitis includes infectious diseases that are generally caused by *Aggregatibacter actinomycetemcomitans*. *A. actinomycetemcomitans* is dominant in aggressive periodontitis with a frequency of around 90% compared to chronic periodontitis which is only 21% and in healthy individuals around 17% (Paju and Susanna, 2000). *A. actinomycetemcomitans* has a number of virulence factors that help the progression of the disease. Virulence determines the strength of the pathogenic potential and also means the relative capacity in both quantity and quality of a bacterium that causes disturbance to the host immunity. Virulence includes the capacity of tissue damage, the invasive level of bacteria, and the ability to avoid host defense responses (Rahman, 2010).

*A. actinomycetemcomitans* act as antigens that stimulate inflammatory cells out of capillary blood vessels so that phagocytic processes occur that involve neutrophil cells and macrophages (Fives-Taylor *et al*, 2000). Neutrophils are primary cells that stimulate the affected regions in response to lesions caused by bacteria and are also part of the most important in the body's immune system (Asturk *et al*, 2012). PMN in cytokines gives rise to an increase in cell Proteinase (Slade *et al*, 2007; Zheng *et al*, 2007), Proteinaseactively contributes to the inflammatory process and has an important function in the regulation of the immune response to microorganisms (Bals and Wilson, 2003). Cathepsin C plays an important role in host defense against bacteria and is an activator of PMN serine proteinases such as elastase and proteinase in humans (Sørensen *et al*, 2001). *A. actinomycetemcomitans* stimulates PMN to increase Cathepsin C activity resulting in an increase in Cathepsin C levels (Korkmaz *et al*, 2008).

Neutrophil cells and macrophages that are stimulated by inflammation caused by *A. actinomycetemcomitans* produce Reactive Oxygen Species (ROS) that are released into the extracellular environment. The ROS does not have a specific target so it can cause tissue damage. The amount of ROS produced is too much, so the tissue in the extracellular environment will experience oxidative stress which can then cause damage especially to the periodontal tissue (Dhotre *et al*, 2012).

Early detection of aggressive periodontitis has an important role in supporting the success of therapy. Early diagnosis and management reduce the severity and possible complications of aggressive periodontitis (Lalla and Papapanou, 2011). Saliva contains many proteins and nucleic acid molecules that reflect physiological status; unlike other body fluids, saliva as a diagnostic offers an easy, inexpensive, safe, non-invasive approach to detecting disease (Lawrence, 2002). Saliva can be used to diagnose and estimate the prognosis of aggressive periodontitis therapy. Saliva contains many proteins and nucleic acid molecules that reflect physiological status; unlike other body fluids, saliva as a diagnostic offers an easy, inexpensive, safe, non-invasive approach to detect periodontitis (Mandel, 2000). This salivary biomarker has often been explored to monitor the health and early diagnosis of a disease. Saliva can detect ROS activity by looking at ROS products in the form of Malondialdehyde (MDA), but it can also monitor the increase in Cathepsin C so that it can be used as a biomarker in detecting Aggressive periodontitis (Miller *et al*, 2010).

**Aggressive periodontitis**

Aggressive periodontitis is a destructive and rapidly developing periodontal disease, characterized by rapid damage from periodontal ligaments and alveolar bone, tooth loss, and minimal response to periodontal therapy (Marcuschamer *et al*, 2009). In this disease, plaque bacteria and calculus appear to be few, not comparable to damage which happens very fast and progressive. Some literature states there is a relationship between damage that occurs with defects in the immune response and genetic factors (Fedi *et al*, 2000).

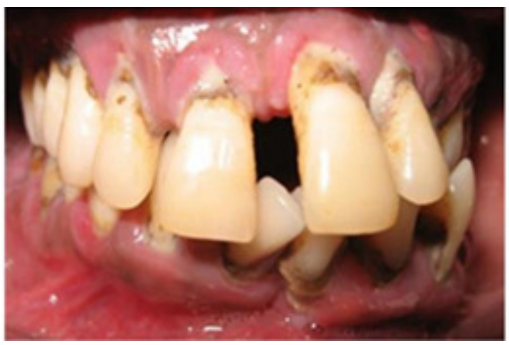

**Fig. 1 :** Aggressive periodontitis appears clinically (Noack *et al*, 2004).

Overall aggressive periodontitis is about age under 30, but sometimes it occurs in older individuals. This disorder is characterized by a complete loss of interproximal attachment, involving at least three other permanent teeth besides the first molar and incisors. The radiographic picture shows bone damage affecting almost all teeth, can be vertical, or horizontal, or both. Clinical features show gingiva with acute and severe inflammation, often proliferation, ulceration, suppuration, and bright red. Spontaneous bleeding or with mild stimulation. This response occurs at the destructive or active stage. This disease can stop spontaneously or after periodontal

therapy. In other circumstances, the damage continues so that the patient loses teeth, despite conventional periodontal treatment (Noack and Thomas, 2004).

