ORIGINAL ARTICLE

IL-18 have a Pivotal Role in Gingivitis Lower Third Molar Impaction and Candidate for Pyroptosis Cells Death Event Marker.

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ABSTRACT

Introduction: Pericoronitis often occure in the gums of the teeth that have just grown. As a result cells dearh will occure, name necrosis, apoptosis or pyroptosis. A cells death coordinate by caspase-1, companied by membrane rupture and express inflammatory protein named pyroptosis. This research to sure that IL-18 as proimflammatory cytokine always expressd in gum inflammation of mandibular third molar impaction. So it going to used as marker candidate of pyroptosis cells death event excluding necrosis and apoptosis programe cells death. **Material and Methods:** Sample collected based on the principle of balance with young adult ages, there is an impact on the third molar teeth. A frozen section biopsy is performed together with the extraction of the tooth by an oral surgeon fulfilling the indication of extraction, Tissue samples were obtained from patients on lower third molar impacted, measuring 1mm in diameter. Protein isolation performed, obtained, and examined by the ELISA method. Data analyzed using a T-test of SPSS for Window 19. **Results:** We collected 21healthy gum and 19pericoronitis patien. The level of IL-18 in healthy gum is 215.75mg/ml lower than pericoronitis (2782.09mg/ml) and with T-test analysis had a significant difference between both of them (p=0.001). **Conclusion:** IL-18 candidate as a marker of pyroptosis of pericoronitis lower third molar impaction

Keywords: IL-18, Marker, Pyroptosis, Pericoronitis

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INTRODUCTION

Pericoronitis often occur in the gums of the teeth that have just grown. As a result cells death will occur. This is a common problem that usually attacks young adults. There are about 95% of cases of pericoronitis that occur in teeth that grow in part. [1, 2] Pericoronitis is inflammation that attacks the gum tissue in the wisdom teeth. The youngest molars are the third deepest and last growing molars. [3, 4] Pericoronitis if left untreated can damage the composition of the teeth and cause bad breath. This disease infects molars that grow abnormally, are embedded, or grown sideways. Pericoronitis symptoms are divided into two based on disease conditions, namely acute or chronic. Signs of pericoronitis are pain, swelling, tenderness, redness and halitosis, Pericoronitis initially caused by the arrangement of the imperfect teeth. The reason could be because the distance between the teeth is too tight or too tenuous. Left over food that is left stuck to the teeth will form a pile of plaque and allow antigenic bacteria to enter the gum tissue so that the appearance of inflammation. [5, 6] These inflammatory events activate antigen presenting cells (APC), which are played by T lymphocyte cells. [7] Th1/Th2 balance is very important in gum disease immunoregulation, which is inflammatory disorders influenced by antigen presenting cells (APC) and T cell interactions. [8]

As a result of infection and inflammation, cell death will occur. Bacteria induce cell death, likes apoptosis, necrosis, and pyroptosis. Apoptosis is non-inflammatory programmed cell death that is triggered by two different pathways, the intrinsic and extrinsic pathway. Apoptosis characterized by caspase-3 activation. Necrosis is triggered by the production of ROS which is caused by bacterial. A type of programmed cell death caused by inflammatory-mediated caspase-1 and companied by membrane rupture, release Interleukine-1betha (IL-1β)

and IL-18 named pyroptosis. As we know athogenic Associated Molecular Pattern (PAMP) and/or Dangerous Associated Molecular Patterm (DAMP) are recognized Node Like Receptor (NLR) protein trigger pyroptosis. [9] Interleukin-18 is a protein that encoded by the IL-18 gene. This protein is proinflammatory cytokines. Both hematopoietic and non-hematopoietic cells potent to produce IL-18. This was first explained in 1989. [10, 11, 12] Interleukine-18 recognized and produced firsth by Kupffer cells, a resident macrophage of the liver and now know constitutively expressed in non-hematopoietic cells, likes as intestinal epithelial cells, keratinocytes, and endothelial cells. [13] Function of IL-18 to modulate innate and adaptive immunity. [14, 15] Th1 immune response express IL-18, tumor necrosis factors and interferon in whereas Th2 immune response express IL-4, IL-5, IL-13, [16] Interleukin-18 now known as a cytokine which triggers the Th1/Th2 response and plays a role in the inflammatory reaction. [17]

The objective of this research to sure that IL-18 cytokine always expressd in gum inflammation (pericoronitis) of mandibular third molar impaction. So it going to used as marker candidate of pyroptosis cells death event excluding necrosis and apoptosis programe cells death.

