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Research Report

Deoxypyridinoline level in gingival crevicular fluid as alveolar bone loss biomarker in periodontal disease

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ABSTRACT

Background: *Periodontal diseases have high prevalence in Indonesia. They are caused by bacteria plaque that induced host response to release pro inflammatory mediator. Pro inflammatory mediators and bacteria product cause degradation of collagen fibers in periodontal tissue. Deoxypyridinoline is one of pyridinoline cross-link of collagen type I that can be used as biomarker in bone metabolic diseases, however, their contribution to detect alveolar bone loss in periodontal diseases remains unclear.* ***Purpose:*** *This study was to evaluate deoxypyridinoline level in gingival crevicular fluid as alveolar bone loss biomarker on periodontal disease.* ***Methods:*** *This study used 24 subjects with periodontal diseases and 6 healthy subjects. Dividing of periodontal disease was based on index periodontal. Gingival crevicular fluid was taken at mesial site of maxillary posterior tooth by paper point and deoxypyridinoline be measured by ELISA technique.* ***Results:*** *We found increasing of deoxypyridinoline level following of the severity of periodontal diseases. There was also significant difference between healthy subjects and periodontal diseases subjects ($p<0.05$).* ***Conclusion:*** *Deoxypyridinoline level in gingiva crevicular fluid can be used as alveolar bone loss biomarker in periodontal disease subjects.*

Key words: *Deoxypyridinoline, gingival crevicular fluid, alveolar bone loss*

ABSTRAK

Latar belakang: *Prevalensi penyakit periodontal di Indonesia cukup tinggi. Ini disebabkan oleh bakteri plak yang merangsang respon tubuh untuk mengeluarkan mediator keradangan. Mediator keradangan dan produk bakteri menyebabkan degradasi serat kolagen jaringan periodontal. Deoksipiridinolin merupakan salah satu ikatan piridinium dari kolagen tipe I yang dapat digunakan sebagai biomarker penyakit metabolisme tubuh. Akan tetapi, penggunaan deoksipiridinolin untuk mendeteksi kehilangan tulang alveolar pada penyakit periodontal masih belum jelas.* ***Tujuan:*** *Tujuan penelitian ini untuk mengetahui bahwa kadar deoksipiridinolin pada cairan krevikular gingival dapat digunakan sebagai biomarker kehilangan tulang alveolar pada penyakit periodontal.* ***Metode:*** *Penelitian ini menggunakan 24 subyek penelitian yaitu 24 orang dengan penyakit periodontal dan 6 orang tidak menderita penyakit periodontal. Pembagian penyakit periodontal berdasarkan indeks periodontal. Cairan krevikular gingival diambil dari bagian mesial gigi posterior atas dengan menggunakan paper point dan diukur kadar deoksipiridinolin dengan menggunakan teknik ELISA.* ***Hasil:*** *Hasil penelitian menunjukkan ada peningkatan kadar deoksipiridinolin seiring dengan tingkat keparahan penyakit periodontal. Hasil statistik juga menunjukkan ada perbedaan rata-rata antara subyek penelitian yang tidak menderita penyakit dengan subyek yang menderita penyakit periodontal ($p<0,05$).* ***Kesimpulan:*** *Kadar deoksipiridinolin pada cairan krevikular gingival dapat digunakan sebagai biomarker kehilangan tulang alveolar pada penderita penyakit periodontal.*

Kata kunci: *Deoksipiridinolin, cairan krevikular gingiva, kehilangan tulang alveolar*

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INTRODUCTION

Periodontal disease and dental caries are the primary causes of permanent tooth loss. In Indonesia, periodontal diseases prevalence was higher than dental caries.[1] Survey Kesehatan Rumah Tangga (SKRT) of Indonesia Healthy Department in 2008 showed that 46% Indonesia's population affected periodontal disease and the prevalence increased as followed the age.[2] Periodontal diseases are inflammation and degeneration of soft and hard tooth supporting tissue that caused by dental plaque bacteria. Progressivity of periodontal diseases involves some factors, such as local, systemic and environment factor. These factors will influence host and bacteria interaction. Bacteria of oral cavity can cause inflammation by host cell activation to produce pro-inflammatory mediator.[3] Pro-inflammatory mediator causes collagen fibers of periodontal tissue degradation, including collagen cross-link of alveolar bone.[3]

