ABSTRACT

Toll-like receptors (TLRs) play a role as a bridge between innate immune system and adaptive immune system, playing an important role in body's defense against invading germs. Thus, the introduction of TLR-4 can be used as a marker for aggressive and chronic periodontitis. This research aimed to analyze the expression of TLR-4 in patients with aggressive and chronic periodontitis. The samples in this study were derived from tissue suffering from aggressive and chronic periodontitis. The number of samples in this research was 40 patients with aggressive periodontitis and 40 patients with chronic periodontitis. Then, TLR-4 expression test was conducted by using immunohistochemistry. Based on the results of T-test, a statistical test, it is known that t value was 5.697 with significant value about 0.000 (α = 5%). It is also known that there was significant difference of TLR4 protein expression between patients with aggressive periodontitis and in those with chronic periodontitis. Moreover, based on a box plot diagram, it is known that there was significant difference of TLR4 protein distribution between in patients with aggressive periodontitis and in those with chronic periodontitis. Besides, it is also known that TLR4 protein expression has an effect on the incidence of patients with aggressive periodontitis and chronic periodontitis, as well as the shape of the regression equation. Furthermore, it is known that OR estimated value for the variable expression of TLR4 protein was 1.275 (sign = 0.000). It indicates that if TLR4 protein expression increased 1 (one) unit, then the risk of aggressive periodontitis could be 1.275 times. Conclusion: The increasing of TLR4 protein expression in patients with aggressive periodontitis indicates that cytokine becomes an indicator of inflammation in aggressive periodontitis.

INTRODUCTION

Toll-like receptors (TLRs) are membrane signaling receptors that play an important role in the body's natural defense against microbes, and also help to bridge between innate immune system and adaptive immune system by inducing a variety of effector and co-stimulator molecules. The mechanism of innate immunity has always responses quickly to infection, and then spread pathogenic bacteria to the effective and well-developed adaptive immune. These molecules actually have the properties of activating pathways mediated by Toll-interleukin-1 receptors (TIRs) to activate proinflammatory transcription, namely Nuclear factor – kB (NF-κB)1,2. Two of the TLR family, namely TLR2 and TLR4, are identified as important signaling receptors for periodontal pathogenic bacterial cell wall components expressed on dendritic cells. The expression patterns of TLR, moreover, depend on the stage of maturation of TLR1-TLR5 3,4. TLR4 is the main receptor recognizing specific bacteria, and also serves as a sensor for lipopolysaccharide endotoxins of gram-negative bacteria. Thus, TLR4 regulation is complex involving specific tissues and different cells5. However, Whitsett et al.6 stated that the important family of TLRs that needed to be researched was TLR4 since this receptor has an important role to the signal response to LPS from gram-negative bacteria, but not against LPS from gram-positive bacteria. The introduction of A actinomycetemcomitans LPS causing aggressive periodontitis by TLR4 actually involves three different extracellular proteins, namely; LPS Binding Protein (LBP), CD14 and Myeloid Differentiation Protein 2 (MD2), which stimulates signaling cascade to activate NF-κB and proinflammatory cytokine products8. A research conducted by Sun et al.9 showed that the pattern of gene expression can become an indication that periodontal pathogenic bacteria will trigger TLR4 signaling and lead to aggressive periodontitis. This condition is a possibility due to the stimulation of protein expression mediated by TLRs producing an abnormal protein that plays an important role in aggressive periodontitis. Thus, disruption caused by the deficiency of TLRs function can induce fatal predisposition disruption activity of NF-κB and cytokine productions. It means that by using ligands corresponding to TLR, the immune response can be controlled leading to a new expected equilibrium9. Inflammation that occurs in periodontitis, furthermore, is a complex process started with tissue damage and

Chiquita Prahasanti1*, Aulanni’am Aulanni’am2

1Department of Periodontic Faculty of Dentistry, Airlangga University, Surabaya – Indonesia
2Biochemistry Laboratory, Faculty of Sciences, Brawijaya University, Malang – Indonesia

Available Online: 25th February, 2017

Keywords: Toll-like receptors-4, aggressive periodontitis, chronic periodontitis.
followed with repairing process. The disease is usually started with acute inflammation, and then becomes chronic one. Damage that occurs in periodontal tissues is actually caused by poor antibody response against periodontal pathogenic bacteria. Next, abnormalities that occur will be seen with attachment loss and connective tissue damage, continued with alveolar bone damage. In addition, the incidence of aggressive periodontitis in various countries around the world shows an increasing tendency. A research conducted by Albdanar et al. reported that 199 (28.8%) of 690 study subjects suffered from aggressive periodontitis, and the prevalence will be increased as the increasing of age. Moreover, a research conducted by Levin et al. also showed that 5.9% of 642 young soldiers found suffered from aggressive periodontitis, and African-Americans known have the risk of aggressive periodontitis fifteen times greater than white American. Based on the research, it is also known that there was positive relationship between smoking and the occurrence of aggressive periodontitis. Finally, oral tissue can be considered as a defense that can react quickly to various periodontal pathogens since it can identify the molecular components of pathogenic material in the mouth, and can also control them well. However, its response to microorganisms is still very low. Therefore, Toll-Like Receptors (TLRs) considered as Pattern Recognition Receptors (PRRs) play an important and very essential role in moving innate immune system, in this case TLR4 as a marker in periodontitis