MATERIALS AND METHODS

This research is observational analytic with cross sectional approach. Design of the research is posttest only with control. The study was conducted at the dental and oral clinic of Moewardi Hospital from July-September 2018.

Sample are balanced between patien men and women. Others criteria are young adults with the possibility of lower mandibular third molars to grown and impaction. The tooth region can be right and or left side and on radiograph images teeth appear impaction or eruption difficulties. Another requirement is that the impacted teeth have been decided by the oral surgeon to be extracted on the based that they have disrupted comfort, such as dizziness, frequent headaches, ringing in the ears, pain when eating.

As an inclution criteria are patien with impaction of mandibular third molar and have inflammation of pericorona clinically. And as exclution criteria is had systemic disease like hepatitis, neoplasm, etc.

When the extraction done as well as a frozen section biopsy done too. From the 1mm (as a half) frozen section, a paraffin block process is performed which is then stained hematoxylin eosin to distinguish healthy or diseased tissue (pericoronitis) by pathologist. Some other part of the frozen section were carried out by protein isolation, which was then performed an ELISA (Enzyme Link Immunosurben Essay) examination using anti-IL-18 monoclonal antibodies (Signal Chem. Specialist in signal proteins). Protein was isolate from tissue performed by Isolation Proteins Protocol by Smits H (1994). [18]

Data obtained in the form of data ratios were analyzed using the T test to determine differences in expression between healthy gum and gingivitis ones. The research protocols handling were approved by the Medical Ethical Committee of Moewardi Hospital, Solo, Indonesia. 2017 by Dr. Hari Wujoso

RESULTS

The samples collected were 20 male samples and 120 female samples. This acquisition is in accordance with the above research plan with balanced sample criteria. Data that is collected 22 samples aged 20-30 years and 18 samples aged 30-40 years. And in accordance with the desired sample of young adults in the presence of teeth that are difficult to grown.

From histopathological examination conducted by a pathologist by staining hematoxylin eosin seen and grouped into 21healthy gum and 19periodontitis tissue samples. From that examination it was seen in healthy gum tissue as mild lymphocytes and little visible fibrous-collagen tissue. and concluded inflammation does not occur. While in pericoronitis tissue it is seen as numerous and extensive lymphocyte cells and the formation of fibrous-collagen tissue is seen. There were showed in figure 1.

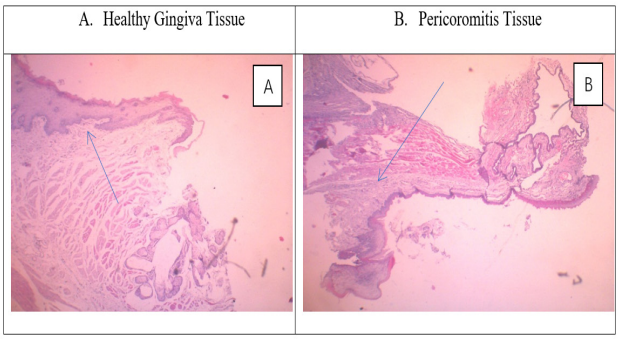

Fig. 1: A. In the healthy gingiva tissue. B. In gum tissue inflammation (pericoroniyis).

From the examination using the ELISA method, the data obtained are presented in table I. The average expression of IL-18 in healthy gum tissue was 215.75mgr/ml. While in pericoronitis is 2782.09mg/ml. By T Test statistical analysis found that there was very significant difference between healthy and pericoronitis in the expression of IL-18 with p=0.001.