Collagen cross-link reinforces collagen fibers of tissue. However, the presence of inflammation in supporting tissue causes collagen fibers degradation and also collagen cross-link destruction. The product of collagen degradation cannot be re-metabolized in body and will be released into bloodstream and excreted in urine.[4] In mature tissue, collagen type I cross-link is formed by pyridinium cross-link, such as pyridinoline and deoxypyridinoline. Pyridinoline is the most collagen cross-link in cartilage and soft tissue, while deoxypyridinoline is the most collagen cross-link in bone and ligament.[5]

Deoxypyridinoline has specificity for bone loss. Deoxypyridinoline can be used to know bone loss in osteoporosis and bone metabolic diseases, such as hyperthiroid, hyperparathyroid, and Paget's disease.[6] Shibutani showed that deoxypyridinoline level in gingival crevicular fluid, urine, and serum can be used to detect periodontitis in beagle dog.[7]

Dentists need information and examination for determining diagnostic of periodontal disease. They used clinical examination such as probing depth, bleeding on probing, loss attachment, plaque index, and radiographic examination. The advantage of this method is easy, cheap and non invasive.[7] However, this examination only can detect alveolar bone destruction in late period or the destruction is more than 3 mm.[8] Recent it is being developed procedures for a more practical examination. It uses biology indicator or biomarker. Indicator or biomarker is more specific because it is related with host resistance to local and systemic factor. Indicator use samples from biofilm plaque, gingival crevicular fluid (GCF) and saliva.[3] Periodontal diseases indicators are usually related to collagen fiber destruction, such as deoxypyridinoline, but it is still unclear and has never been proven on human. We measure deoxypyridinoline level in GCF of healthy and subjects with periodontal diseases to indicate alveolar bone loss, as a biomarker.

MATERIALS AND METHODS

This research was admitted and approved by agreement from ethical commission of Dentistry Faculty, Gadjah Mada University. Thirty patients consecutively recruited for the study at the Periodontic Department of Prof. Soedomo Dental Hospital Gadjah Mada University. All of patient must signed inform concern as agreement legally of research subject.

There were 24 patients with periodontal disease and 6 healthy subjects. Inclusion criteria: man or woman 30–50 years old, had 20 teeth minimally in oral cavity, did not have systemic disease, non smoker, did not use oral rinse, antibiotic, or drug that had calcium metabolic effect for 6 months, did not get periodontal treatment for 6 month, and were not pregnant, menstruation, or menopause. All patients also were examined loss attachment degree, probing depth, and bleeding on probing. Subjects were divided into 5 groups: patients with gingivitis, mild, moderate, and severe periodontitis, and healthy subject (as control). Clinical criteria of periodontal index used Russel's modification for determining diagnostic of periodontal diseases. Determination of tooth sample that was taken the GCF was depended on probing depth and loss attachment. Healthy subject (control) showed no loss attachment, pocket and bleeding on probing. Gingivitis showed no loss attachment and pocket, but there is bleeding on probing. In mild periodontitis, there is loss attachment less than 3 mm, periodontal pocket 3–4 mm, and bleeding on probing. In moderate periodontitis, there is loss attachment more than 3 mm, periodontal pocket 4–5 mm, and bleeding on probing. In severe periodontitis, there is loss attachment more than 3 mm, periodontal pocket more than 5 mm, and bleeding on probing.[7,9]

Having taken GCF from teeth was based on clinical examination and radiographic, particularly first molar of maxilla. If first molar was missing, it could be substituted by second molar or second premolar. However, the site of collecting sample must not be near residual ridge. GCF samples were collected using a paper point. Paper point #25 was inserted into pocket periodontal for 30 seconds gently. Previously selected tooth was isolated with sterile cotton rolls, and the supragingival plaque was removed with sterile cotton pellets.[10,11] Paper point was removed into 0.5 mL eppendorf tube and closed by paraffin tape. Then, eppendorf tube was inserted into ice box and kept in deep freezer -20 °C until deoxypyridinoline test. When paper point inserted into periodontal pocket, paper point must not make injury in gingival sulcus, because it made bleeding and influenced the result.

That eppendorf tube was holed under the tip of tube by sterile needle and given 50 µL 0.02 M phosphate buffer solutions (PBS) (pH 7.0–7.2) as solvent. Then, that eppendorf tube was put in a new 1.5 ml eppendorf tube and centrifuged in 1000xg for 20 minutes at room temperature 18–25 °C. Its procedure was for getting

GCF+PBS solutions. Eppendorf tube was centrifuged again in 1000xg for 20 minutes. Deoxypyridinoline test used ELISA technique (USCN life, China).[12]

The result were analyzed by Kruskal-Walis-H test and followed by Mann-Whitney-U test with 5 % ($p<0.05$) significant degree.