Aggressive periodontitis can be classified into localization and generalization such as localization of the first molar, or incisors with proximal attachment loss in at least 2 permanent teeth, one of which is the first molar. Aggressive periodontitis characteristics as usual for patients under 30 years of age, generalized proximal attachment loss of at least 3 other teeth besides the first and incisors, Pronounced episodic nature of periodontal destruction, the response of serum antibodies to the infectious agent is poor (Nkem, 2011).

The etiology of aggressive periodontitis is gram-negative bacilli such as *A. actinomycetemcomitans, Capnocytophaga* spp. *Porphyromonas gingivalis*, *Prevotella intermedia*. The pathogenic ability of bacteria to cause periodontal disease is very complex. Some important pathogenic mechanisms, namely the entry/ invasion of bacteria or bacterial products into the periodontal tissue are thought to be important for the disease process. Clinical studies show that *A. actinomycetemcomitans* can penetrate the gingival epithelium. *A. actinomycetemcomitans* and *Campylobacter rectus* produce leukotoxins which can kill neutrophils and monocytes. Gram-negative bacterial walls contain Lipopolysaccharides (LPS, endotoxins) which are released after the bacteria die. Aside from being a trigger for the inflammatory process, LPS can also cause tissue necrosis. Plaque bacteria produce enzymes that play a role in periodontal disease. These enzymes include collagenase, hyaluronidase, gelatinase, aminopeptidase, phospholipase and alkaline and acid phosphatase (Mustaqimah, 2007).

Subgingival gram-negative bacteria use protein as their nutrient and have proteolytic enzymes to break down proteins into peptides and amino acids to be absorbed. A number of periodontal pathogens can produce proteases that can degrade the structure of proteins and periodontal tissues involved in immune and inflammatory reactions in chronic periodontitis. *A. actinomycetemcomitans* produce collagenase enzymes such as matrix metalloproteinases (MMPs) that can damage type 1 collagen. Collagenase enzymes encourage collagen degradation and disruption in the periodontal connective tissue. In order to survive in the periodontal environment, bacteria must be able to neutralize or avoid the host immunity mechanism to get rid of and eliminate bacteria. A number of mechanisms possessed by periodontal pathogens in avoiding or disturbing host immunity, including the direct destruction of PMN and macrophages. Leukotoxins produced by several strains of *A. actinomycetemcomitans* can destroy PMN and macrophages or inhibits the PMN chemotaxis. Some bacterial species such as *A. actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia* and *Bacteriodes forsythus* are increasing in number. This bacterial growth is associated with impaired immune system regulatory mechanisms, *i.e.* there are functional defects in PMN, monocytes, or both. This defect can damage both PMN chemotaxis against the area of infection or the ability of phagocytosis and eliminate microorganisms. Defects in PMN, monocytes, and genetic factors allow bacterial infections. The most common interleukins associated with aggressive periodontitis are IL-1, IL-6 and IL-8, which are produced by mononuclear phagocyte cells, T cells, B cells, epithelial cells, endothelial cells, fibroblasts, keratinocytes, platelets, and synovial cells (Lalla and Papapanou, 2011; Ferry and Richard, 2005).

The diagnosis of aggressive periodontitis is based on history, clinical features and supporting examinations. From the history obtained symptoms of gingival bleeding, tooth shake. From investigations to ensure the bacteria that can cause culture and for radiological examination, radiological picture. An initial examination needs to be done such as the patient's oral hygiene examination, measurement of the plaque index and calculus index (Fedi *et al*, 2000; Ferry and Richard, 2005).