Table I: The expression of IL-18 (mg/ml) in healthy gum and pericoronitis.

No.of Sample	Healthy gum	Pericoronitis A
Total	4315.05	55641.99
Mean	215.75	2782.09
SD	159.7	602.85

Table II: Paired Samples Test Analysis between group and IL-18 expression.

	Mean	Std.Error	t	df	Sig. (2-tailed)
Group –IL18	-1475.14175	230.77284	-6.392	39	.0001

DISCUSSION

Inflammation of the gum occurs as a result of complex interactions between bacteria and immunocompetent cells named pericoronitis. Inflammation will produce cytokines IL-18 and play a role in the pathogenesis of gum disease. [19, 20, 21, 22] Cellular responses are characterized by the presence of T cells into the gum tissues which are affected due to the presence of cytokines. [16, 23] Th2 is the most common in gingivitis. Th1/Th2 balance is very important in gum disease immune-regulation and influenced by antigen characteristics, antigen presenting cell and T cell interactions. [24] In gingivitis always mediated by Th1 cells, whereas the development of lesions reflects shift towards Th2 subset. [24, 25] Th1 immune response produce interferon gamma, tumor necrosis factor alpha, interleukin-18 that involve iand enhances to humoral immunity. [17, 26]

Third molar teeth experience the most impact, and extractions are a common procedure performed by an oral and maxillofacial surgeon. Patients aged over 20 years, vertical impaction is the most frequent. Class 1 impaction will increase with age, because the prevalence of grade 1 is 0%:50% at the age of 18years : 26years. Extraxtion of impacted tooth has the most frequently performed procedures for dentist. Third molar are most occur, with an average impact rate of 24%. Prophylactically, 50% of mandibular third molars impacted are extracted without any subjective symptoms, and clear indications. [27]

Important histopathological picture to see in inflammation of gum is shown the presence of epithelial ulceration, blood vesselproliferation and chronic inflammation cells. In this study the healthy gum tissue is seen as little inflammation cells like lymphocytes and vessel formation normal blood and gingivitis tissue is seen as full inflammatory cells like lymphocytes and blood vessels are enlarged with an increased number of erythrocytes. [28]

From this research we found the level of IL-18 in healty pericorona and pericorona had a significant difference between both. It's means that Interleukine-18 play a role in the pathogenesis of pericoronitis with pyroptosis occurrence. Cytokines IL-18 contribute to increasing body defense. It is evident that neutralization of IL-18 plays an important role in mediating inflammation. [20] Interleuin-18 most produced by APC. [29, 30] IL-18 plays an important role in the development of gum disease due to its proinflammatory properties. [31] Regardless of its physiological role, IL-18 is also capable of triggering chronic inflammatory reactions, eq. where interleukin-18 increased significantly in Adenomyosis, Hashimoto's thyroiditis and autoimmune hypothyroidism. [32, 33] IL-18 has also been found to increase the production of amyloid-beta associated with Alzheimer's in human neuron cells. Now known, antibodies that neutralize IL-18 can reduce the severity of the disease. Anti-IL-18 also protects the liver against cellular injuries caused by toxins. [34]

Interlein IL-18, which has recently been reported be higher in both gingival tissue and gingival crevicular fluid in patients with periodontitis [5]. Similarly, blood serum IL-18 was significantly elevated in patients with chronic periodontitis. [36] and increasing IL-18 in gingival were correlated directly with pocket depth [37]. As a result of infection and inflammation, cell death will occur. Bacteria induce cell death, including pyroptosis. Apoptosis is non-inflammatory programmed cell death that is triggered by two different pathways, the intrinsic and extrinsic pathway. Apoptosis characterized by caspase-3 activation. Necrosis is triggered by the production of ROS which is caused by bacterial. Pyroptosis is a type of programmed cell death caused by inflammatory-mediated caspase-1 companied by membrane rupture and release Interleukine-18. [9]

CONCLUSION

IL-18 candidate as a marker of pyroptosis of pericoronitis lower third molar impaction.

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