RESULTS

There was increasing of deoxypyridinoline level following the severity of periodontal diseases. The increasing of deoxypyridinoline level in periodontal diseases subjects was 4–60 times from healthy subjects (Table 1). Based on Kruskal–Wallis–H test, there was significant different in deoxypyridinoline level of subject with and without periodontal disease ($p<0.05$). Then, the result was analyzed by Mann–Whitney–U test to know mean differences between groups. It showed there is significant different of deoxypyridinoline level between healthy, gingivitis, mild, moderate and severe periodontitis subjects ($p<0.05$) (Table 2).

DISCUSSION

Based on research result, there was increasing of deoxypyridinoline level following severity of periodontal disease. The highest of deoxypyridinoline level was in severe periodontitis subjects. It showed that there was alveolar bone destruction in severe periodontitis subject more than the other groups. Research result also showed there was significant different of deoxypyridinoline level in subject with and without periodontal diseases ($p<0.05$).

Table 1. Mean and standard deviation (SD) of deoxypyridinoline level in healthy and periodontal diseases subjects (nmol/ L)

Variable	n	Mean	SD
Control	6	6.38	1.29
Gingivitis	6	25.43	3.92
Mild Periodontitis	6	142.71	27.31
Moderate Periodontitis	6	270.13	53.99
Severe Periodontitis	6	388.61	21.39

Periodontal diseases are inflammation disease that causes connective tissue and bone surrounding tooth destruction. The inflammation is started from gingival then spread into periodontal tissue. There was different pattern of tissue destruction between gingivitis and periodontitis. In gingivitis, there was just gingival inflammation surrounding the tooth and without loss attachment. In periodontitis, there were loss attachment and alveolar bone loss.[13]

In gingivitis subject, there is significant different of deoxypyridinoline level. Because gingival is formed by collagen fibers of type I, III, and V. Although, the composition of type I collagen fibers is lesser than type III and V. Alveolar bone and ligament periodontal are formed by collagen fibers of type I and III. The type I collagen fibers is higher than type III.[14] Gingival inflammation in gingivitis subjects caused type III collagen fibers degradation. De Coster research showed on immunofluoressence test, the most type III collagen fibers and some type I and V collagen fibers of gingivitis subjects was lost. There was no type III collagen fibers and less of type I collagen fibers in gingivitis.[7] Deoxpyridinoline was type I collagen cross-link and the most in ligament and bone. Deoxypiridinolin cross link is support link of type I collagen fiber.[3] It caused significant different of deoxypyridinoline level between gingivitis and peridontitis subjects.

Based on research result, deoxypyridinoline level changing was found in GCF of periodontal disease patients. GCF is inflammatory exudates fluid that seeps out into the gingival crevicular or periodontal pockets around teeth with inflammation gingival. GCF and serum contains local materials, such as tissue breakdown products, inflammatory mediators, antibodies to kill bacteria of dental plaque.[15]

In bone loss, deoxypyridinoline will be released into bloodstream and excreted into urine. Initial events are triggered by lipopolysaccharides (LPS). LPS from Gram negative plaque biofilms on the periodontal tissues. As a first line of defense, PMNs are recruited to the site. Monocytes and activated macrophages respond to endotoxin by releasing cytokines (TNF and IL-1) that direct further destruction processes. MMPs, which can act as powerful collagen-destroying enzymes, are produced by fibroblasts and PMNs. TNF, IL-1, and receptor activator of nuclear factor-kappa B ligand (RANKL) are elevated in active sites and mediate osteoclastogenesis and bone breakdown. Bone-specific markers, such as I-carboxy-telopeptide pyridinoline (ICTP) and deoxypyridinoline,

Table 2. Result of mann–whitney–u deoxypyridinoline level

	Control	Gingivitis	Mild periodontitis	Moderate periodontitis	Severe periodontitis
Control	0.001*	-	-	-	-
Gingivitis	-	0.001*	-	-	-
Mild periodontitis	-	-	0.001*	-	-
Moderate periodontitis	-	-	-	0.001*	-
Severe periodontitis	-	-	-	-	0.001*

Explanation: *: there is significant different between groups ($p<0.05$)

are released into the surrounding area and transported by way of GCF into the sulcus or pocket and serve as potential biomarkers for periodontal disease detection. When deoxypyridinoline is into bloodstream, it will penetrate and go out from blood vessel to gingival tissue through GCF (Figure 1). Pyridinium cross link such as deoxypyridinoline and ICTP was found in GCF because they were collagen cross-link that released when collagen of periodontal tissue in periodontal diseases was degraded.[16]