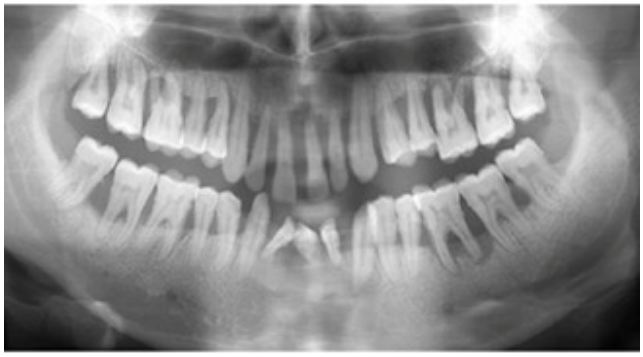

**Fig. 2 :** Radiographic Aggressive Periodontitis (Nagy *et al*, 2002).

Treatment plans undertaken for aggressive periodontitis sufferers include Dental Health Education (DHE), Scaling Root Planning (SRP), Splinting if tooth decay is found, Gingival Curettage if 3-4mm pockets are found and Periodontal Flap Surgery if pockets> 4mm are found. Host Modulation Therapy (HMT) is sometimes needed to support treatment in aggressive periodontitis patients because the host condition is susceptible to bacterial injury (Yoshinari *et al*, 2004).

The prognosis of aggressive periodontitis patients tends to be poor, questionable, to hopeless due to a large

number of rocking teeth that result from alveolar bone support or periodontal tissue adhesion is no longer adequate (Lalla and Papapanou, 2011; Ferry and Richard, 2005).

**Saliva as aggressive periodontitis Biomarker**

The multifactorial component in saliva not only protects the integrity and homeostasis of oral tissues, but also provides clues to the occurrence of disease or systemic and local conditions. This salivary biomarker has often been explored to monitor the health and early diagnosis of a disease. Saliva has been extensively studied with periodontal disease because it is very easy to collect and allows analysis (Miller *eta l*, 2012). Early detection of aggressive periodontitis has an important role in supporting the success of therapy. Early diagnosis and management reduce the severity and possible complications of aggressive periodontitis (Lalla and Papapanou, 2011).

## DISCUSSION

Aggressive periodontitis is a destructive and rapidly developing periodontal disease, characterized by rapid damage from periodontal ligaments and alveolar bone, tooth loss and minimal response to periodontal therapy (Carranza *et al*, 2002). The prevalence of aggressive periodontitis in the world from 5-15% including Indonesia has increased in the last few decades (Slade *et al*, 2007; Melok, 2009). In aggressive periodontitis, plaque and calculus bacteria appear to be few, not comparable to damage that occurs very quickly and progressively. Some literature states there is a relationship between damage that occurs with defects in the immune response and genetic factors. Aggressive periodontitis can stop spontaneously or after periodontal therapy. In other circumstances the damage continues so that the patient loses teeth, despite conventional periodontal treatment. Aggressive periodontitis is an infectious disease that is