MATERIALS AND METHODS
This research can be considered as an analytical observational research with case-control design involving patients with aggressive periodontitis and chronic periodontitis. Population of the research was patients with a diagnosis of aggressive periodontitis and chronic periodontitis who came to Periodontology Clinic of RSGM, Faculty of Dentistry, Universitas Airlangga. Samples were then derived from abnormal periodontal tissue during treatment with periodontal flap surgery. Next, TLR4 protein expression was immunohistochemically examined. Finally, TLR4 protein expression in patients with aggressive periodontitis and chronic periodontitis was then analyzed by using biotin-labeled antibody and then visualized with DAB-deminobenzidine.

This research was conducted on patients at Periodontology Clinic of RSGMP - Faculty of Dentistry, Universitas Airlangga with a diagnosis of aggressive periodontitis or chronic periodontitis. Based on inclusion and exclusion criteria, there were 40 samples of patients with aggressive periodontitis and 40 samples of patients with chronic periodontitis.

RESULTS
The expression of TLR-4 protein in patients with aggressive periodontitis and chronic periodontitis can be seen in the Table 1.

The mean of protein expression in those with aggressive periodontitis was of 20.65, while that in those with chronic periodontitis was 14.48. It means that the mean of TLR4 protein expression in those with aggressive periodontitis was much higher than that in those with chronic periodontitis. Next, based on the results of t-test, it is known that the variance of data obtained in those with aggressive periodontitis patients and in those with chronic periodontitis was homogeneous. T-value obtained was 5.697 (p=0.000). Thus, it can be said that the expression of TLR4 protein in patients with aggressive periodontitis was different from that in patients with chronic periodontitis.

By using simple logistic regression analysis, furthermore, it is known that the expression of TLR4 protein affects the occurrence of aggressive periodontitis and chronic periodontitis with the value of the sign about 0.000. It is also known that the risk for aggressive periodontitis was 1.275 times higher than for chronic periodontitis since the estimated OR value for the expression of TLR4 protein was 1.275 (p = 0.000). The difference of the data distribution of TLR4 protein expression between in patients with aggressive periodontitis and chronic periodontitis can be seen in the following box plot figure 2. In Figure 2, it can be known that the distribution of TLR4 protein expression between patients with aggressive periodontitis and chronic periodontitis patients was very much different.

DISCUSSION
Toll-like receptors (TLRs) can be considered as an introduction key to the structure of innate immunity. Thus, TLRs will be stimulated to release antimicrobial peptides and proteins as a result of pathogen-associated molecular patterns derived from the periodontalpathogenic bacteria. In other words, the innate immunity will be quickly recognize the infection and spread of pathogenic bacteria. Therefore, Toll-like receptors (TLRs) can also be considered as the starting point of the immune system, in which extracellular environment factors continuously give information on the cells to respond to infection and facilitate cellular responses through the top of signaling pathways in new gene transcription.

Innate immunity, acting as a sensor or the first elimination of pathogens that are selective and discriminatory, actually can identify both microbes living in the body itself and newcomer microbes, while adaptive immunity then will cause memory immunity. Consequently, TLRs will help to bridge the innate immune system and the adaptive immune system by inducing a variety of effector and costimulator molecules although the main function of TLRs is to recognize pathogens. However, TLRs unwittingly are also involved in the pathogenesis of the disease. Ferocity of natural immune system ironically can injure the host in order to maintain the host.

Furthermore, Whitsett et al. stated that the family of TLRs important to be researched was TLR4 because this receptor has an important role to the signal response to LPS from gram-negative bacteria, but not against LPS from gram-positive bacteria. TLR4 expression can be
found in various tissues and cells. The locations of TLR4 expression are: (1). in tissue including heart, lung, skin of the fetus, brain of the fetus, placenta, ileum and many others; (2), in immunocompetent cells including fetal intestine enterocytes, gastric cells, osteoblasts, endothelial cells, adipocytes, fibroblasts gingiva, periodontal ligament and gingival epithelium, smooth muscle cells, Kupffer cells, hepatic stellate cells, keratinocytes. Thus, the expression and activity of periodontal ligament can be used to see the development of aggressive periodontitis.7,13

Based on TLR4 protein expression between in patients with aggressive periodontitis and chronic periodontitis in this research, it can be said that TLR4 protein expression affects the occurrence of aggressive periodontitis, in which the risk to be aggressive periodontitis was 1.275 times greater. This statement was supported by a research conducted by Mori et al.14 showed that TLR4 ratio was higher in severe periodontitis than in mild periodontitis. Moreover, a research conducted by Beklen et al.15 proved that TLR4 expression in the connective tissue samples derived from patients with periodontitis increased compared to that in the healthy tissue. In other words, aggressive periodontitis was caused by the interaction between the host and the bacteria in the oral cavity, and this is a fundamental understanding of the pathogenesis of periodontal disease.