Deoxypyridinoline levels in GCF can be used as early marker of alveolar bone destruction in periodontal disease. Several reasons deoxypyridinoline levels can be used as early marker of alveolar bone destruction in periodontal disease are a) deoxypyridinoline is the result of type I collagen degradation and alveolar bone constituent; b) deoxypyridinoline will be released during the bone loss and will not be re-metabolized; and c) deoxypyridinoline on GCF is a safe, non-invasive and efficient biological sample to see inflammation or alveolar bone loss.[17] GCF also has good value for diagnosis of periodontal disease. GCF contains protein as periodontal tissue destruction product, one of them is deoxypyridinoline.[10]

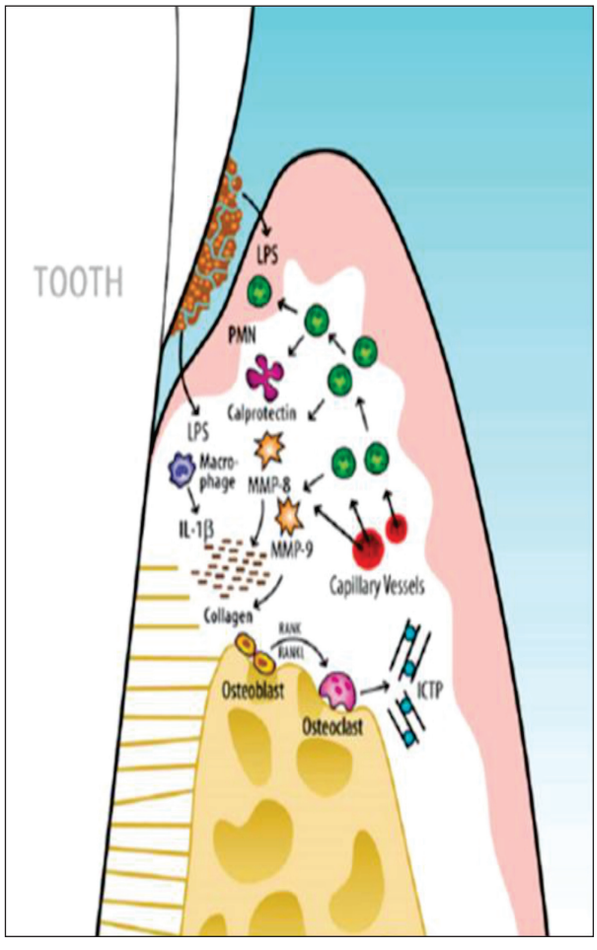

Figure 1. Schematic overview of pyridinium cross link such as ICTP and deoxypyridinoline was released into gingival crevicular fluid.[14]

Deoxypyridinoline level in periodontal disease patients is higher than control (Table 1). This indicates that since the beginning of periodontal disease is gingivitis to chronic periodontal disease, there are damage collagen fibers, which continues on dental alveolar bone destruction. Results of animal studies showed that deoxypyridinoline level increased in GCF and serum of experimental animals suffering from periondontitis.[7]

Deoxypyridinoline level was different significantly between mild, moderate and severe periodontitis subjects. There was loss attachment ad alveolar bone destruction, but the severity of destruction is different. In mild periodontitis, alveolar bone destruction just was in interdental septum of alveolar bone. In moderate periodontitis, alveolar bone destruction was less a third of bone of tooth support, and in severe periontitis, alveolar bone destruction was more than third of bone of tooth support.[17] Severity of alveolar bone destruction was related with collagen degradation of alveolar bone that was manifested in deoxypyridinoline level changing.

Deoxypyridinoline is potential agent for periodontal diseases biomarker, although, it need further research. It was caused deoxypyridinoline is pyridinium crosslink that formed between type I collagen molecules and will be released into circulation when collagen is catabolized or degradation.[17] Furthermore, deoxypyridinoline could detect progressivity of periodontal disease, since early stage (gingivitis) to advanced stage (severe periodontitis), so it could be called as biomarker. Although it need validation and qualification test, before it used widely. The tests are functioned to be accurate and good standardization and have clinical end point.[19]

This research could be concluded that deoxypyridinoline level in GCF could be used as alveolar bone loss biomarker in periodontal disease. Besides, it needed further research about the changing of deoxypyridinoline level longitudinally, so it could be used as biomarker or alveolar bone loss.

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