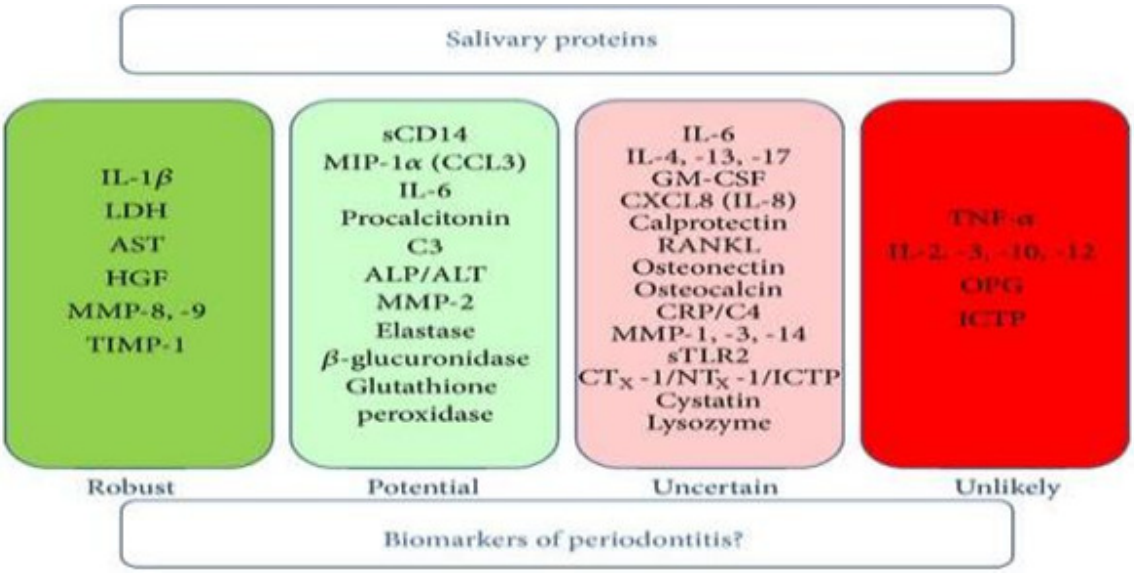


**Fig. 3 :** Various types of proteins in saliva that can be used as biomarkers of periodontitis (Miller *et al*, 2010).

Based on Andrej A. research, biomarkers in aggressive periodontitis and chronic increase in IgG, IgA, and IgM compared to healthy periodontal (Andrej *et al*, 2005). CRP, C3, Cathepsin C, MDA, and á-2M are higher in aggressive periodontitis than chronic periodontitis and healthy periodontal. Alkaline phosphatase is increased in aggressive periodontitis and chronic periodontitis. Interleukin (IL) 1â, MMP-8, Prostaglandin E2 increase in chronic periodontitis and aggressive periodontitis. Esterase and lactoferrin increase in periodontitis and lysozyme which decreases in periodontitis. Mucin will decrease in aggressive periodontitis. Histatin is increased in chronic periodontitis and aggressive periodontitis.

generally caused by bacteria *A. actinomycetemcomitans* (Paju and Susanna, 2000). *A. actinomycetemcomitans* is dominant in aggressive periodontitis with a frequency of about 90% compared to chronic periodontitis which is only 21% and in healthy individuals about 17%. *A. actinomycetemcomitans* has a number of virulence factors that help the progression of the disease (Carranza *et al*, 2002).

*A. actinomycetemcomitans* produces leukotoxins, which can eradicate neutrophils and monocytes. The walls of gram-negative bacteria contain LPS, which is endotoxins released after the bacteria die that lead to tissue necrosis. Plaque bacteria produce enzymes that play a role in periodontal disease. These enzymes include

collagenase, hyaluronidase, gelatinase, aminopeptidase, phospholipase, and acid and base phosphatase. *A. actinomycetemcomitans* produce collagenase enzymes (MMP) that can damage type 1 collagen (Fives-Taylor *et al*, 2000). Collagenase enzymes encourage collagen degradation and disruption in the periodontal connective tissue. Leukotoxins produced by several strains of *A. actinomycetemcomitans* can destroy PMN and macrophages. This bacterial growth is associated with impaired immune system regulatory mechanisms, *i.e.* there are functional defects in PMN, monocytes, or both. *A. actinomycetemcomitans* act as antigens that stimulate inflammatory cells out of capillary blood vessels resulting in the process of phagocytosis. Neutrophils are primary cells that stimulate affected areas in response to lesions caused by bacteria and are also the most important part of the body's immune system. Neutrophils produce antimicrobial peptides including defensins, Human Neutrophil Peptides (HNP) and Cathelicidins as AMP which work to maintain homeostasis. AMP may secreted by endogenous mesenchymal stem cell within the tissue (Nugraha *et al*, 2019b). Proteinase 3 is 29 kDa serine proteinase stored in PMN[11] PMN exposure to cytokines results in an increase in Proteinase 3 cells (Zheng *et al*, 2007). Proteinase 3 has many functions, such as degradation of extracellular matrix proteins, platelet activation, induction of apoptosis, and increased TNF-á and IL-1â (Bals and Wilson, 2003; Sørensen *et al*, 2001; Korkmaz *et al*, 2008). Proteinase 3 and antimicrobial peptides are involved in the non-oxidative elimination of microorganisms (Pham, 2008). Proteinase 3 actively contributes to the inflammatory process and has an important function in the regulation of the immune response to microorganisms (Sugawara *et al*, 2001; deHaar *et al*, 2004). Cathepsin C plays an important role in host defense against bacteria and is a central component of coordination in PMN serine proteinases such as elastase and Proteinase 3 in humans, so it functions as an activator of Proteinase 3 in the immune response (Pham, 2004; deHaar *et al*, 2004). *A. actinomycetemcomitans* stimulates PMN to increase Cathepsin C activity resulting in an increase in Cathepsin C levels (Noack *et al*, 2008; Soell *et al*, 2002).