In addition, the main component of periodontopathogenic bacterial products, (LPS) A actinomycetemcomitans, will actually start assault and occur repeatedly causing immune response seen in the expression of TLR4. Meanwhile, polymorphisms that occur will increase the likelihood and severity of occurrence of an abnormality. According to Takahashi et al.16, genetic variants can produce variations in the structure of the tissues (innate immunity), antibody responses (adaptive immunity), and inflammatory mediators (non-specific inflammation). Therefore, susceptibility to periodontitis is actually influenced by genetic and environmental factors, such as the influence of periodontopathogenic bacteria. In other words, it can be said that genetic factors influence the immune response to bacterial infections. It means that those factors play an important role in affecting a person's vulnerability reflected in the increasing of TLR4 protein expression.

The results of this research indicated that TLR4 protein expression was directly associated with aggressive periodontitis since TLR4 was a ligand of Aggregatibacter actinomycetemcomitans germs often found in patients with aggressive periodontitis and considered as the potential bacteria stimulating the expression of TLR4. After bonding with its ligand, TLR4 will induce the translation of proinflammatory cytokines, and also bridge the innate immune system to the adaptive immune system by inducing activation of Th1 lymphocytes through molecular biology signal. The introduction of TLR4 to LPS involves several additional proteins, such as lipopolysaccharide-binding protein (LBP) which is an
acute phase protein, LBP will facilitate LPS A. actinomycetemcomitans bind to LPS receptor complex containing CD14, TLR4 and MD2. MD2 factors have an important role in the binding of LPS to TLR4 leading to more specific and more powerful binding. The results showed in this reaction indicated the increasing of the expression of TLR4 protein. The expressions of LBP and MCD14 will actually be less expressed in healthy tissue indicating an important role of periodontal pathogens as a cause of abnormality.5,17

This study was conducted on a sample of patients with aggressive periodontitis and chronic periodontitis whose oral cavity has a variety of periodontal pathogenic bacteria. Like in other infections caused by gram-negative bacteria such as in this aggressive periodontitis, TLR4 plays an important role in mediating the effects. It is because A. Actinomyetemcomitans bacteria plays an important role in aggressive periodontitis to produce endotoxin lipopolysaccharide (LPS). As a result, the cell contaminated by LPS will induce immune responses through TLR4. Thus, TLR4 expression will be increased in macrophages as well as in gingival fibroblasts experiencing inflammation. The increasing occurred is possibility because TLR4 is involved in the innate immune response against periodontal pathogens.

Furthermore, inflammatory mediators play a relevant role in the pathogenesis of the disease. Therefore, the bacteria must exist for the occurrence of periodontal disease followed by host susceptibility. The existence of germs, Aggregatibacter actinomycetemcomitans, in patients with aggressive periodontitis in this research has actually been proven by a research conducted by Thiha et al.15 who stated that the prevalence of Aggregatibacter actinomycetemcomitans found in the tissue samples was 63% in patients with aggressive periodontitis, and that was 16% in chronic periodontitis. TLR4 expressed on the periodontal ligament exposed to LPS causing damage to the periodontal ligament gives information that this situation is the beginning of the periodontal pocket case causing tooth loss.17 The mechanism of TLR4 activation is LPS as the cause of periodontal defects with the help of LBP and CD14, which then acts as a catalyst for LPS binding to MD2, so LPS/MD2 complex will be formed to interact with TLR4. The expressions of LBP and MCD14 are actually less expressed in healthy tissue indicating an important role of periodontal pathogens as a cause of abnormality.5,14,17

CONCLUSION

The increasing of TLR4 protein expression in patients with aggressive periodontitidis indicated that cytokine becomes an indicator of inflammation in aggressive periodontitis.

Table 1: The descriptive value of TLR4 protein expression in patients with aggressive periodontitis (AP) and chronic periodontitis (CP).

<table>
<thead>
<tr>
<th>Protein TLR4</th>
<th>Periodontitis</th>
<th>N</th>
<th>Mean</th>
<th>Std Deviation</th>
<th>t-test</th>
<th>t =</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP</td>
<td>40</td>
<td>20.65</td>
<td>4.912</td>
<td></td>
<td>5.697</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>40</td>
<td>14.48</td>
<td>4.782</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

REFERENCES

14. Mori Y, Yoshimura A, Ukai T, Lien E, Espevik T Har A. Immunohistochemical localization of Toll-like receptors 2 and 4 in gingival tissue from patients