Neutrophil cells and macrophages that are stimulated by inflammation caused by A. *actinomycetemcomitans* produce reactive oxygen species (ROS) that are released into the extracellular environment. The ROS does not have a specific target so it can cause tissue damage. The amount of ROS produced is too much, so the tissue in the extracellular environment will experience oxidative stress which can then cause damage especially to the tissue one of which is the periodontal tissue (Ochiai and Yamamoto, 2014). ROS is a hydroxyl free radical that can react with fatty acid components of cell membranes so that a chain reaction occurs, known as fat peroxidation, forming malondialdehyde (MDA), which will cause the breakdown of fatty acid chains into various toxic compounds and damage to cell membranes. MDA is a compound that can describe the activity of free radicals in cells, so it is often used as an indicator of oxidative stress due to free radicals (Amalina, 2007; Türkoðlu, 2014).

Early detection of aggressive periodontitis has an important role in supporting the success of therapy. The prognosis of aggressive periodontitis patients tends to be Poor, Questionable, to Hopeless due to a large number of rocking teeth that result from alveolar bone support or periodontal tissue adhesion is no longer adequate. Early diagnosis and management reduce the severity and possible complications of aggressive periodontitis (Lalla and Papapanou, 2011).

Saliva contains many proteins and nucleic acid molecules that reflect physiological status (Nugraha *et al*, 2019c). Unlike other body fluids, saliva as a diagnostic offers an easy, inexpensive, safe, non-invasive approach to detect disease. Saliva can be used to diagnose and estimate the prognosis of aggressive periodontitis therapy (Mandel, 2000). This salivary biomarker has often been explored to monitor the health and early diagnosis of an illness (Miller *et al*, 2010). Saliva can detect ROS activity by looking at ROS products in the form of a specific protein, Malondialdehyde (MDA). MDA is the result of a reaction released by macrophages due to the body's immune reaction in the form of ROS. Besides that, it can also monitor the increase in Cathepsin C so that it can be used as a biomarker in detecting aggressive periodontitis (Ochiai and Yamamoto, 2014).

The examination of Cathepsin C in saliva using salivary samples was analysed by Enzyme-Linked Immunosorbent Assay (ELISA). Before the quantization of Cathepsin C, saliva samples were eluted from the strip by placing 2 mL of buffered saline phosphate by shaking the ELISA tube for 45 minutes. Cathepsin C levels were tested with commercially available ELISA kits (Human Cathepsin C, Uscn Life Science Inc., Wuhan, China) according to the manufacturer's instructions. The minimum limit of Cathepsin C detected was 0.113 ng / mL. Cathepsin C levels in each sample were calculated based on dilution, and the results were expressed as the total amount and concentration in the Saliva sample. A calculation of the concentration data for each enzyme was carried out by dividing the amount of each mediator

by the volume of sample (Türkoðlu, 2014). Previous research conducted, there was a difference of 1.65ng / µL on examination of Cathepsin C levels in the normal group and aggressive periodontitis group. The normal group was 0.8 ng/µL and in the aggressive periodontitis group it was 2.45 ng/µL.

ROS examination uses the reaction of Lipid Peroxidase (LPO) products. LPO was tested by measuring MDA, which is the final product of fatty acid peroxidation and reacting with thiobarbituric acid (TBA) to form a color complex that has a maximum absorption at 532 nm. In the TBA reaction test, MDA substances and TBA react to produce pink pigment and have a maximum absorption at 532 nm. The reaction was carried out at pH 2-3 at $90^0$C for 15 minutes. The sample was cooled for 10% (w/v) trichloroacetic acid to precipitate the protein. The pellet precipitate is centrifuged and the supernatant aliquot reacts with a volume equal to 0.67% (w/v) TBA in boiling water for 10 minutes. After cooling, the absorbance was read at 532 nm The MDA concentration was calculated by the MDA-TBA absorbance coefficient of the complex $1.56 \times 105$ $cm^{-1}$ $M^{-1}$ and expressed as nmoles of MDA per milliliter of saliva. The minimum levels of MDA and cathepsin C that can be detected are 0.068 ng/mL and 0.113 ng/mL. Previous studies conducted, there were differences of more than 0.0068ng/µL on examination of MDA levels in the aggressive periodontitis group. The normal group was 0,00013 ng/µL and in the aggressive periodontitis group was 3.45 ng/µL so that there were significant differences in MDA levels in the control group and aggressive periodontitis (Ochiai and Yamamoto, 2014).

## CONCLUSION

An increase in MDA as a product of ROS and an increase in Cathepsin C due to *A. actinomycetemcomitans* can be detected in saliva. Thus, further research is still needed to examine Cathepsin C and ROS as a biomarker in detecting aggressive periodontitis.

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